Initial Biodegradation Potential of Microcystin-LR by Biofilters Experiencing Sudden Cyanobacterial Blooms in Drinking Water Treatment

by

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AUTHOR'S DECLARATION

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

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Abstract

Cyanobacterial blooms have become a major cause of drinking water problems because certain metabolites from cyanobacteria are toxic to human health. Microcystin-LR (MC-LR) is one of the most commonly detected cyanotoxins. In this study, a green water treatment technology, biofiltration, was tested for its potential in removing MC-LR from lake water that was used as a source of drinking water. Previous research has shown that biofilms growing in biofilters may have the ability to biodegrade MC-LR. However, there still lacks evidence of the validity and response mechanisms of rapid flow biofilters for toxin removal when cyanobacterial blooms occur in drinking water sources.

In this study, two types of experiments were conducted: 1) bench-scale biofilter column testing, and 2) bottle shaker tests. In both experiments, liquid chromatography-mass spectrometry (LC-MS) was used to analyze the concentration of microcystin-LR in water samples, and liquid chromatography with organic carbon detection (LC-OCD) was used to monitor if natural organic matter can interfere with the removal. ATP values on the media were also measured, which indicated the biological activity of the biofilm.

In the column testing, rapid-flow anthracite/sand biological active biofilters were acclimated without MC-LR in the lake water feed. During the testing, natural water was spiked with MC-LR, and removal through the biofilters was tested. Control filters (biologically inactive) were used to test if the treatment for MC-LR is a physical process or a result of biodegradation. In addition, two different empty bed contact times (EBCTs) (9 min and 18 min) were tested to determine the effect on the removal rate. Results showed that the acclimated bench-scale columns did not remove MC-LR in the two 6-hour biofiltration tests. The biofilter with double the contact time (18 min EBCT) did not improve the performance, and the effluent concentration of MC-LR remained at a similar level to the inlet concentration. ATP results indicated that the biofilm on the biofilter media was active. However, there was no measurable removal of MC-LR when water was flowing through the columns. According to the ATP measurements, biofilter media had a more active biofilm in summer than in winter. However, this higher activity did not affect the ability of

biofiltration to remove MC-LR, and there was no removal in the summer or winter testing. Control filters did not remove MC-LR in the two filtration tests, showing that there was no removal by adsorption. This experiment suggested that the MC-LR could not be removed immediately by the bench-scale biofiltration systems if the biofilters had never contacted with MC-LR. EBCT, water temperature and seasonal water quality changes are known to affect biofilter performance, but they were not the primary influencing factors for MC-LR removal.

In the shaker testing, biofilter media was collected from three full-scale water treatment biofilters, and the media used in bench-scale biofilters was also tested. Anthracite media from 2 locations and GAC media from 2 other locations were tested. The media was mixed with water containing MC-LR in a bottle, to determine if biofilms on the media and media itself could remove MC-LR. Different types of water were tested to determine if MC-LR removal was affected by water quality changes. Autoclaved media was used as a control to test for non-biological (i.e. adsorption) removal mechanisms. Anthracite testing results showed that the biofilms on the biofilter media had the capability for removing MC-LR, but the rate was very slow. Anthracite with high ATP values from a full-scale water treatment biofilter could degrade MC-LR from 20 μg/L to 1 μg/L in approximately 40 h. Anthracite from the bench-scale columns had lower ATP values and also showed biodegradation. However, the removal rate was low, and an unknown adsorptive mechanism influenced the results. Both types of GAC media had a very strong adsorptive capacity for MC-LR, which masked the effects of biodegradation. In conclusion, this experiment showed that biofilter media revealed the potential for removing MC-LR by biodegradation. Four types of water including biofilter feed water with varying levels of nutrients, including biofilter influent and effluent water, synthetic spring water, and synthetic spring water with acetate, were tested, but results showed that nutrient supplementation did not affect biodegradation rates.

Overall, results showed that although the biofilms on the biofilter media could biodegrade MC-LR in the shaker testing, biofilter columns did not show removal in the biofiltration tests. The biodegradation rate was very slow, which could be a contributing factor to the fact that no removal was found in the filtration test. Longer contacting time (two to three days) in the shaker testing might be the main factor that influenced

the removal in the two experiments, either due to a slow rate of biodegradation or the long contact time that allowed the biofilter media to acclimate to the MC-LR and induce biodegradation of the compound. Recommendations for future work include a study of the mechanisms by which the anthracite media could biodegrade MC-LR. Future studies could also focus on pre-loading MC-LR to the biofilters to determine the potential for biodegradation in columns that have been continuously exposed to the compounds.

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

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List of Abbreviations

ATP	Adenosine triphosphate
AOP	Advanced oxidation process
DBP	Disinfection by product
DOC	Dissolved organic carbon
DOM	Dissolve organic matter
EAWTP	Elgin area water treatment plant
EBCT	Empty bed contact time
EPS	Extracellular polymeric substances
GAC	Granular activated carbon
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography mass spectrometry
LC-OCD	Liquid chromatography organic carbon detector
LMWA	Low molecular weight acids
LMWN	Low molecular weight neutrals
MAC	Maximum acceptable concentration
MC-LR	Microcystin-LR
MCs	Microcystins
NOM	Natural organic matter
NTU	Nephelometric Turbidity Units
OC	Organic carbon
ON	Organic Nitrogen
PAC	Powdered activated carbon
SSE	Sum of squared errors
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV254	Ultraviolet light absorbance at 254 nm
WTP	Water treatment plant

Chapter 1 Introduction

1.1 Problem Statement

Rapid industrial development, vast technological breakthroughs, and continuously increasing needs of humans have caused a mass of problems in water resources. These problems matter because water is necessary for human, animals, plants, and ecosystems. Although people have realized the problems, and actions have been taken to protect water for many years, water concerns are still serious, such as warmer water temperatures, hazardous chemicals, nutrient reloading, and a shortage of available drinking water.

The Great Lakes are one of the most important freshwater resources on earth since they have 18% of the world's freshwater (USEPA and Government of Canada, 1995). However, due to intensive human activities around the lakes, water quality has changed. A major problem in the Great Lakes is the occurrence of seasonal algal and cyanobacterial blooms, particularly in Lake Erie. Industrial and agricultural activities release nutrients like phosphorus into the lake, which can cause rapid growth of photosynthetic microorganisms including algae and cyanobacteria. When the cell concentration reaches a critical level, the blooms can be easily seen, and cyanobacteria in particular have a distinct appearance. Cyanobacterial blooms not only affect the aesthetic quality of water but can also release hazardous toxins, named cyanotoxins, which can be difficult to remove by traditional water treatment processes (Hoffmann, 1976).

Cyanobacteria, historically referred to as blue-green algae, are a group of aerobic photoautotrophic bacteria that easily survive in water requiring only carbon dioxide, nutrients and light. With the right environment, cyanobacteria can reproduce quickly. Different strains of cyanobacteria produce specific types of cyanotoxins as a by-product of their growth. The most investigated cyanotoxins include anatoxin-A, microcystins, nodularins, cylindrospermopsins, saxitoxins, lyngbyatoxin-a, and aplysiztoxin, which have toxic effects in mammals (Bláha et al., 2009).

One of the most commonly occurring cyanotoxins is microcystin-LR (MC-LR) (Carmichael, 1992), which can affect liver function in animals and humans (Bláha et al., 2009). MC-LR is soluble in water and non-volatile, and the major exposure path for most people is through consumption of water (Health Canada, 2002). Conventional water treatment processes are reported to have limited ability for removing microcystin-LR, and additional treatment will be needed if the toxin blooms within the source water (Hoffman, 1976; Himberg et al., 1989). Research is being done to study and identify effective and efficient treatment methods. Biofiltration is an innovative water treatment process in drinking water treatment that has been used to remove particles, organic matters and trace contaminants. The application of this technology to remove cyanotoxins has shown potential from previous studies (Cousins et al., 1996; Ho et al., 2006), but its full capacity is still unknown, particularly for seasonal blooms. As a result, biofiltration could be a potential water treatment method that removes MC-LR with the advantages of effectiveness, efficiency, and energy saving.

1.2 Research Objectives

The primary goal of this research is to determine if biofiltration is an effective barrier to MC-LR when a sudden cyanobacterial outbreak occurs at the source water of a drinking water treatment plant. This project investigated the effectiveness and efficiency of biofilter media for removing MC-LR in different types of water. Specifically, this research project aimed to:

1. Construct a bench-scale biofiltration column system at a municipal drinking water treatment plant in Ontario that is fed with water from Lake Erie.
2. Test the capability of the bench-scale biofilters in removing MC-LR from the feed water without prior MC-LR exposure.
3. Investigate the influence of acclimating water temperature on the performance of biofilters, and their ability to remove MC-LR.
4. Explore if a longer contact time can enhance the removal of MC-LR by the biofilter.

5. Test the ability of biofilter media from a full-scale water treatment plant to biodegrade MC-LR using bottle shaker tests. The removal rate and effect of different types of water with various water qualities were evaluated.

1.3 Thesis Structure

This thesis contains five chapters in total. Chapter 1 provides the problem statement and research objectives. Chapter 2 contains a literature review and identifies the research gaps. It summarizes the current knowledge of algae blooms, toxic cyanobacterial metabolites, and drinking water regulations to these toxins. Biofiltration is also introduced in Chapter 2, as well as the mechanisms and principles behind the biofiltration technologies. Applications and benefits are listed to demonstrate how biofiltration is used in water treatment processes. In addition, Chapter 2 also presents published studies to date that have assessed cyanotoxin biodegradation and removal using biofiltration. Chapter 3 introduces the methods and approaches that this research used to reach the goals. This chapter also includes the methodologies and equipment used to build experimental equipment and measure samples to collect data. Chapter 4 shows experimental results, and it is divided into two main parts, as the project involves two major types of laboratory experiments. The first part of Chapter 4 presents the results of bench-scale biofiltration column tests, and the outcomes of testing different types of biofilter media in removing MC-LR in the bottle shaker tests are in the second part. This chapter also compares the results of this project to similar experiments done in other research studies. Chapter 5 summarizes the results of the experimental data and makes conclusions to the project based on the results. Recommendations are also made for future studies and water treatment practice. Appendices are included in this thesis for supportive information.

Chapter 2 Literature Review

2.1 Biofiltration

2.1.1 General concepts in biofiltration

Filtration is a commonly used surface water treatment process for the production of drinking water. It removes particles when water passes through the pores between or in the filter media, and it is usually placed after coagulation, flocculation, and sedimentation in conventional water treatment processes. Filtration has been found to be an efficient drinking water treatment technology since the 19th century when filtration was installed in a centralized system in Scotland for industrial and domestic use (Basu et al., 2016). Sand, granular active carbon (GAC), and anthracite are commonly applied as filter media. By the rate of water passing through the filter, it can be a slow-flow filter or a rapid-flow filter.

Slow-flow filters are also called slow sand filters, which operate with a flow rate below 1 m/h and often at 0.2 m/h to 0.4 m/h. Slow sand filtration is widely applied in small treatment facilities because it removes particles through simple filtration or adsorption mechanism and removes organic matters through biological degradation (USEPA, 1997). These filters have been used for treating drinking water for more than 200 years, and have always been widely used because of low cost for maintenance and benefits for removing microorganisms and organic matters (Grützmacher et al., 2002).

Large municipalities with high drinking water demands use rapid-flow filters after coagulation/flocculation/sedimentation because of its short contact time. Comparatively, conventional rapid-flow filtration often involves disinfection, such as adding chlorine before water flows through the filter to keep the filter non-biological. Disinfectants (e. g. chlorine) depress the biofilter performance since they kill bacteria when water passes. Consequently, treatment plants that apply conventional rapid media filtration can only rely on coagulation and flocculation to remove organic matters, which contributes to the

production of disinfection by-products and bacterial regrowth in the distribution system (Basu et al., 2016). However, since the 1980s, filters with biological activities, also called biofiltration, have gained more and more attention because of the ability to solve these problems by removing organic matter (Uhl, 2008). For example, studies have found that the rapid flow biofiltration could remove organic carbon from raw natural water, and could remove contaminants including disinfection by-products (Chuang et al., 2011), phosphorus and biochemical oxygen demands (BOD) (Mitchell et al., 2016).

Biofiltration uses biofilms growing and attaching on filter media without any disinfectant. When water passes through the filter media, a certain amount of bacteria in the water attach to the media surface (Bryers, 2000; Bryers, 2008; Elhadidy, 2015). Since the flowing water continuously provides the necessities of life for bacteria, such as water, nutrients, minerals, and oxygen, bacteria reproduce fast on the media surface (Elhadidy, 2015). Meanwhile, assimilation and catabolism in bacteria remove biodegradable materials in water. The processes produce cellular and extracellular material, including organic and inorganic compounds, which become the extracellular polymeric substances (EPS) that, with bacterial communities, are the main components of biofilms (Wingender et al., 1999)

Biofiltration takes advantages of both filtration and biodegradation. In detail, filter media screens particles, in the same way as non-biological filters, which mainly involves two transportation mechanisms including diffusion and sedimentation, and also attachment mechanisms including electrostatic interactions, Van Der Waals forces, or surface chemical interactions (Amirtharajah, 1988). Meanwhile, the biofilms can remove smaller particles such as organic compounds, mineral ions. These particles diffuse into the biofilms when water is flowing through the pores. Bacteria in the biofilms consume the biodegradable organic matter, inorganic compounds, or minerals to support their growth and metabolic activities.

2.1.2 Biofilter Biofilms

Biofilms are a combination of bacterial cells and cell secretions that are also called extracellular polymeric substances (EPS). EPS, water, and various communities of bacteria together become a small

ecosystem (Sigee, 2004). EPS exchanges substances with flowing water and provides nutrients for bacterial cell survival. In the meantime, the gelatinous structure of EPS binds the bacterial communities, provides living spaces and protects them from antimicrobial substances in the environment (Flemming and Wingender, 2010).

Formation of biofilms on biofilter media surface develops in three stages (Bryers, 2008; Elhadidy, 2015) as follows:

(1) Water transports cells and nutrients to the media surface. Then, bacterial cells attach to the surface. Nutrients that deposit on the surface provide a preliminary environment for cell growth (Morton & Surman, 1994).
(2) Bacterial communities start to grow and reproduce on the surface. Biofilms develop and cellular activities excrete substances to the surrounding area. Nutrients from flowing water provide energy and other necessities for cells (Bryers, 2008).
(3) When the biofilms are growing, the thickness will increase until it reaches a maximum. The biofilm is then too thick to let nutrients and oxygen diffuse to the bacteria at the base, which weakens the biofilm so that flowing water flushes them away. When the rate of growth and detachment of biofilms reaches equilibrium, the biofilter starts into a plateau phase (Flemming, 1997).

EPS can be 50%-90% of the total organic matter in biofilms (Flemming and Wingender, 2001). According to the summary by More et al. (2014), the main components of EPS include polysaccharides, proteins, nucleic acids, lipids and humic substances, and their roles are described in Table 2.1. Polysaccharides typically take up more than half of the EPS contents (Flemming and Wingender, 2010; Wingender et al., 1999; More et al., 2014)

Table 2.1. Major components of EPS, and their significance to biofilms (Flemming and Wingender, 2010; Wingender et al., 1999; More et al., 2014).

Major components	Significance
Polysaccharides	Bind bacterial cells, attach biofilm on the media surface, adsorb organic and inorganic matter, provide nutrients, retain water
Proteins	Aggregate cells, supporting structures, adsorb organic and inorganic matters, act as electron donor or acceptor, protect cells
Nucleic acids	Transfer genetic information (e.g. DNA) from cell to cell, provide nutrients, bind bacteria.
Lipids and biosurfactants	Attach cells to surface, help bacterial communities grow.
Humic substances	Attach the biofilm to media surface, support redox activities in biofilm matrix.

2.1.3 Biodegradation in biofilters

In general, biodegradation is the process that microorganisms use to break down materials. Biodegradation is the way in which cells obtain the energy and nutrients needed for living. Since a range of aquatic toxic organic compounds are biodegradable including pesticides (Vandermaesen, 2016), cyanobacterial metabolites (Ho et al., 2012a), disinfection by-product precursors (Chen et al., 2009; Liao et al., 2015), pharmaceuticals, and personal care products (Reungoat et al., 2011), biodegradation processes can potentially be used to remove this material from water and wastewater through biofiltration processes. Furthermore, there are various environmental factors that can affect biodegradation in water biofiltration, including media type, temperature, hydraulic conditions, and backwashing (Basu et al., 2016).

2.2 Factors affecting biofiltration

2.2.1 Biofilter media

Media selection is an important factor because parameters such as composition, uniformity coefficient, effective size, specific surface area, roundness, and density can affect filter performance (Suthaker, 1996). Conventional water treatment filters often apply granular media such as sand, anthracite, granular activated carbon (GAC) as filter media. In general, uniformity coefficient and effective size are the primary factors when a conventional drinking water filtration system is designed, as they can affect the filtration performance and headloss (Suthaker, 1996). However, additional parameters are taken into consideration when a biofilter is designed because the main mechanism is not only physical and chemical but also biological. As a result, some other factors, such as the available room for biofilm attachment and the better conditions for biofilms are also important in biofiltration.

Granular activated carbon (GAC), anthracite and sand are commonly used biofilter media, and sometimes they are used in combination as dual media filters. Anthracite/sand and GAC/sand are common combinations for dual media filters. The benefit of using dual media is to reduce the effects of headloss (Crittenden, 2012). An alternative type of filter media, ceramic, has been suggested more recently because of its lower density and higher effective size, which can improve the effectiveness and efficiency of biofilters (Sharma et al., 2018). Several studies have demonstrated that GAC is more effective in removing organic matters in biofiltration than anthracite and sand as biofilter media (Wang et al., 1995; Thiel et al., 2006). Thiel et al. (2006) conducted biofiltration tests using pilot-scale biofilters to verify the performance using GAC and anthracite, as they concluded that the GAC biofilters could remove 11% to 14% of DOC, compared to only 1% to 3% removal by anthracite biofilters. However, GAC takes advantage of its adsorptive capacity for organic matters and, therefore, this may result in part account for the higher DOC removal. Wang et al. (1995) compared GAC, anthracite/sand in different biofilters having the same EBCT, and they found a 21% to 29% removal for TOC within GAC biofilters, while only 16% in the other, since GAC adsorption played a major role for the enhancement of the removal. However, they also suggested a

possible reason for better performance within GAC filters was the larger amount of biomass on GAC than on anthracite/sand, when a nearly exhausted GAC filter was compared to anthracite/sand filters. Moreover, the more porous structure of GAC can provide a larger contact area with water, and allow more biofilm to attach than the non-porous structured anthracite and sand, which can be a factor that enhances biological degradation (Basu et al., 2016). Exhausted GAC can also have better removal of organic matter than the other filter media. Emelko et al. (2006) used biofilters containing exhausted GAC and found that BOM removal was more likely due to biodegradation since the adsorptive capacity was minor within exhausted GAC. Unlike the work by Wang et al. (1995) and Thiel et al. (2006), Huck et al. (1990) showed that anthracite/sand biofilters had similar organics removal compared to GAC biofilters, even though sand and anthracite did not adsorb organic matter. Huck et al. (2000) also suggested that the filter media (GAC or anthracite) didn't affect the BOM removal when the water temperature was lower than 10 °C. Research by Sharma et al. (2018) compared two types of ceramic media, exhausted GAC and anthracite as drinking water biofilter media, and the research revealed that ceramic media could achieve the same level of biomass (measured as ATP) as GAC and two times higher than anthracite biofilters. Also, the ceramic biofilters had less organic matter removal, similar run time to the biofilters with exhausted GAC, while they had better organic matter removal, longer run time, and less backwashing requirements when it was compared to anthracite filter.

2.2.2 Empty bed contact time (EBCT)

Empty bed contact time (EBCT) is a parameter describing residence time of water in an empty bed filtration system, which represents the duration of water contacted with the filter media. It is defined as the ratio of water loading rate divided by the filter bed depth. In biofiltration, EBCT is a commonly accepted parameter influencing the biofilter performance, especially the organic matter removal which will increase when EBCT increases (Lechevallier et al., 1992; Huck et al., 1998; Wu and Xie, 2005; Zhang et al., 2017). In addition, although there was an approximate linear relationship found in increasing EBCT and increasing

the biodegradable organic matters by Zhang and Huck (1996), they also indicated that the effects by increasing EBCT could be minimized beyond a certain value of EBCT. Elhadidy (2015) investigated EBCT from 8 to 24 min and found EBCT had a significant impact on the biofilter performance. Peldszus et al. (2012) compared biofilters with different EBCTs and found a biofilter with a 15 min EBCT had greater removal of organic matter and turbidity than 5 or 10 min EBCT biofilters. Zhang and co-workers (2017) compared biofilters with 10 and 18min EBCT to remove 11 types of emerging concerning contaminants in drinking water and found that the biofilter with 18 min EBCT had much better ability to remove organic compounds than the biofilter with 10 min EBCT. A possible reason could be that slowly biodegradable natural organic matter requires a longer contact time for biodegradation (Yavich et al., 2004). However, Elhadidy (2015) found BOM removal was not directly proportional to EBCT. Comparatively, several studies found that EBCT in certain ranges had little impact on the removal of organic matter including BOM (Price, 1994), TOC (Hozalski et al., 1995) and AOC (Wer et al., 2008). Elhadidy (2015) suggested biofilter EBCT could be determined by the requirements of applications, and a shorter contact time might be sufficient for high-pressure membranes pretreatment to control biodegradable compounds important in biofouling, while longer EBCT could be applied when biofilters were designed to enhance biological stability in water.

2.2.3 Biomass

Biomass indicates the amount of growing biofilm or bacteria on the biofilter media. Viable biomass can be monitored to measure biological activities on the filter media. Acclimation is the period when the biomass develops until it is in a steady state when there is a steady removal rate of DOC (Basu et al., 2016). However, acclimation time can vary from 20 days to 16 months, and it depends on water temperature, media type, backwash and water quality (Liu et al., 2001; Basu and Huck, 2004). Velten et al. (2011) investigated attached biomass when developing a pilot-scale GAC filter, and they found a stabilized biofilm concentration and a stable effluent concentration of DOC were reached after 3-months of acclimation. Liu

et al., (2001) compared favorable and unfavorable conditions for biofilters, and results showed a significantly longer time for biofilters to acclimate in an environment with colder water, backwashing with chlorine, and less biodegradable organic matter in the feed water.

Measurements of adenosine triphosphate (ATP) have been used in recent years to represent viable biomass on the biofilter media and as a monitoring and controlling tool for biological activities in biofiltration (Velten et al., 2011; Evans et al., 2013; Pharand et al., 2014). Since research has shown that an insufficient biomass concentration could limit the DOC removal in biofilters (Carlson and Amy, 1998), the amount of biomass may be a good indicator for the performance of a biofilter. However, Pharand et al. (2014) found that in acclimated biofilters operating without any disinfectant in the feed or backwash water, the bulk ATP on biofilter media was not directly related to DOC removal by biofilters. In addition, Elhadidy (2015) also investigated the relationship between ATP and performance of biofilters removing organic matter, and the study suggested bulk ATP was not useful for monitoring performance of a biofilter since it did not have a relationship to DOC or AOC removal. Similarly, Huck et al. (2000) found that biomass level, measured by phospholipid method, was not directly related to BOM removal.

2.2.4 Backwashing

Backwashing is one of the most important procedures in biofilter operations. Backwashing directly determines the success of biofiltration because it affects the biomass growth, attachment, detachment, and accumulation. Backwashing forces media to float, move and collide and directly removes the excess biomass including bacteria and EPS. The movement of particles and flowing water also removes accumulated nonbiological particles to achieve headloss recovery. The removal of particles may have a positive impact on the biofilm attached to the media surface, for the reason that the particles may become a barrier for biodegradable compounds diffusing into the biofilms (Niquette et al., 1998). As a result, proper backwashing conditions are necessary for biofilters, especially for long-term operations (Prevost et al., 1995). Some backwashing involves air scour and chlorine to enhance the particle and biomass removal. Air

scour has been demonstrated to be effective in removing particles and headloss development in filters, and it has shown to not affect TOC removal (Emelko et al., 2006). Several studies have examined the effects of using disinfectants in backwash water (Miltner et al., 1995; Miltner et al., 1996; Liu et al., 2001; Wer et al. 2008). Miltner et al., (1995) found a significant removal of biomass with the use of chlorine in backwashing water, with few effects on BOM removal. Liu et al., (2001) found chlorinated backwashing affected BOM removal only at a lower temperature in anthracite filters and, therefore, effects depend on various conditions including water temperature, chlorine dosage, and media type.

2.2.5 Water temperature

Water temperature is an important condition to be monitored in biofiltration. Lower water temperature may hinder biological activities, which can result in reduced biodegradation and decreased organic matter removal. However, studies have found that the effects of temperature vary widely. Specifically, Huck et al., (1998) found a significant reduction of organic matter removal when water temperature was below 5 ^{o}C, and Peldszus et al., (2012) found a reduction up to 15% for DOC removal when water temperature was below 10 ^{o}C. Also, Liu et al., (2001) compared biofilters at 20 and 5 ^{o}C, and found higher water temperature led to the faster achievement of steady-state performance than lower water temperature. Comparatively, Emelko et al. (2006) found that TOC removal was not affected by water temperature when using GAC and anthracite biofilter media at the water temperature of 1 to 3 ^{o}C and 21 to 24 ^{o}C. Nishijima et al. (1998) found that temperature changed seasonally from 5 to 30 ^{o}C did not apparently affect DOC and trihalomethanes removal when the EBCT was as long as 15 min and under ozone pre-treatment. In general, other factors such as EBCT, ozonation and certain types of media can influence the temperature effects on the reduction of organic matter removal.

2.3 Contaminant removal by biofiltration

As stated earlier, biologically active filters take advantage of both filtration and biodegradation since they involve particle filtering, degradation of organic matter and in some cases adsorption (e.g., GAC). In addition to the removal of organic matter, biofilters can also be used to remove contaminants from drinking water. Liu et al. (2017) discussed the capacity of biofiltration in removing disinfection by-product (DBP) precursors. Biofiltration can also remove trace contaminants such as pharmaceuticals and personal care products, endocrine-disrupting compounds and some micropollutants in drinking water (Zearly and Summers, 2012). Hallé et al. (2015) verified the high effectiveness of biofilters in removing DEET (diethyltoluamide), naproxen and ibuprofen that were commonly present in a local river. As well, taste and odour compounds including 2-methyl isoborneol and geosmin can be effectively removed by biofiltration (Ho et al., 2007; Elhadi et al., 2006), but not by conventional treatment processes (Bruce et al., 2002). Biofiltration also has potential to remove cyanobacterial toxins, as will be discussed in more detail in the next section.

2.4 Cyanobacteria and cyanotoxins

Cyanobacteria are considered to be the earth's oldest known bacteria that produce oxygen, which can also be one of the main attributes to the oxygen-rich atmosphere on earth (Schopf, 2000; Rasmussen et al., 2008). They were previously also known as blue-green algae as the color was often blue and green, but have been identified as prokaryotic bacteria. In addition, most cyanobacteria are aerobic photoautotrophs, as their energy comes from photosynthesis (WHO, 1999). The cells possess chlorophyll a, which makes them perform like plants and generates oxygen and gain energy via photosystems I and II (Castenholz and Waterbury, 1989). The highly adaptable cyanobacteria mean that they are distributed all over the world, even at the polar regions (Paerl and Paul, 2011). They can be found in various environments such as lakes, oceans, rivers, rocks, or soil (Whitton, 2012). Cyanobacteria require some common elements in nature for

growth (nitrogen, phosphorous, iron, and trace elements) (Paerl and Paul, 2011; WHO, 1999). They can also adapt to the environment by changing cell buoyancy, which enables them to sink to deeper water levels to gain nutrients or float to the surface to receive light (Reynolds, 2006; WHO, 2011).

The commonly found genera of cyanobacteria include *Anabaena, Aphanizomenon Cylindrospermopsis, Lyngbaya, Microcystis, Oscillatoria, Phormidium, and Planktothrix* (WHO, 2015). Cyanobacteria's basic morphology includes unicellular, multicellular filamentous or colonial forms (Catherine et al., 2013; WHO, 1999). Cyanobacteria can reproduce very fast in a short period (called cyanobacterial blooms or algal blooms) when environmental conditions are favorable, for example when water contains excessive nitrogen and phosphorous, low UV radiation, and high water temperature (Paerl and Paul, 2011). With the development of industrialization, common usage of fertilizers, and fast urbanization, cyanobacterial blooms are becoming more frequent. This situation raises many problems for aquatic ecosystems and sometimes threatens human health. Blooms directly cause a shortage of oxygen for aquatic animals and plants in water and can result in increases in fish death and decreases in water quality (Robarts et al., 2005). As well, a high density of bacterial cells increases turbidity, which interferes with light penetration and negatively affects benthic habitats (Paerl and Paul, 2011).

Cyanobacteria blooms can be toxic because some species produce organic toxins (cyanotoxins) while growing (Codd, 1995; Carmichael, 2001; Chen et al., 2011). These toxins are either released to the environment or held inside the cells (WHO, 2015), however, when the cells die, the intracellular toxins are released into the surrounding water. Cyanotoxins are very toxic to aquatic organisms including fish, invertebrates, some mollusks, plants, and animals (Zanchett et al., 2013), and can directly impact the aquatic ecological equilibrium. Also, human beings can be exposed to cyanotoxins in drinking water, by activities in recreational water facilities or by eating fish or shellfish products that accumulate cyanobacteria and their metabolites (WHO, 1999).

Cyanotoxins are the metabolites of cyanobacteria, which have a variety of effects on human health. The cyanotoxin group can be divided into several categories based on the impacts on organisms: 1) neurotoxins, 2) hepatotoxins, 3) dermatoxins, and 4) toxic to other systems (e.g. kidney, cardiac,

reproductive system.) (USEPA, 2014). For example, microcystin-LR and cylindrospermopsin are toxic to the liver system, and exposure can cause liver inflammation, hemorrhaging and other symptoms such as kidney damage, tumor growth (WHO, 2015). Saxitoxins are intensely toxic to human nervous systems by blocking sodium signals in nerve axons, which causes loss of sensation and paralysis (Falconer, 2008).

Increasing occurrence and frequency of cyanobacteria blooms has become a worldwide concern. Lake Erie in North America, Lake Winnipeg in Canada, and Lake Taihu in China are examples that have a history of severe cyanobacterial blooms in which toxic cyanotoxins were found in the water (Michalak et al., 2013). Over one hundred people were affected in Brazil by a mixture of microcystin and cylindrospermopsin in 1996, and it caused 76 deaths because of the insufficient treatment for drinking water (Jochimsen et al., 1998; Carmichael et al., 2001). Algal blooms have caught much attention in North America, especially in the Great Lakes. Toxic cyanobacterial metabolites have been found in Lake Erie (Michalak et al., 2013), Lake Ontario (Makerewicz et al., 2009), and parts of Lake Michigan (Rediske, 2015). Lake Erie is the shallowest among the Great Lakes, and the water temperature is typically higher than other one of the Great Lakes. Also, many nutrients, mainly agricultural fertilizer, enters the lake from the connecting river watershed, especially the Maumee River, which contributes to the favorable environment for cyanobacteria blooms to develop (Miller et al., 2017). In 2011, a large cyanobacterial (algal) bloom took place in Lake Erie, with bloom area of about 5,000 km^2, and was over seven times greater than any previous algae blooms (Michalak et al., 2013). The maximum microcystin concentration reached was over 4,500 μg/L in early August 2011 (Michalak et al., 2013). As millions of people are relying on the water in the Great Lakes, cyanotoxins are regarded as a severe threat to human health (Miller et al., 2017).

Since there is an increasing number of cyanotoxin concerns to human drinking water resources, guidelines and regulations have been developed by governments and institutions around the world to monitor and control cyanotoxin concentrations in drinking water. Table 2.2 shows the guidelines for microcystin-LR in different countries. The regulations are typically set for MC-LR because it is one of the most detected microcystins, and it also has sufficient available toxicology data to set up guideline values for drinking water (WHO, 2017). Regarding other types of cyanotoxins, Australia is the only country that

has set a 1.3µg/L for saxitoxin as a guideline concentration for drinking water. Another two common detected cyanotoxins, cylindrospermopsin and anatoxin-a, have a suggested guideline value of 1µg/L in drinking water based on Falconer (2005) and Fawell et al. (1999).

Table 2.2. Official guidelines values for microcystin-LR concentrations in drinking water.

Organization	Value name	Value	Source
Government of Ontario	Maximum Acceptable concentration (MAC)	**1.5 µg/L**	O. Reg. 169/03: Ontario Drinking Water Quality Standards under Safe Drinking Water Act, 2002, S. O. 2002, c. 32
Health Canada	Guideline value: Maximum acceptable concentration (MAC)	**1.5 µg/L**	Health Canada, 2002
USEPA	Ten-day Health Advisory	**0.3 µg/L** (for bottle-fed infants and young children of pre-school age) **1.6 µg/L** (for school-age children through adults is 1.6 µg/L)	USEPA, 2017
Government of Australian	The Australian Guidelines	**1.3 µg/L** Microcystins (as microcystin-LR toxicity equivalents)	National Health and Medical Research Council/Natural Management Ministerial Council, 2004
WHO	Guideline value	**1.0 µg/L**	World Health Organization, 2017
Minnesota Department of Health	Drinking water advisory	**0.1 µg/L**	USEPA, 2017
New Zealand Ministry of Health	Maximum acceptable value	**1.0 µg/L** Microcystins (as microcystin-LR toxicity equivalents)	New Zealand Ministry of Health, 2006

2.5 Municipal drinking water treatment processes for removing MC-LR

Cyanobacterial or algal blooms in surface water sources have become a severe concern in recent years because they can adversely affect aesthetics of raw water, clog filtration systems, which may result in cyanobacterial toxin outbreaks that may enter water supply through insufficient treatment or the release of toxins from dead cells in water collection and treatment (WRF, 2010). One of the most commonly found cyanotoxins, MC-LR has been shown to have limited removal in the conventional water treatment train including coagulation, sedimentation, and filtration (Hoffmann, 1976; Himberg et al., 1989; Şengül et al., 2018). Other drinking water treatment processes including activated carbon, oxidation processes, and

membranes have been investigated to determine their ability to remove cyanotoxin variants, and some of them can work effectively. The removal of cyanobacteria cells and intracellular cyanotoxins are not discussed in this review.

2.5.1 Activated carbon

Many cyanotoxin variants can be effectively removed by granular activated carbon (GAC) (Keijola et al., 1988; Newcombe, 2002; Huang et al., 2007) and powdered activated carbon (PAC) (Keijola et al., 1988; Cook et al., 2000; Ho et al., 2010). Activated carbon is made from different materials, commonly wood, coal and coconut and have demonstrated different levels of effectiveness in adsorption of cyanotoxins (Westrick, 2010). Pore sizes influence adsorption of the toxins as well (Ho et al., 2008)

GAC is commonly used as a filter media in water treatment processes. In terms of MC-LR, Huang et al., (2007) compared different types of GAC, and they found that the coconut shell based GAC with the highest density of pores could adsorb most MC-LR and have the fastest removal rate compared to coal-based and wood-based GAC, even if the latter two also had very effective removal. Chennette (2017) verified virgin GAC could have the ability to remove MC-LR by adsorption completely, and the study examined the kinetics of the adsorption and found they could be better described by pseudo first-order reaction than the pseudo second-order reaction. However, GAC was demonstrated not to be an effective adsorber when continuous MC-LR is treated, and a breakthrough of the toxin at effluent was found in a study by Newcombe (2002) after 60 days of treatment. The results suggested that GAC could not be utilized for a long-term MC-LR treatment, and replacement or regeneration of GAC was necessary to ensure proper treatment. Chennette (2017) compared preloaded GAC and virgin GAC and found the adsorption of MC-LR could be hindered if the GAC was preloaded in natural water. Differences of MC-LR adsorption through preloaded and virgin GAC was also found by Ho and Newcombe (2007) because NOM could compete with MC-LR for adsorption sites on GAC.

Water treatment utilities often regard PAC as a flexible adsorptive treatment method compared with GAC, but it also has some challenges for operation and, therefore, trends to be used as needed (Vlad et al., 2014; Chennette, 2017). For example, PAC dosage could be managed and controlled when emergency contamination happens in raw water. Ho et al. (2011) investigated microcystin variants and cylindrospermopsin and found that PAC was very effective at removing these two cyanotoxins. Results of the study also showed that water quality (two different raw water with distinct UV absorbance) and contact time (30, 45 and 60 min) didn't have effects on removal of the toxins. Donati et al. (1994) investigated eight different types of PAC in ultrapure water and natural river water to adsorb 2.5 mg/L MC-LR, and the study found that PAC with the largest amount of mesopores had the greatest removal. It also showed that natural water could importantly reduce the removal rate at the beginning and the maximum removal rate when it was compared to the testing with pure water. In addition, Liu (2017) did batch testing to examine the capacity of different types of PAC adsorbing MC-LR in natural water and pure water, and the study found the fastest adsorber can reach an equilibrium at 1.5 h with a nearly 100% removal (initial 100 μg/L MC-LR) in natural water. The same type of PAC adsorbed MC-LR fastest in ultrapure water with equilibrium time of 1 h and 100% removal. Further, the study suggested the adsorption of MC-LR was hindered by the natural water compared to the ultrapure water, possibly due to the dissolved NOM competed in the adsorption processes.

2.5.2 Oxidation processes

Commonly applied disinfection agents, such as chlorine, can effectively degrade cyanotoxins including microcystins (Nicholson et al., 1994; Newcombe and Nicholson, 2004), cylindrospermopsin (Rodríguez et al., 2007) and saxitoxins (Nicholson, 2003). Although by-products were discovered following the chlorination of microcystins and cylindrospermopsin, they were determined to be non-toxic, which were tested by Nicholson et al. (1994) and Banker et al. (2001), respectively. However, some types of cyanotoxin such as anatoxin-A are resistant to chlorine (Newcombe and Nicholson, 2004). Other oxidants such as ozone

are very effective at degrading cyanotoxins. Ozone has shown to be a more effective oxidant than permanganate and chlorine, to remove MC-LR, cylindrospermopsin and anatoxin-A (Rodríguez et al., 2007).

Other strong oxidants also have the potential to be an effective treatment for cyanotoxins. Ultraviolet/hydrogen peroxide (UV/H_2O_2) is an advanced oxidation process (AOP) which combines a powerful oxidant and hydroxyl radical with UV light to oxidize contaminants such as cyanotoxins (He et al., 2015). UV/H_2O_2 has been demonstrated to have a high degradation rate for microcystins (Li et al., 2009; Qiao et al., 2006). UV/H_2O_2 could also remove other cyanotoxins including cylindrospermopsin (He et al., 2013; He et al., 2015) and anatoxin-A (Afzal et al., 2010). Chang et al. (2015) found that a combination of UV and ozone were more effective in removing microcystin-LR than only UV or only ozone. Zhu et al. (2015) found a similar result, and they also found that two other common microcystin variants (microcystin-RR and microcystin-YR) were also degradable by UV/ozone.

2.5.3 Membranes

Membrane filtration is a newer technology for drinking water treatment, where highly pressurized water is filtered by the pores within the membrane system because of a pressure differential, in order to separate unwanted particles, ions and contaminants. However, maintenance, fouling, and high energy demands are the main barriers to wider application. Membrane filtration can be useful for removing cyanobacteria and cyanotoxins including MC-LR (Gijsbertsen-Abrahamse et al., 2006). For example, nanofiltration was demonstrated to effectively remove more than 90% of microcystins and cylindrospermopsin in a bench-scale membrane test by Dixon et al. (2011). Additionally, this study discovered that the concentration of NOM did not affect the removal of microcystins. Teixeira and Rosa (2006) suggested that nanofiltration can be an effective treatment to remove microcystins from drinking water and found that more than 95% removal of MC-LR (initial concentration of 10 μg/L) was achieved at a 90% water recovery rate. Gijsbertsen-Abrahamse et al. (2006) also found the removal of 99% dissolved

MC-LR when they used nanofiltration membrane at a viable influent concentration range from 5.5 to 9.0 μg/L.

2.5.4. Biofiltration

2.5.4.1 Biodegradation of MC-LR

MC-LR was found to be a biodegradable compound in the 1990s (Jones and Orr, 1994; Cousins et al., 1996). Cousins et al. (1996) conducted MC-LR biodegradation tests with natural water and found that a low concentration (10 μg/L) MC-LR could be completely degraded in a week. Another experiment confirmed that both aerobic and anaerobic microorganisms in drinking water sediment slurries could degrade MC-LR, and anaerobic bacteria could quickly remove MC-LR with the addition of nitrogen sources (Holst et al., 2003). Microcystin-LR was shown to be more easily biodegraded compared with other types of cyanotoxins. The ability of bacteria found in natural water to biodegrade various types of cyanotoxins including microcystin-LR, cylindrospermopsin, geosmin, and saxitoxins was investigated by Ho et al. (2012b). Their work showed MC-LR was the most easily biodegraded cyanotoxin, with a rate at 24 °C of 1.9 μg L^{-1} d^{-1}, and that cylindrospermopsin (0.6 μg L^{-1} d^{-1}) and geosmin (7.1 ng L^{-1} d^{-1}) were less easily biodegradable. Saxitoxins were the only species that could not be biodegraded (Ho et al., 2012b).

Microcystins have a common structure of cyclic heptapeptides, and all of them contain a unique β-acid named Adda and two variable residues, that result in the different microcystin variants. For MC-LR, the variable residues are leucine and arginine (Carmichael, 1992). Cousins et al. (1996) provided the evidence that Adda side chain in the MC-LR structure was the site that is initially attacked during biodegradation, and this finding was also verified by Bourne et al. (1996). A strain of *Sphingomonas* was isolated from natural water that could biodegrade MC-LR (Bourn et al., 1996; Harada et al., 2004; Ishii et al., 2004). Bourn et al. (1996) found that at least three types of enzymes were identified in the cell extracts, and they were responsible for the degradation of MC-LR. The enzyme activities are directly involved in peptide hydrolysis, damage to the cyclic heptapeptide, and linearization of the structure. The linearised

intermediate substances are then decomposed to smaller molecular weight substances by the presence of other enzymes, and the intermediate compounds are found to be non-toxic (Bourn et al., 1996). *Morganella morganii* was another recognized microcystin-degrading bacterium isolated in the water from Lake Mead, Nevada, USA (Eleuterio & Batista, 2010). Yang and co-workers (2014) also found that *Bordetella* species from Taihu, China, could biodegrade MC-LR, where cyanobacteria blooms have occurred several times. This strain also had the *mlrA* gene that was found in *Sphingomonas*, and the gene was confirmed to participate in MC-LR biodegradation (Bourne et al., 2001; Yang et al., 2014; Li et al., 2012). The research of biodegradation for MC-LR by bacteria is significant for water treatment because it shows potential for biofiltration removing MC-LR in drinking water biofilters.

2.5.4.2 MC-LR removal by biofiltration

Several studies have found that slow-flow biofilters can remove cyanotoxins including MC-LR with the help of biofilms growing on the filter media (Grützmacher et al., 2002; Ho et al., 2006; Bourne et al., 2006). Grützmacher et al. (2002) conducted full-scale experiments using slow sand filtration to treat water with 5.9 to 12.8 µg/L total microcystins using a filtration rate of 0.033 m/day. The results showed that the slow sand filter could effectively remove 43% to 99% microcystins, with 43% removal found in low temperatures (less than 4 °C) and more than 90% removal detected at moderate temperatures (10 to 20 °C). The reduction of removal ability was possibly due to the low temperature because it might hinder the biodegradation of microcystins (Grützmacher et al., 2002). In addition, Ho et al. (2006) tested slow sand filters with and without 12-month pre-exposure to MC-LR, and they found that there were no MC-LR detected (100% removal) in the effluent of the pre-exposure filter with 22 µg/L MC-LR in the influent of the filter. However, toxin breakthrough was detected at the effluent of the biofilters that had not been pre-exposed to MC-LR in the first three days after the toxins were added, but after that, they were able to eliminate MC-LR. Consequently, results of this study showed that biofilters might need acclimation time for removing MC-LR. Bourne et al. (2006) investigated slow sand filters with a flow rate of 0.017 m/h in

order to treat river water with an initial MC-LR concentration of 50 μg/L, and they found that the filter reached 100% removal of MC-LR at 12 days. They also found that inoculating a bacterium (*Sphingomonas* sp.) to the media could enhance the biofilter removing MC-LR with 90% removal in the first two days, while the uninoculated filter did not remove any MC-LR until the fourth day. Somdee et al. (2014) conducted a flask testing and found that *Novosphingobium* sp. KKU15 could completely degrade 5 mg/L MC-LR in 3 days after it contacted with MC-LR in a mineral salt medium. They also investigated a slow sand filter inoculated river sand as the filter media with the same bacteria used in the flask test and fed with the same mineral salt medium at a flow rate of 0.19 m/h. The results suggested that no breakthrough of MC-LR can be achieved (100% removal) after seven days with an influent MC-LR concentration of 5 mg/L, while no removal was detected in the uninoculated control filters (Somdee et al., 2014).

There are only a few studies that investigated MC-LR removal through rapid flow biofilters. Ho and co-workers (2006) showed that rapid flow biofiltration could also remove MC-LR. In their experiments, bench-scale columns were filled with biologically active sand from a drinking water treatment plant in Australia. They tested EBCTs that ranged from 7.5 to 30 min (filtration rate of 0.3 to 1.2 m/h) and used raw water spiked with 20 μg/L MC-LR. They found the almost complete removal of MC-LR over the range of EBCTs tested following an initial acclimation period by using water spiked with MC-LR. Moreover, Eleuterio (2007) also tested rapid flow biofilters for removing MC-LR. The EBCT and hydraulic loading rate were 7.5 min and 2.5 m/h, respectively, and the biofilter was acclimated with nutrients but without MC-LR. After feeding the biofilter with 20 μg/L MC-LR, the results showed a maximum of 80% removal was achieved six days after spiking. Consequently, biofiltration has shown the capability of removing MC-LR through biodegradation. However, these studies all show that acclimation of the biofilters with microcystin pre-exposure was necessary in order to be an effective treatment method dealing with emerging MC-LR outbreaks.

2.6 Research needs

Biofiltration has gained much interest because it can remove not only particles in water, but also organic matter including NOM, DBP precursors, pharmaceuticals, and micropollutants and, therefore, it has the potential for removing cyanobacterial toxins. Biofiltration has been shown in previous studies to remove MC-LR through biodegradation. However, research needs were identified through the reviewing of the current literature. Specifically, there is only limited evidence that shows biofilter performance in removing microcystins under various conditions (Ho et al. 2006; Eleuterio, 2007). Little study has been done that focuses on investigating the biofilters capacity to deal with sudden outbreaks of MC-LR at the source water of a drinking water treatment plant. In particular, previous studies have mostly used biofilters that have been pre-exposed to cyanotoxins. However, toxin acclimation is not practical in a real situation where cyanobacterial blooms only occur seasonally, as is the case in Ontario. Therefore, studies that assess the ability of biofiltration at full-scale drinking water treatment plants to respond to seasonal cyanotoxin occurrences are needed. Furthermore, factors that influence biodegradation of MC-LR in biofilters remain unknown, and studies have not reached consistent conclusions.

Chapter 3 Materials and Methods

3.1 Microcystin-LR preparation and analysis

Microcystin-LR (MC-LR) was obtained from Cedarlane (Ontario, Canada), and the original supplier was Cayman Chemical Co. (MI, USA). The pure microcystin-LR (MC-LR) was solid, and it was stored in a freezer at -20℃. MC-LR stock solutions (20 mg/L) were prepared by dissolving 1 mg of MC-LR in 50 mL ultrapure water. Ultrapure water was obtained using a Millipore Milli-Q® PLUS water system. Stock MC-LR solutions were stored in the freezer at -20℃.

A Shimadzu 8030 Liquid Chromatography-Tandem Mass Spectrometer (LC-MS/MS) system was used to analyze MC-LR concentration in water samples. The method was previously described by Chennette (2017). The system consisted of two Shimadzu LC-20 ADXR pumps, a Shimadzu DGU-20A3R degassing unit, and a Shimazu SIL-20AC XR auto-sampler. The analytical column was the 50 mm × 2.1 mm Pinnacle DB C18 column with 1.9 µm packing manufactured by Restek (PA, USA). The column was protected with a Trident TM in-line guard cartridge holder containing a 10 × 2.1 mm Pinnacle DB C18 (5 µm packing) guard cartridge. HPLC grade acetonitrile (Sigma-Aldrich, Oakville, ON) with 0.1% formic acid was used as mobile phase B. The analytical column temperature was 35℃. Ultrapure water with 0.1% formic acid was mobile phase A. An internal standard, Cyclo (Arg-Ala-D-Phe-Val) was obtained from Peptides International (KY, USA) and was used as described by Chenette (2017). 100mg/L stock solution of the internal standard was prepared at the working concentration of 50 µg/L and stored at -20℃. MC-LR calibration curves were established using 7 points that ranged from 0.1 to 40 µg/L, and a sample calibration curve is shown in Appendix A. Before LC-MS/MS analysis, water samples were filtered with Clarinert™ 0.45 µm pore size, 13 mm diameter nylon syringe filters (Agela Technologies, DE, USA). For each water sample, 20 µL was injected into the analyzer.

3.2 Water quality analysis

The turbidity of water samples was measured by Hach 2100P Turbidimeter (Loveland, CO, USA). pH was measured by an Orion 410A pH Meter (MA, USA) during the experiment. Additionally, pH and turbidity data were also collected by the online monitoring systems at full-scale treatment plants in this study and provided to us by the plant staff.

3.3 LC-OCD analysis

NOM components were measured by LC-OCD (DOC-Labor Dr. Huber, Karlsruhe, Germany). The primary mechanism of the system is to separate organic matter by sizes in a column, and three detectors are placed online to measure organic carbon (OC), organic nitrogen (ON), and the ultraviolet light absorbance at 254 nm (UV_{254}) (Huber et al., 2011). Within the LC-OCD analyzer, five major components can be separated by size or by the apparent molecular weight including biopolymers, humic substances, building blocks, low molecular weight acids (LMWA), and low molecular weight neutrals (LMWN) (Huber et al., 2011). The LC-OCD was also used to measure DOC.

Water samples analyzed by LC-OCD were collected at the Elgin Area Water Treatment Plant during the time that the biofilters were acclimated at the plant. LC-OCD water samples were also collected during the two biofiltration tests at the University of Waterloo. Raw water samples were collected from the intake water tank, and treated water samples were collected from the three biofilters effluent sample ports. Additionally, water samples used for batch shaker testing from three of Ontario's water treatment plants were also analyzed by LC-OCD, which were collected from the sampling ports immediately before and after the biofilters at the plants. All water samples were placed in 500 mL glass bottles, transported in a cooler with ice packs and stored in the fridge (4 ºC) at the University of Waterloo lab. Water samples to be analyzed by LC-OCD were filtered using Supor® 450, 47 mm diameter, 0.45 μm pore size membrane filters (PALL Life Science, MI, USA) and then stored at 4 ºC before analysis. The 0.45μm membrane filters were

rinsed with 300 mL ultrapure water before filtering each water sample. The LC-OCD is housed and operated by Dr. Huck's research group under the guidance of Dr. Sigrid Peldszus. Rachel Trowel, Andrea Steuer, and Lin Shen were the operators of the system during the current research, and they were responsible for calibration, analyzing water samples, maintenance of the system, and providing raw data. Quantitative analysis of the data was done using the software ChromCALC, DOC-Labor (Karlsruhe, Germany).

A typical chromatogram for LC-OCD analysis of the raw water taken in Lake Erie is shown in Figure 3.1. The first identified fraction separated by LC-OCD was the biopolymers that have the high molecular weight of more than 10 kDa and are mainly composed of proteins, amino sugars, and polysaccharides (Huber et al., 2011). The biopolymer fraction elutes at 25 to 35 min. Eluting at around 45 min is the humic substances, which have an intermediate molecular weight greater than 2 kDa but less than that of biopolymers (Huber et al., 2011). In most of the natural water samples, building blocks appear after the humic substances peak, which may be composed of breakdown fragments of humic substances according to Huber and his co-workers (2011). Low molecular-weight organic matter elutes last and can be identified as low molecular-weight acids and low molecular-weight neutrals. Huber et al. (2011) found that some small molecular weight humic substances can elute at the same time with low molecular-weight acids. In this experiment, the low molecular-weight acids fraction was calculated to include the low molecular weight humics.

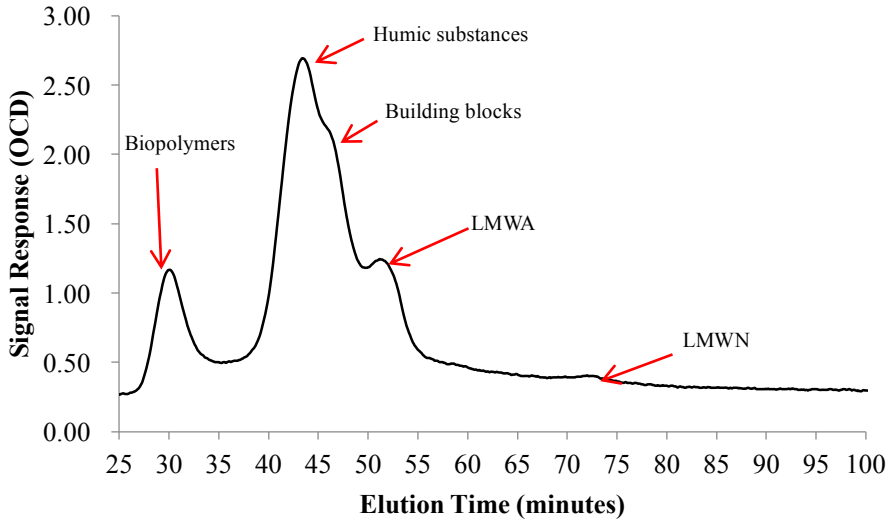

Figure 3.1. A sample LC-OCD chromatogram for the natural water (untreated) from Lake Erie with the different fractions identified. LMWA refers to low molecular weight acids, and LMWN refers to the low molecular weight neutrals.

3.4 ATP analysis

Total microorganisms on media samples were measured by detecting the concentration of adenosine 5'-triphosphate (ATP). A LuminUltra^{TM} DSA test kit was used to measure ATP (LuminUltra, Fredericton, New Brunswick, Canada). The procedures for measuring ATP were as follows. Three grams of media samples were collected and stored in sterile 10 mL plastic vials. Media was then rinsed by gentle inversion using water collected from the biofilter to remove loose particles. One gram of rinsed media was added to the 5 mL Ultralyse 7 tube, and they were mixed well for 5 min. Then, 1 mL of the lysis solution was added to a 9 mL Ultralute (dilution) tube and mixed. Both 100 µL from the Ultralute tube and 100 µL of Luminase were transferred to a clear 1.5 mL assay tube, mixed and measured using a Modulus^{TM} Luminometer (Turner Biosystems, CA, USA). The ATP standard provided by the kit was tested as described by the manufacturer. The formula for calculation of ATP values on media samples is shown in Appendix B. ATP concentration was expressed as ng ATP/cm^3 by converting ng ATP/g by the density of media samples.

3.5 Biofilter column experiments

3.5.1 Bench-scale biofiltration set-up

The bench-scale biofiltration set-up was made for the biofilters to acclimate at the Elgin Water Treatment Plant. It had three main components including three biofilters, a roughing filter, and a driving pump. Specifically, three 2.54 cm ID, 60 cm long glass columns (Biofilter 1, 2, and 3) were filled with new filter media. Biofilter 1 (BF1) and Biofilter 2 (BF2) were duplicates, and they were filled with a depth of 22 cm anthracite at the bottom and 8 cm sand on the top. Biofilter 3 (BF3) was an extended biofilter for BF1, which doubled the contact time. BF3 was filled with 30 cm sand. The anthracite used in this study had a uniformity coefficient (UC) of 1.5 with an effective size (ES) of 1 mm, while the sand had a UC of 1.5 and ES of 0.5 mm. Two flow meters (VWR® FR4000 Series Water Flowmeters, PA, USA) were placed before BF1 and BF2 to monitor the flow rates, and flow was controlled by a flow-rate adjustment knob on the flow meter and the driving pump. Sampling ports were placed after each column. A MasterFlex® L/S® Peristaltic Pump, manufactured by Cole-Parmer® (QC, Canada) was used to drive water flowing through the bench-scale system. All the filters were operated in an up-flow pattern during acclimation to reduce the backwashing requirements. Appendix C shows the pictures of the settings at the Elgin Area Water Treatment Plant.

The bench-scale biofilters were operating using raw water from the Elgin Area Water Treatment Plant (EAWTP). The use of raw water instead of treated water is because (1) Elgin Area Water Treatment Plant applied chlorination after sedimentation, which could eliminate bacteria in biofilters, (2) the chlorine content fluctuated, and it was difficult to apply dechlorination, (3) there was no access to the water before dosage of chlorine and after sedimentation. Lake Erie has historically experienced intensive cyanobacteria blooms because of warmer water temperature, a shallower depth than the other Great Lakes, and plenty of nutrient sources (Miller, 2017). However, the full-scale filters at the Elgin plant were not operating

biologically. The bench-scale biofilters in this study contained the same media as the filtration system in the plant, which consisted of dual-media filters (anthracite and sand in a ratio of 11:4). Additionally, a low-concentration of chlorine (from May to December) was dosed at the intake of the EAWTP to prevent zebra mussels. Therefore, as discussed below, a GAC roughing filter was included as a pre-treatment filter before the water entered the biofilters during acclimation. The roughing filter also acted to remove some turbidity from the influent water before the biofilters. A similar method was used in the study of Chennette (2017) when she needed to preload GAC filters without any disinfectant. The GAC roughing filter was a 5 cm inner diameter and 60 cm high glass column with a media bed depth of 20 cm giving an EBCT of 9 min. Exhausted coal-based GAC (Calgon Carbon, PA, USA) was acquired from the Region of Waterloo. Complete removal of chlorine by the GAC was tested using tap water at the University of Waterloo. Chlorine removal was also tested on-site during biofilter acclimation and the roughing filter could effectively remove the low-dosage chlorine during seasonal prechlorination. Two sampling ports were placed before and after the GAC filter for water quality and chlorine content monitoring during the acclimation process. The chlorine concentration before and after the GAC roughing column was tested twice a week by a Tijana Rajic (Elgin and Huron Area Water Supply Systems personnel).

At the Elgin DWTP, the biofilters were mounted on a PVC board (Figure C.1). Starting on November 3, 2016, the biofilters were fed from a 1,893-Litre feeding tank (Figure C.2) to provide continuous input of natural water. The system was continuously operated from November until August with an average flow rate of 20 mL/min. The system was flushed with a rate of 100 mL/min every two weeks to eliminate particles clogging the system. When a new biofilter starts to operate, there is a period for the biofilter to acclimate to build mature biofilms and biomasses until it is in a steady state when organic matter removal rate is stable (e.g. Basu et al. 2016). The primary purpose during acclimation was to make media and bacteria in contact with natural water instead of trying to accomplish filtration.

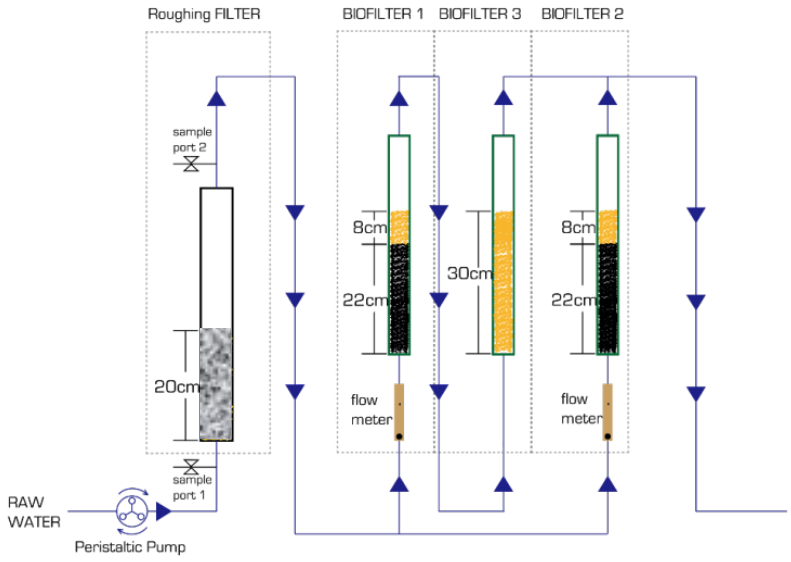

Figure 3.2. Design of the biofilters' acclimation system. Grey particles within the roughing filter were GAC, while black particles in the biofilters represent anthracite and yellow ones represent sand. Arrows show the flow direction, all filters were run with up-flow.

Raw water samples, and biofilter influent and effluent water samples from each column were collected every month, and they were transported on ice packs to the lab at the University of Waterloo for LC-OCD analysis. pH, temperature and turbidity of the raw water during biofilter acclimation were recorded every 4 h by the plant, and the original data were provided by Tijana Rajic.

3.5.2 MC-LR removal by bench-scale biofilters

Testing for MC-LR removal by the bench-scale biofilters was done at the University of Waterloo. Design of the setup is shown in Figure 3.3. BF1, BF2 and BF3 that had been continuously acclimated at the EAWTP were taken back from the plant on March 27, 2017. Appendix D shows the experimental settings at the University of Waterloo Lab. After the experiment to test for MC-LR removal on March 27, the filters were sent back to the Elgin plant and continued to run with raw lake water until the second MC-LR removal experiment on August 17, 2017. During transport, the columns were filled with biofilter feed water to keep

the biological activities, and they were also wrapped with sponge pads and bubble wrap to keep the temperature constant. At the University of Waterloo, the columns were mounted on a metal frame and operated with raw water collected from the EAWTP at a flow rate of 18 mL/min (EBCT=9 min, hydraulic loading=1 m/h) immediately after they were in the lab (Figure D.1 and Figure D.2). The raw water (40 L) was collected from the raw water sampling port at the plant. Chlorine residual was also tested, and results showed that no free chlorine or total chlorine was detected in the raw water. A down-flow pattern was used in the MC-LR removal experiment instead of up-flow used during acclimation to better simulate operations and because the filtration test ran for only several hours so that clogging would not interfere with the operation.

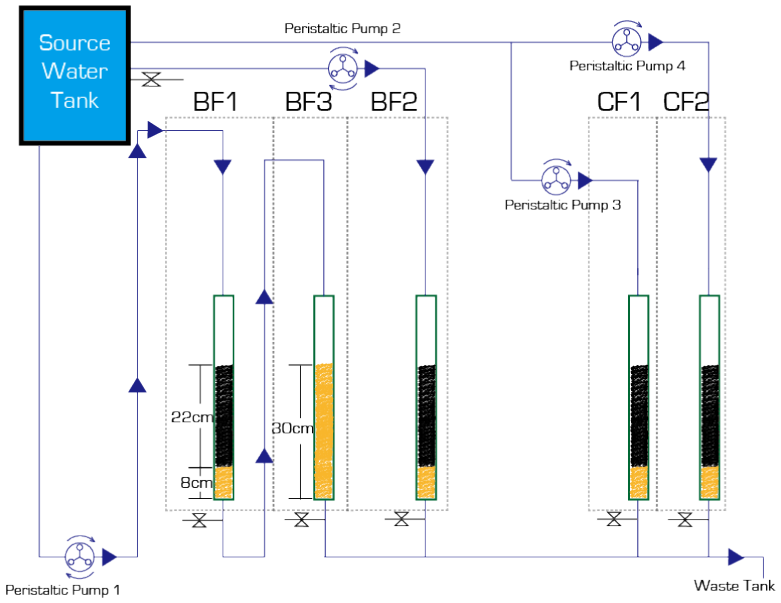

Figure 3.3. Design of the biofilter experimental setup during MC-LR removal testing. Black particles in the biofilters were anthracite and yellow ones were sand. Arrows show the flow direction.

The set-up at the University of Waterloo also included control columns. Control filter 1 (CF1) and Control filter 2 (CF2) are the same filters with BF1 and BF2, but they were filled with washed new media before the experiment started (Figure D.1 (b)). Each flow line (BF1/BF3, BF2, CF1, CF2) was

driven by an individual peristaltic pump (Cole-Palmer, USA) with a static flow rate of 18 mL/min. Sampling ports were set after each filter. A 20 L water tank was used as a source water tank, and a 30L water tank was used as a waste tank. The intake tank was covered by two layers of black film to prevent the light effects on MC-LR (Figure D.3).

Before the experiment started, 200 mL samples were taken from the intake tank and sampling ports after each column to test water quality including LC-OCD, pH, and temperature before the experiment started. Forty mL of a 20 mg/L stock MC-LR solution was added to the feed tank with 20L Lake Erie raw water and a timer was started to record the sampling time. A radial blade impeller was used to mix the solution every 15 min. Triplicate 10 mL samples were collected every hour at the intake tank and sampling ports after each column. The MC-LR biofiltration test was operated for 6 hours after the MC-LR was injected into the source water. After 6 hours, the filters were fed with raw water without MC-LR for 2 hours to remove any MC-LR residue in the columns, to make sure they were suitable to be sent back to the EAWTP for further operation. After the experiment, 2.0 g of media samples were collected from each column to measure the ATP values.

3.6 Batch tests

3.6.1 Biofilter media

Biofilter media was collected from 3 full-scale DWTPs in Ontario. Water Treatment Plant A (WTP A) used a river as its raw water, and water was pre-treated by flocculation/coagulation, sedimentation, and ozonation before the biofilters. The plant uses anthracite/sand as biofilter media. An anthracite media sample was taken from one of the biofilters in WTP A on June 5, 2017. Top layers were scooped using a sampler, and the media sample was collected, then stored in a sterile sample bag and transported in a cooler immediately to the lab at the University of Waterloo. Media ATP was measured within several hours after the media sample was collected. Part of the collected media sample was autoclaved at 121 ℃ for 15 min

and then washed with milli-Q water to remove the biological activities on the media. The sterilized media was used as a control for this experiment to test if MC-LR can be removed by media without biological activities. The comparison between sterilized media and biologically active media was the main assessment of biodegradation in this experiment.

Biofilters using granular activated carbon at Water Treatment Plant B (WTP B) were also investigated. This plant treats water from an inland lake in Ontario. The water treatment plant applies screening and ultrafiltration membranes before biofiltration. Water Treatment Plant C (WTP C) treats surface water from a river in Ontario. Raw water is treated by coagulation/flocculation, sedimentation, and ozonation before biofiltration. The plant also uses GAC as biofilter media. Media from WTP B was collected on June 26, 2017, and media from WTP C was collected on July 17, 2017. Media from WTP B and C was sampled, transported and stored as described for WTP A, and ATP was similarly measured. Anthracite from the bench-scale biofilters used in this project was used as a 4th media type for shaker tests. Autoclaved controls were prepared as previously described.

3.6.2 Source water

Biofilter media was incubated in 4 different types of water containing MC-LR for the shaker tests including biofilter influent and effluent water taken from the full-scale or bench-scale biofilters. For each full-scale biofilter, 1L of influent water and 1L of effluent water was collected and they were transported in a cooler on ice. Also, two types of spring water (spring water and spring water with acetate) were tested. The chemical composition and the preparation procedure are shown in Appendix E.

3.6.3 Experimental setup and sample collection

Media and water samples were added to 500 mL glass bottles within several hours after they were collected from the biofilters. Each bottle contained 20 grams wet media sample and 100 mL of each water

type. Similarly, sterilized biofilter media was also combined with the four types of water. In addition, each combination was done in duplicate bottles. Then, 0.2 ml of 20 mg/L MC-LR stock solution was spiked into each bottle to achieve an initial concentration of 40 µg/L. The initial concentration of MC-LR was tested by collecting 1 mL of water sample from each bottle immediately after MC-LR was spiked. The bottles were then put on a rotary shaker at 100 rpm at room temperature (20 ± 2 ℃). Furthermore, two layers of black plastic film covered all the bottles to prevent light effects.

1.5 mL samples were taken from the bottles every 24 h. The samples were filtered with 0.45 µm syringe filters, 1 mL of which were then added to 1 mL amber glass sample vials (Chromatographic Specialties Inc., ON, Canada) together with 100 µL internal standard solution. Samples were stored at -20 ℃, and they were measured within 3 days of sampling.

3.6.4 Reaction kinetics modeling

This study modeled the removal kinetics using pseudo-first-order kinetics model to compare the reaction rates observed in the batch bottle testing. Non-linear regression was completed using least sum of squares, and the modeling was completed using Solver in Microsoft® Excel. Initial values for parameter estimates were obtained by preliminary linear regression analysis.

Chapter 4 Results and Discussion

This chapter is divided into two major parts. The first part includes the results and discussion of bench-scale biofiltration column tests in removing MC-LR. Biofiltration bench-scale column tests at the University of Waterloo used biologically active filters running with raw water from Lake Erie spiked with MC-LR to test if biofiltration could remove MC-LR. Non-biologically active media was included as control filters and operated with the same type of water as the biofilters. The first biofiltration test was done in March 2017 when the biofilters had been acclimated at the Elgin Area Water Treatment Plant (Elgin Area WTP) for four months (November 2016 to March 2017) in the winter, and the second biofiltration test was done in August 2017 when the biofilters had been operated at the water treatment plant for a total of 9 months (November 2016 to August 2017) including the spring and summer. The second section describes research done using batch shaker tests. In these experiments, biofilter media was incubated with water in bottles for a longer period of time (3 days), to determine if biological activity on the filter media showed potential for removing MC-LR. Batch shaker tests used biofilter media from 3 full-scale DWTPs and also media from the bench-scale columns presented in Chapter 3. The media was incubated with different types of water including full-scale biofilter influent and effluent and spring water without and without acetate, to test if nutrients in the water could affect MC-LR removal.

4.1 Biofiltration column tests for removing MC-LR

4.1.1 Water quality measurements

Water quality of the raw water, including pH, turbidity, and temperature, was monitored by online analyzers at the Elgin Area Water Treatment Plant. Average values in each month from November 2016 to August 2017 are shown in Table 4.1. The maximum water temperature reached was 17°C in July, while the minimum water temperature was below 1°C in January. pH stayed stable at around 8.

Table 4.1. Water quality monitored by Elgin Water Treatment Plant while the three biofilters were acclimating at the plant.

Month (2016-2017)	pH			Turbidity (NTU)			Water Temperature (°C)		
	Monthly Average	Standard Deviation	n	Monthly Average	Standard Deviation	n	Monthly Average	Standard Deviation	n
Nov	8.16	0.08	164	44.8	51.8	160	11	2	51
Dec	8.05	0.08	144	66.1	37.9	134	5	2	58
Jan	8.08	0.10	128	70.9	148.8	125	1	1	43
Feb	8.21	0.12	132	46.5	29.6	131	1	1	45
Mar	8.18	0.07	136	51.1	43.1	137	2	1	48
Apr	8.14	0.09	128	22.6	16.9	129	7	2	41
May	7.95	0.14	120	24.5	42.3	121	10	1	41
Jun	7.54	0.10	136	15.3	22.4	134	12	2	44
July	7.87	0.22	172	6.5	5.3	174	17	3	61

However, turbidity varied greatly, as the water level is shallow in Lake Erie especially near the intake therefore, water quality is easily disturbed by the weather conditions. Average turbidity was shown higher in the winter months (November 2016 to March 2017) compared to the summer months (April 2017 to August 2018). Turbidity can affect biofilter performance. The bench-scale biofilters in this experiment used untreated lake water as the influent water instead of water after sedimentation that is most often used by full-scale biofilters. However, we could not use post-sedimentation water during this experiment due to the fluctuating chlorine content. The lower chlorine (seasonal) dosage applied to the raw water was removed by a GAC prefilter as described in Section 3.5.1. The untreated lake water can have a large amount of suspended particles, and this can cause clogging of the spaces between biofilter media and can cause a reduced flow rate. In this experiment, a roughing filter was added prior to the biofilters, and also an up-flow direction was applied to the three biofilters to minimize the effects of particles. However, there was still frequent clogging that occurred in the biofilter set-up.

During the MCLR removal testing, the columns were brought back to the University of Waterloo laboratory together with raw water from the Elgin plant. Before the experiment started, the water was adjusted to room temperature (16℃) for each experiment. The turbidity of the raw water was different in the two biofiltration tests done in March and in August. Raw water samples used in the March experiment had turbidity of 52 NTU, but was lower at 3.65 NTU in the second experiment in August. The high turbidity in March was possibly caused by the rain on the sample day with moderate windy weather.

Turbidity removal by the biofilters during the MCLR removal experiments is shown in Table 4.2.

Table 4.2. Turbidity (NTU) measured in the biofiltration column testing.

Water Sample	First filtration test in March	Second filtration test in August
Influent of all filters	52.0	3.7
Effluent from BF1	37.5	2.3
Effluent from BF2	35.4	2.2
Effluent from BF3	18.8	2.0
Effluent from CF1	37.9	2.0
Effluent from CF2	36.2	1.9

BF3 was the second stage to BF1, and had double the EBCT (18 min) compared with BF1 and BF2 (9 min). In the biofiltration column testing in March, the results showed that the two-stage filter (BF1/BF3) had the greatest turbidity removal (64%) as would be expected. BF1 and BF2 had less turbidity removal, at 28% and 32%, respectively. The control filters (C1 and C2) using new media had similar removal of 27% and 30%, respectively. In the column testing conducted in August, turbidity levels were lower and there was less difference in removal between the different filters. The effluent water from CF1, CF2 and BF3 had a similar level of turbidity that was around 2 NTU, and the percent removal was 47%. However, two single-stage biofilters had slightly higher turbidity effluent (2.2 NTU), and the removal for turbidity in BF1 and BF2 was 40%. The two-stage filter increased the contact time, which can explain why it had greater removal than the single-stage biofilters. Control filters used new media, so it is possible that higher removal was due to greater particle attachment, although the differences with the other filters are quite small.

4.1.2 Organics removal in biofilters

LC-OCD analyzed the concentration of organic matter in the water of samples collected at the effluent of each biofilter and the influent water tank. Water samples were collected while the biofilters were being acclimated at the water treatment plant, as well as during the biofiltration column testing for MCLR removal. Data collected from January to August 2017 during the acclimation of biofilters is presented in Appendix F, and removal of each organic fraction is summarized in Figure 4.1.

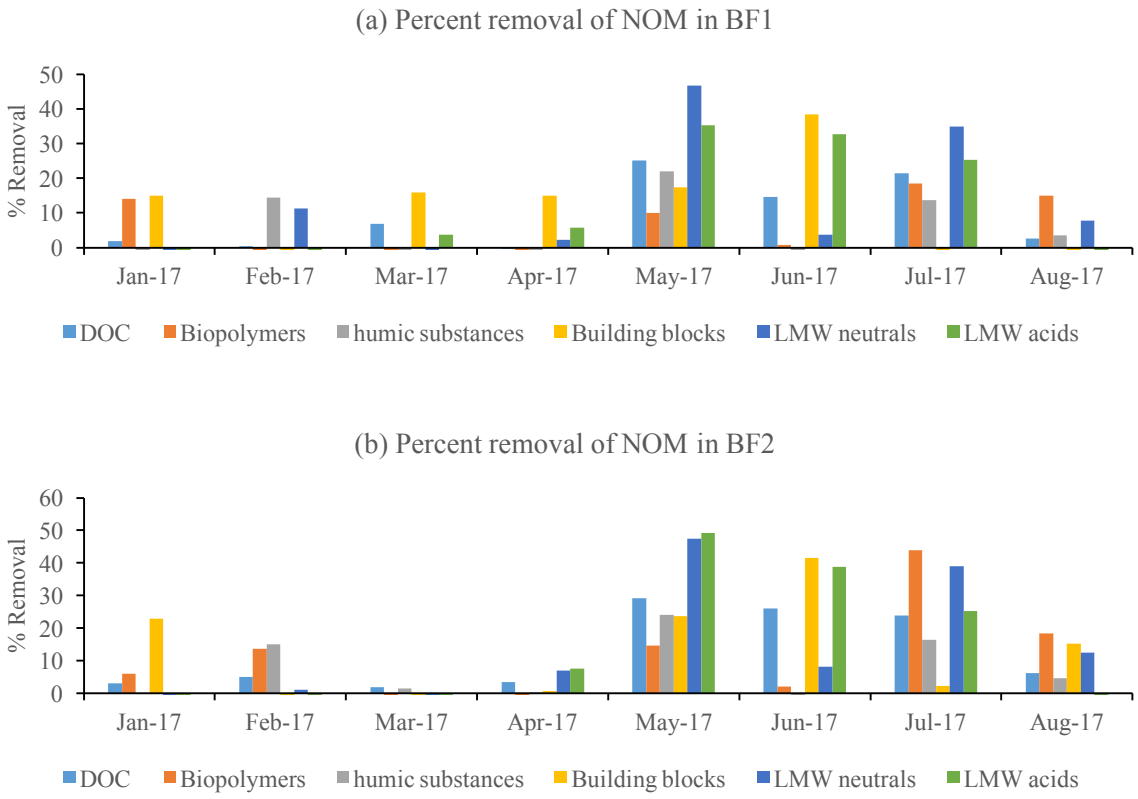

Figure 4.1. Organic matter percent removal detected by LC-OCD. The columns show removal through BF1 (a) and BF2 (b) LMW neutrals and LMW acids refers to low molecular weight neutrals and low molecular weight acids.

Between January to April, before the first column testing, results showed that the removal of organic matter was very low through the three biofilters, with removal of total DOC and other fractions between 0% to 7% However, DOC removal increased starting in May, and the maximum removal reached 25% in summer (May 2017). Removal of biopolymers and low molecular weight neutrals also increased from May

2017, and a maximum of 31% (July 2017) and 47% (May 2017) were achieved, respectively. Other fractions also showed increased removal compared to the results from January 2017 to April 2017, but the data was variable. Consequently, the longer acclimation time and the warmer water temperatures (Table 4.1) could effectively enhance the performance of the biofilters. Hallé et al. (2009) demonstrated that better acclimation and warmer water temperature could effectively enhance the organic matter removal, and significant drop of biopolymers and humics removal was found during winter with cold water temperature.

LC-OCD fractions were also measured during the biofiltration column testing for MCLR removal conducted in March and August, and results are shown in Figure 4.2 and Figure 4.3.

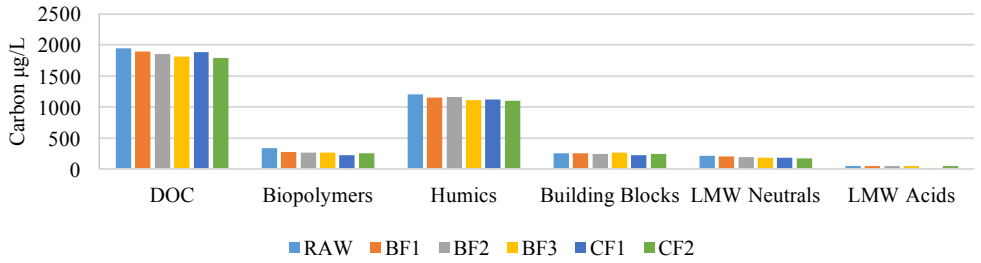

Figure 4.2. LC-OCD results for the first biofiltration test for MCLR removal in March 2017 for the influent water and effluent water of each filter. LMW neutrals refer to low molecular neutrals and LMW acids refer to low molecular weight acids.

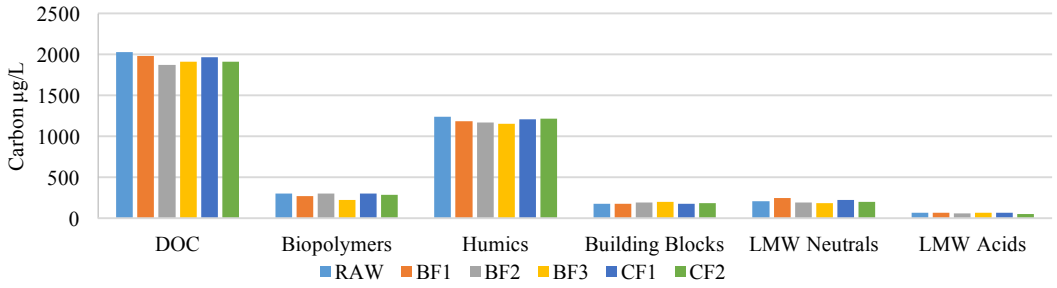

Figure 4.3. LC-OCD results for the second biofiltration test for MCLR removal in August 2017 for the influent water and effluent of each filter. LMW neutrals refer to low molecular neutrals and LMW acids refer to low molecular weight acids.

Organic components of DOC are listed including biopolymers, humics, building blocks, LMW acids/humics and LMW neutrals. Even though the turbidity was substantially higher in March, the total

DOC in the raw water was 1.9 mg/L in the biofiltration column testing in March and 2.0 mg/L in the second column testing in August. There was very little difference in organic fractions in raw water when data in August was compared to the data in March, except for biopolymers. The biopolymer concentration in August test was 306 µg/L, while the biopolymer concentration in March was 343 µg/L.

The percent removal of DOC by the biofilters and control filters during the 2 MC-LR removal test periods is are shown in Table 4.3 and Table 4.4.

Table 4.3. Organic components removal (%) in each filter in the first column testing for MC-LR removal done in March 28, 2017.

Filters	DOC	Biopolymers	Humics	Building Blocks	LMW Neutrals	LMW Acids
BF1	2.8	18.9	4.5	2.9	7.5	2.7
BF2	4.8	22.0	3.5	4.7	12.6	5.5
BF3	6.8	22.0	8.1	-2.4	17.6	10.8
CF1	2.9	34.1	7.3	13.9	14.7	5.1
CF2	7.8	24.3	9.2	6.6	21.3	1.2

Table 4. 4. Organic components removal (%) in each filter in the second column testing for MC-LR removal done in August 5, 2017.

Filters	DOC	Biopolymers	Humics	Building Blocks	LMW Neutrals	LMW Acids
BF1	2.0	11.6	3.9	0.7	-15.0	1.9
BF2	7.4	1.7	5.4	-10.3	7.9	3.7
BF3	5.8	26.5	6.6	-13.0	11.3	-4.7
CF1	3.1	1.0	2.3	-1.3	-4.9	0.8
CF2	5.6	5.1	1.9	-5.6	7.1	23.7

BF1, BF2 and BF3 removed 2.8%, 4.8% and 6.8% DOC in the first biofiltration test, and CF1 and CF2 had 2.9% and 7.8% removal. A similar pattern was found in the second biofiltration test (2.0%, 7.4%, 5.8% in BF1, BF2 and BF3, and 3.1%, 5.6% in CF1 and CF2). The amount of DOC in the water samples taken from the effluent of BF1, BF2 and BF3 showed little differences compared to control (non-biological) columns. For the DOC fractions, in the March experiment the biopolymers fraction was the most removed

organic component, and BF1, BF2 and BF3 removed 18%, 22%, 22% biopolymers, respectively. However, CF1 and CF2 also showed 34.1% and 24.3% removal of biopolymers, which was slightly greater than the removal found in the biofilters. A possible reason for the high removal of DOC and biopolymer by the controls columns might be the use of new anthracite/sand media, and they were able to adsorb some organic matter. In contrast, the second column testing did not show an apparent removal for biopolymers within CF1 and CF2, and BF1, BF2 and BF3 showed variable removal (11.6%, 1.7% and 26.5%, respectively). BF2 was the duplicate biofilter column to BF1, which was expected to have little difference to the BF1. Consequently, the difference between the two duplicate filters possibly indicate that the biofilters had somewhat variable performance on biopolymer biodegradation. However, the absolute difference are not large and the overall removals are small. BF3 (EBCT=18 min) had more than 2 times greater removal than its first-stage biofilter BF1 (EBCT=9 min), which indicated the longer EBCT enhanced the ability to remove biopolymers. EBCT has been previously shown to have a positive effect on biopolymers removal (Hallé et al., 2009).

In both filtration tests, humics showed no more than 10% removal, although the humics made up a large percentage of the DOC. In the first filtration test in March, BF1, BF2 and BF3 showed 4.5%, 3.5% and 8.1% removal of humics, while the CF1 and CF2 showed slightly higher removal (7.3% and 9.2%). However, in the second filtration test, the removal of humics (3.9%, 5.4% and 6.6%) didn't change much as was shown in the first filtration test, but CF1 and CF2 showed little removal (2.3% and 1.9%). Other fractions including building blocks, LMW neutrals and LMW acids showed highly variable removal for both the biofilter and control columns and, therefore, conclusions on the ability to remove/biodegrade these fractions could not be made.

As a result, both biofiltration column experiments revealed some removal of NOM. However, only the second test in August showed biodegradation of NOM (biopolymers and humics) due to the difference of removal compared to the non-biological filters CF1 and CF2. It is likely that performance of the biofilter in March was affected by the low water temperature and possibly by the high turbidity in the raw water at that time. As well, the potential for adsorption of NOM by the unused media in the control columns required

verification, since this will affect the comparison of removals by the biofilter columns. In addition, the reason for the high variability of NOM removal by the duplicate biofilter columns is unknown, although the absolute difference is relatively small. Among the NOM fractions, biopolymers had the best removal. Some studies have also found that biopolymers can be more easily removed by biofilters than DOC and humics, which was due to better biodegradability of the biopolymers in water (Hallé et al., 2009; Huber et al., 2011). Tashi (2016) found that the biofilters (anthracite/sand as filter media) with 8 min EBCT could remove up to 46% biopolymers, while only 10% of humics and 16% DOC could be achieved.

4.1.3 Biomass measurements

Before the column testing started, biomass on the biofilter media was analyzed. As shown in Figure 4.4. BF1, BF2 and BF3 were acclimated biofilters and ATP values showed active biological activities during the two biofiltration column experiments. In contrast, CF1 and CF2 used new anthracite/sand media, and small ATP values represented background (blank) values and showed that the media was non-biological.

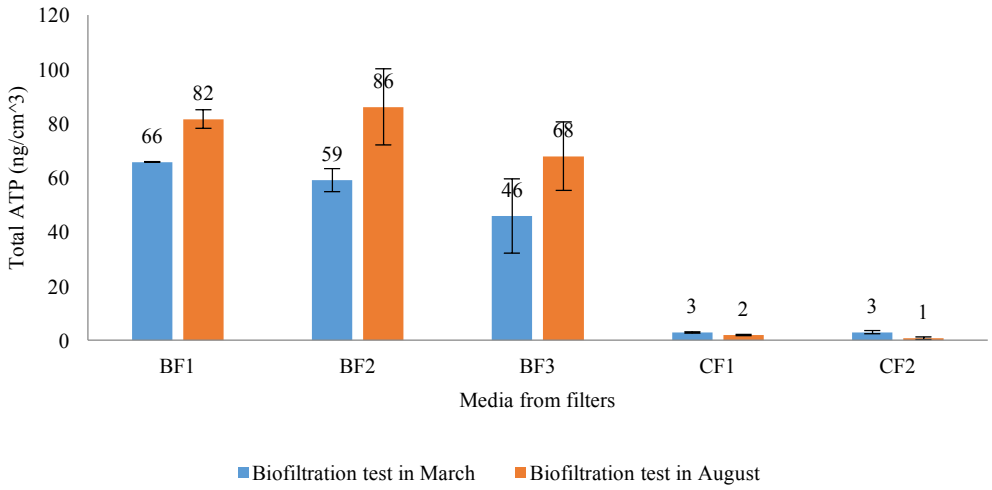

Figure 4.4. ATP measured for the media at the top of each biofilter in the two biofiltration tests. Data points show the average of duplicate measurements, and error bars represent standard deviations from duplicate measurements.

Pharand et al. (2014) investigated various GAC and anthracite biofilters and found that active biofilters typically had ATP values in a range of 10^2 to 10^3 ng/cm^3 when the top layer of media was measured. Consequently, CF1 and CF2 could be defined as non-biological filters in the two column test, while BF1 and BF2 were closer to the range of active biofilters in the second biofiltration test 82 and 86 ng/cm^3) than in the first biofiltration test (70 and 59 ng/cm^3). BF3 had lower ATP (46 to 68 ng/cm^3) than BF1 and BF2, since it was the second stage of BF1 and it could contact with less organic matters compared to the first-stage filter. Other studies also verified ATP decreasing with increasing bed depth. Elhadidy (2015) found about half of the ATP values found in the middle of the filter (550 ng/cm^3) compared to the ATP on the top (1,000 ng/cm^3). Results also showed that the three biofilters had greater ATP values in August than they had in March (Figure 4.4). There are two possible reasons for this increase of ATP. A primary influencing factor is the acclimating time. The biofilters started to run with raw water in the Elgin Area Treatment Plant in November 2016. After four months of continuous operation, the biofilters were then taken to the University lab on March 2017. They were sent back to the treatment plant after the first column testing, and it continued to run from March to August. Five additional months of additional acclimation time could have resulted in an increase in the biomass levels in the biofilms on biofilter media. The increasing of ATP over time was also found in the experiment by Elhadidy (2015), and the anthracite/sand biofilters had increased ATP from 1,000 to 3,500 ng/cm^3 within 7-month operation with river water. In addition, the acclimating water temperature in February was from 0.5 to 3 °C, while the water temperature in July was from 13 to 22 °C. The effects of water temperature on the ATP values on biofilter media was variable in published literature. In Huck et al. (2000), biomass levels measured by phospholipid method were found to decrease when water temperature dropped from 21 to 25 °C to 1 to 3 °C. For the common understanding of biological systems, a low water temperature would be expected to reduce biological activities including bacterial growth and metabolism in biofilters (Moll et al., 1999). The water temperature effects on biomass activities were also investigated by Terry (2017), and the study found that ATP values increased from 510 ng/cm^3 to 1392 ng/cm^3 with increasing the water temperature from 5 to 22 °C. However, Pharand et al. (2013) found that ATP and temperature didn't have a direct relationship in a full-scale system that ranged from 3 to 28 °C.

Other research also found similar results that increasing water temperature didn't result in higher ATP at the surface of filter media (Evans et al., 2013; Fonseca et al., 2001). An experiment conducted by Elhadidy (2015) showed that bulk ATP values on media did not relate to the water temperature. Their biofilters were operated from August to February with higher water temperature in the first three months and lower water temperature in the next three months. ATP values on the biofilter media continued to increase even in the lower water temperature as the biofilters were running. As a result, even if the water temperature influenced biomass on the biofilter media, acclimating time was likely a more significant factor that affected the ATP on the media surface than the temperature.

4.1.4 Biological filtration of MC-LR

Biofilters were tested for the removal of MC-LR that was spiked into water that was collected from Lake Erie at the Elgin Area WTP's raw water sampling port. The biofilters were not exposed to any MC-LR (as far as could be measured) during the time they were acclimated at the WTP until the time the experiment started. MC-LR content in the raw water was monitored by the Elgin Area WTP. Data provided by the plant showed that MC-LR was below the detection limit of 0.1 µg/L from November 2016 to August 2017 while the biofilters were acclimating at the plant.

After MC-LR was added to the feed water of the biofilter columns, samples were collected every hour in the first experiment in March 2017. Triplicate samples were collected for LC-MS analysis from each sample location including the biofilter influent water and the effluent water from each biofilter to measure the removal of MC-LR from the biofilters. The results of the experiment in March are shown in Figure 4.5 and Figure 4.6, and data is presented in Appendix G. Similar sampling procedures were used to collect the water samples for the second filtration experiment in August, except the sampling intervals were 2, 3, 4, and 6 hours in order to reduce the number of samples to be analyzed.

In the first filtration test, the influent concentration was measured by sampling water from the influent water tank. Through the 6-hour experiment, the influent concentration ranged from 30 to 36 µg/L (Figure

4.5). The influent tank was covered with non-transparent (black) plastic film to minimize the possible influence of light, and then the water was mixed every 20 min using a metal mixer. In the first four hours, the MC-LR concentration decreased a little bit, possibly due to the degradation effects of natural water. At four hours, additional lake water spiked with MC-LR was added to the influent tank to maintain the water level, which caused an increase in the MC-LR concentration in the feed tank. However, because this experiment measured MC-LR removal through the biofilters, these small changes in influent concentration would not affect the results.

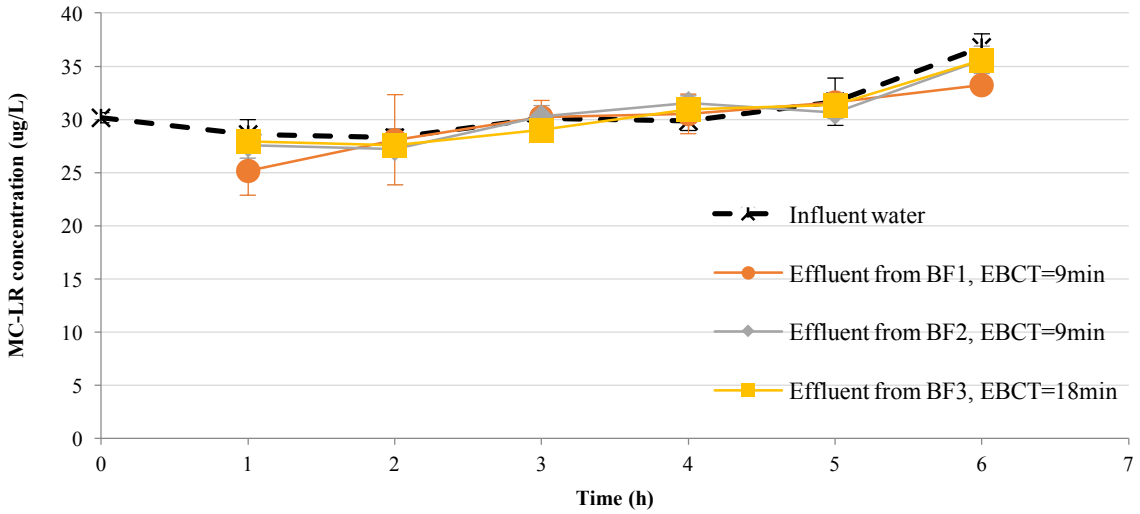

Figure 4.5. MC-LR concentration in the influent water and effluent water of biofilters measured during the first biofiltration test in March. Data points show average values, and error bars represent the standard deviation from triplicate collected samples.

The concentration of MC-LR in the biofilter effluent samples increased in the first hour until it reached a similar level as the influent at 1 h. Then, the MC-LR concentration in the effluent of BF1, BF2 and BF3 stayed at a similar level as the concentration in the influent water (Figure 4.5). Consequently, the biofilters were not removing MC-LR in the 6 h experiment. BF3 was the second stage filter to BF1 and had double the contact time compared to BF1 and BF2. The EBCT of the BF1/BF3 was 18 min while BF1 and BF2 had only 9-min EBCT. Therefore, a longer EBCT did not improve MC-LR removal by the biofilters during this relatively brief experiment.

Two control filters were also run with the same influent water as the three biofilters, and the results of the controls filters in the first column test are shown in Figure 4.6. Compared to the BF1, BF2, and BF3, the control filters used similar media, but it was new and washed with clean water before being added to the columns. Consequently, the clean media did not have any biofilm on the media surface. At 0 h, the MC-LR had just entered the column and had not yet reached the effluent. However, at 1 h the MC-LR concentration was less than the influent concentration. After 2 h, the MC-LR concentration was similar to the influent concentration and remained so until 6 h. So the control filters without any biologically active biofilms did not remove any MC-LR in the experiment over the 2-6 h period.

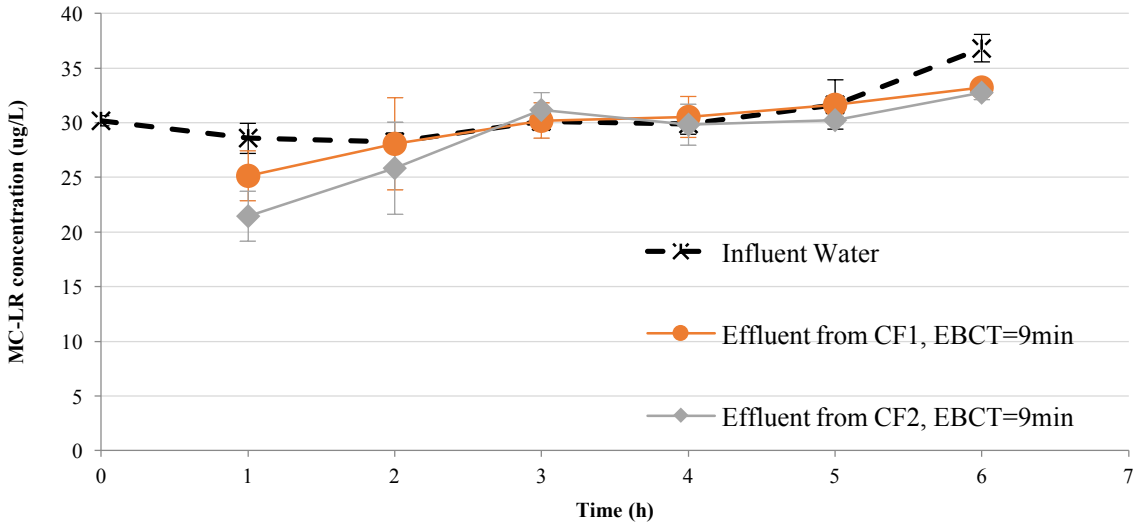

Figure 4.6. MC-LR concentration in the influent water and effluent water of control filters measured during the first biofiltration tests in March. Data points show average values, and error bars represent standard deviations from triplicate collected samples.

The lower concentration of MC-LR in the biofilter effluent compared to influent for the control columns at 1 h (Figure 4.6) was in contrast to the results from the BF1 and BF2. For the biofilter columns (Figure 4.5), MC-LR concentrations in the effluent reached the same concentration as the influent at 1 h. This indicated that there was some MC-LR removed in CF1 and CF2 in the first 2 h. A t-test was done to compare if the difference at 1 hour was significant using a 95% confidence interval between the effluent concentration of MC-LR from BF1, BF2, CF1 and CF2.

The P values are shown in Table 4.5. The MC-LR concentration in CF1 effluent at 1 hour was significantly different than BF1 and BF2 effluent samples, while the CF2 effluent concentration of MC-LR was significantly different than BF2 but not to BF1. As a result, at 1 hour, control filters did have a significantly lower MC-LR concentration in the effluent than the biofilters, which indicated some MC-LR was retained in the control filters. A possible reason for the difference was the adsorptive capacity to the MC-LR by new anthracite media. However, the potential adsorption of MC-LR by the control filters did not occur after 2 h, which indicated that the adsorptive capacity was saturated by 2 h after continuous contact with water containing MC-LR. Likewise, Drogui et al. (2012) also found non-biologically active anthracite filters could remove MC-LR, but removal was more efficient than it was in this study. Their work showed that with an initial concentration of 20 to 26 μg/L MC-LR, the control filter could remove 35% to 46% in the first hour, and 77-94% at 18 hours, while the biofilters did not show removal for MC-LR after 2 hours. The specific mechanism for the removal of MC-LR by the new anthracite was unknown and still needs to be determined. A further discussion of the adsorption by new anthracite media is presented in the second experiment, the bottle tests, where another experiment was conducted to investigate if the spiked MC-LR in raw water could be removed by the control filter media.

Table 4.5. t-test to compare the effluent concentration of MC-LR from control filters and biofilters at 1 hour of the experiment. t and P values were calculated to find out if the difference was significant in 95% interval.

Effluent concentration compared	t value	P value	Result
CF1 VS. BF1	4.27	0.013	Significant
CF1 VS. BF2	22.19	$2.4x10^{-5}$	Significant
CF2 VS. BF1	1.79	0.14	Not significant
CF2 VS. BF2	9.89	$6.0x10^{-3}$	Significant
CF1 VS. CF2	1.22	0.32	Not significant

Consequently, the first column experiment demonstrated the non-biological filters and biologically active filters could not respond effectively to the sudden occurrence of MC-LR in the source water of a treatment plant in winter. Despite some removal found through control filters at the beginning of the experiment, it was too little and of too limited duration to be effective in practice.

The second biofiltration column test was done in August 2017. In this test, the biofilters had been acclimated for another five months after the first column testing, with a warmer water temperature than the filtration test done in March. Also, ATP results suggested higher biological activities on the biofilter media in August compared with March (Figure 4.4). To reduce the number of samples to be measured, the sampling time intervals were changed to collecting water samples at 2, 3, 4, and 6 h after the MC-LR was spiked. The influent concentration of MC-LR was measured at the intake tank, and was 35 µg/L to 40 µg/L over the 6 h experiment. The effluent concentration of MC-LR in the three biofilters was similar to the concentration detected in the influent water (Figure 4.7). Data of this experiment is presented in Appendix H.

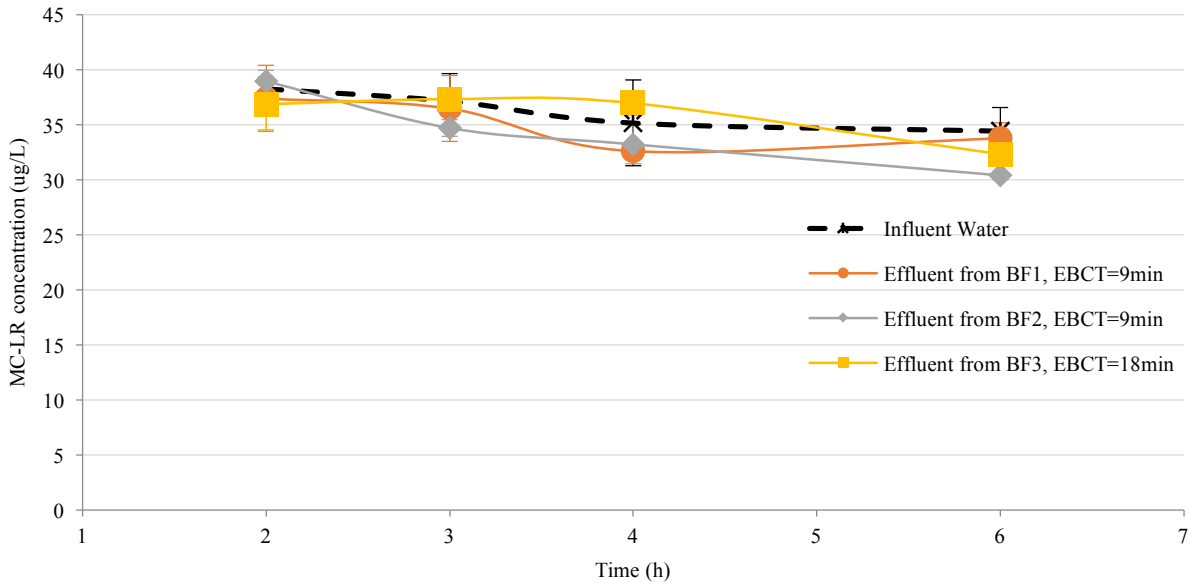

Figure 4.7. MC-LR concentration in the influent water and effluent water of biofilters measured during the second biofiltration tests in August. Data points show average values, and error bars represent standard deviations from triplicate collected samples.

Hence, the three biofilters were not removing MC-LR even after acclimation at warmer water temperatures. Similar to the experiment in March, EBCT of the biofilter did not affect MC-LR removal, and BF3 did not show a difference compared to BF1 and BF2. Since MC-LR wasn't degraded by the three biofilters in either of the two tests, this indicated that an increase in bulk biomass as shown by the higher ATP values in August did not correspond with higher biodegradation of MC-LR.

The control filter did not remove MC-LR, since effluent MC-LR concentration showed no difference compared with the influent concentration (Figure 4.8). A similar result was found in the first filtration test in March based on 2 to 6 h data. No removal of MC-LR by the control filters in this experiment showed that the biofilter results were not influenced by toxin adsorption to the media.

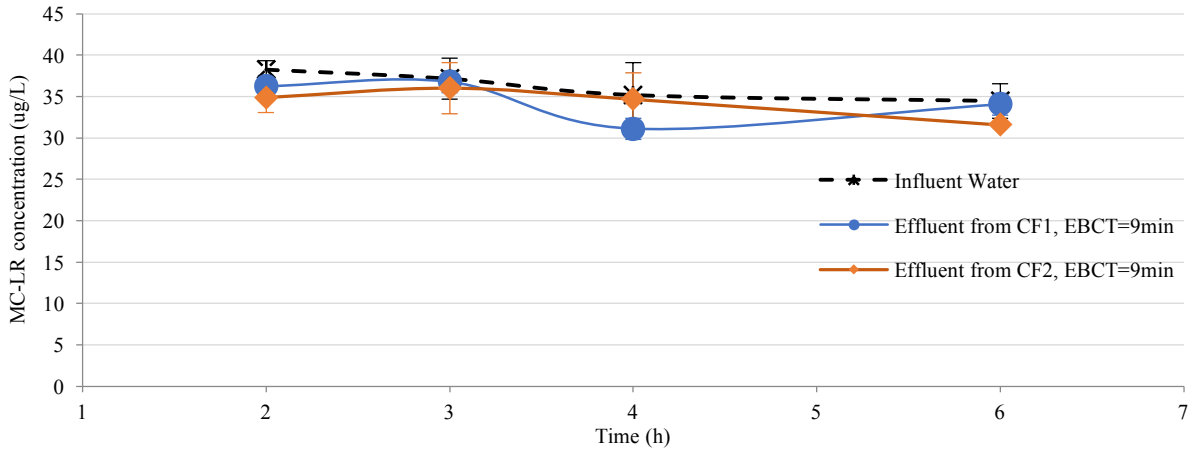

Figure 4.8. MC-LR concentration in the influent water and effluent water control filters measured during the second biofiltration tests in August. Data points show average values, and error bars represent standard deviations from triplicate collected samples.

Currently, limited data have been reported to show degradation of MC-LR under rapid flow conditions in biofilters. Ho et al., (2006) found that biofilters could completely remove 20 µg/L MC-LR at a 7.5-min EBCT using natural water as influent water. However, the biofilters had previously been operated at slow-flow condition (EBCT from 15 to 30 min) during which they were pre-exposed to MC-LR for more than 30 days before removal through the biofilters was tested (Ho et al., 2006). However, cyanobacterial blooms would need to be able to respond quickly. Therefore, the results of experiments in which biofilters are pre-

exposed to MC-LR are not applicable to the real situation in Ontario WTP's biofilters. Ho and his co-workers (2006) also found that the use of full-scale biofilter media that have never exposed to MC-LR couldn't completely remove MC-LR following initial exposure when they did bench-scale biofiltration testing, and the removal was less than 5% in the first day. However, MC-LR removal increased to 63% at day 3 and after 4 days the bench-scale biofilters could completely remove MC-LR. The biofilter was operated in very slow-flow condition with 15 to 30 min EBCT, and was fed with natural water containing 20 μg/L MC-LR. So, they suggested that there must be a lag time for the biofilms in the biofilters to develop the ability to remove MC-LR. Since the main purpose of the experiment done in the current study was to test the immediate response of the biofilters to the sudden outbreak of MC-LR in the source water, the duration of the experiment was shorter than Ho et al (2006). However, a hypothesis can be made that, if the biofilters could have run with natural water containing MC-LR for several days, a noticeable reduction of MC-LR concentration might have occurred. The lag period before MC-LR biodegradation may vary, potentially due to flow conditions, water quality, media type, or water temperature. Several other studies have found that slow sand biofiltration needed 4 to 16 days before MC-LR degradation was detected (Miller and Fallowfield, 2001; Bourne et al., 2006). However, no data was found in reference to the lag period on rapid flow biofilters. Other studies also found a lag period in biodegradation of MC-LR in biofilters, but a potential method to reduce the lag period or acclimation time for MC-LR degradation is to inoculate biodegrading bacteria into the biofilter. Bourne et al. (2006) successfully inoculated *Sphingomonas* sp. into a slow sand filter and reduced the lag period MC-LR biodegradation. With an initial MC-LR concentration of 50 μg/L, the inoculated biofilters operated at an EBCT of 30 h could reach 90% removal in two days, while non-inoculated biofilters needed more than ten days to reach a similar level of removal (Bourne et al., 2006). However, the practicality of, and potential regulatory issues associated with, inoculation of full-scale biofilters would make this approach questionable in practice.

In summary, the first phase of this study evaluated the ability of acclimated biofilters treating raw water from Lake Erie to remove MC-LR by biodegradation. The purpose of the experiment was to discover the capacity of biofilters responding to a sudden water source contamination by a cyanotoxin, MC-LR, at a

water treatment plant. Two biofiltration column tests were done in March 2017 and in August 2017. In both experiments, acclimated biofilters could not remove any MC-LR during the 6-hour experiment after the MC-LR was added to the influent water. The results corresponded with other studies that have shown that pre-exposure of biofilters to MC-LR was important to stimulate biodegradation of the toxin through the filter (Ho et al., 2006), and that pre-exposure times longer than 6 h, for example 4 days found by Bourne et al. (2006) and up to 16 days found by Miller and Fallowfield (2001), are required before MC-LR biodegradation. Consequently, it was necessary to investigate the biodegradation with extended incubation times, which was the main purpose of the next phase of this study.

4.2 Batch tests to evaluate removal of MC-LR by biofilter media.

Since there was no MC-LR removal found in the column testing using bench-scale biofilters, another experiment was designed and conducted to focus on the biodegradation processes happening on the biofilter media using a batch testing method. Batch testing was used because for practical reasons relating to water supply, the bench-scale biofilters could not be run for more than 6 h. Therefore, the primary purpose of the experiment was to study if the biofilms from different types of biofilter media, including the column test studied earlier, could biodegrade MC-LR over extended time periods (2-3 days). Besides media from the bench-scale column tests, biofilter media from three different drinking water treatment plants in Ontario were also collected and tested by the same batch-testing method. Additionally, this experiment tested the effects of different water types and nutrient levels on the biodegradation of MC-LR.

4.2.1 Characteristics of full-scale biofilter from which media was obtained

In addition to the bench-scale biofilter columns previously described, media tested in this experiment was from three full-scale biofilters in Ontario described in this study as Water Treatment Plant A (WTP A), Water Treatment Plant B (WTP B) and Water Treatment Plant C (WTP C). WTP A and WTP C use source

water from a river, and both plants apply ozone before the biofilters. However, the media types of the two biofilters are different. WTP A used anthracite and sand as the filter media, while WTP B's biofilters and WTP C's biofilters used granular activated carbon (GAC). But the source water of the WTP B was from a lake, while WTP C used river water. Additionally, WTP B employs an ultrafiltration system before the water enters the biofilters. Consequently, each water treatment plant had a different treatment train and, thus, the biofilters treated different types of water with different water quality.

Water samples and media samples were taken from the full-scale filters in the plants. The biomass on each type of filter media was investigated by measuring ATP. Additionally, influent water and effluent water of each filter were tested by LC-OCD and turbidity analysis, to have information on the ability of the biofilter to remove organic matter (e.g. DOC, biopolymers) and suspended particles, which also indicated the performance of the biofilters. Analysis of these parameters can also provide information on the general biodegradative activity of the filters to determine if this was related to the ability to remove MC-LR.

Figure 4.9 shows the turbidity measurements of the biofilter influent and effluent water measured by taking water samples at the full-scale plants. Turbidity values of the bench-scale biofilters were retrieved from the second biofiltration column testing in the previous section (Figure 4.9). WTP A, WTP B and WTP C all had low turbidity values entering the filters that did not exceed 1 NTU, while turbidity values entering the bench-scale biofilter was much higher. This is because all the WTPs had pretreatment systems before the biofilters, while the bench-scale biofilters were fed using raw lake water. Turbidity of spring water used in this experiment as control was also measured, and the spring water and spring water with acetate showed very low turbidity (0.11 and 0.15 NTU).

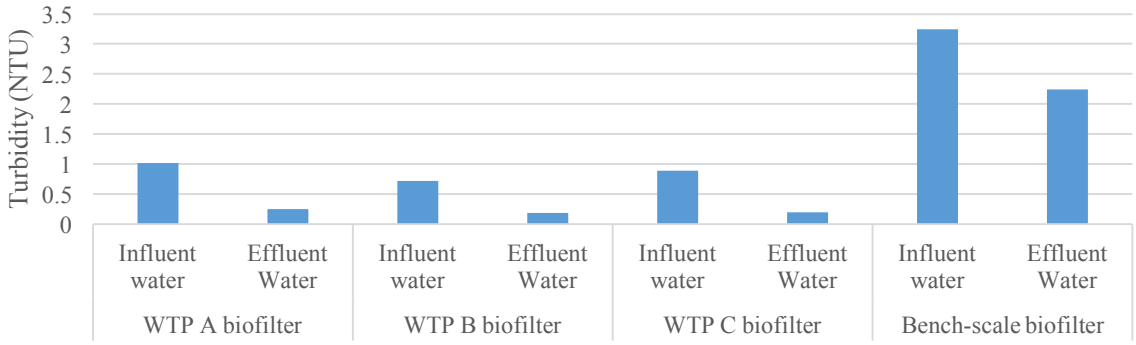

Figure 4.9. Turbidity measurements in the influent water and effluent water of the full-scale biofilters from WTP A, WTP B, WTP C and bench-scale columns used in the batch scale biofiltration tests.

More than 70% removal of turbidity was reached by biofilters at WTP A (75% removal), WTP B (74% removal) and WTP C (78% removal), and the turbidity of the full-scale biofilter effluent water samples were all less than 0.2 NTU. Although the biofilters were using different types of media in different treatment plants, they were all very effective in removing turbidity. Turbidity removal by the bench-scale biofilters acclimated in Elgin Area WTP were not as effective, likely due to the high turbidity in the influent and the lack of prior coagulation. Coagulation and flocculation before the three full-scale biofilters can enhance the turbidity removal because they neutralize the charges of particles, and form large-size masses to make it more easily filtered. In contrast, bench-scale biofilters took in raw lake water rather than treated water, and the particles were too small to be screened by the media, and so more turbidity particles could pass through the filters.

Influent and effluent water from each full-scale biofilter was also analyzed for organic matter content and removal. The water samples were analyzed by LC-OCD, and results are shown in Figure 4.10. LC-OCD results from the bench-scale biofilters used data from the second biofiltration column testing done in August. Organic components including DOC and LC-OCD defined fractions including biopolymers, humic substances, humic building blocks, LMW neutrals, and LMW humics/acids, are shown in Figure 4.10.

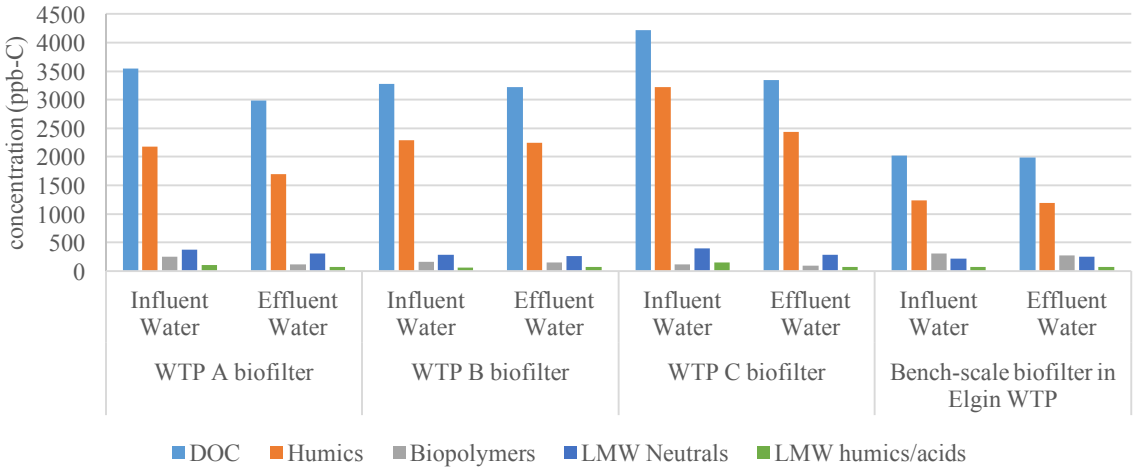

Figure 4.10. LC-OCD results tested for biofilter influent and effluent from WTP A, B and C. Data for the bench-scale biofilter were from the column testing experiment in August.

Removal of each organic component detected by LC-OCD including DOC, humic substances, biopolymers, LMW neutrals, and LMW humics/acids in each biofilter was calculated from the data of influent and effluent water (Table 4.6). WTP C's biofilters had the greatest removal for DOC (21%), humic substances (24%), LMW neutrals (29%), and LMW humics/acids (50%), while WTP A's biofilters remove the most biopolymers (53% removal). WTP B and bench-scale biofilters had low level of removal for each component. 12% removal for biopolymers was obtained by the bench-scale biofilters, while only 5 % removal for biopolymers was achieved by the WTP B's biofilter.

Table 4.6. DOC, humics, biopolymers percent removal from the four water treatment plants' biofilters.

Plant	Organic components percent removal (%)					
	DOC	Humics	Biopolymers	Building blocks	LMW neutrals	LMW humics/acids
WTP A	16	22	53	-23	18	24
WTP B	1	2	5	0	5	-31
WTP C	21	24	19	-79	29	50
Bench-scale biofilter	2	4	12	1	-15	2

DOC removal ranges from 1 to 21% among the 4 tested water treatment biofilters. The lower removal compared to the LC-OCD detected fractions was also found in Elhadidy (2015), where 10-20% removal

was observed compared to more than 50% removal of biopolymers. DOC was often regarded as an indicator for the removal of bulk NOM (Elhadidy, 2015). However, not all the fractions of DOC were related to biological degradation since the biodegradability of DOC fractions were variable (Chen et al., 2016). Among the five components, biopolymers were the most easily removed component since each biofilter tended to have higher level of removal than for the other fractions. Elhadidy (2015) and Tashi (2016) demonstrated that biopolymers had high biodegradabilities within biofilters. Tashi (2016) found biopolymers had higher (51%) removal in the anthracite/sand biofilter, while only 17% DOC, 8% humics, 10% building blocks and 24% LMW neutrals were removed. Biopolymers were demonstrated to be important substances that support the formation of biofilms in drinking water biofilters (van der Kooij et al., 2015). Consequently, biopolymer removal tends to be associated with biological removal. Humics were found to be less removed than biopolymers, since humics were less biodegradable than other fractions (e.g. Elhadidy et al., 2015; Huber, 2002).

DOC removal was consistent with the removal of humics, since humics take up the biggest components of DOC as shown in Figure 4.10. However, it was surprising to have more removal of humics than biopolymers in WTP C's biofilter, possibly due to the high concentration of humics found in WTP C's biofilter influent water (3.2 mg C/L) and low biopolymer concentration (0.12 mg C/L). Building blocks showed no removal through the bench-scale biofilter and WTP B's biofilter and negative removal through WTP A's and WTP C's biofilters. Huber et al. (2011) suggested that building blocks were originated from the breakdown of humics. Consequently, a possible reason of the negative removal was biodegradation of humics by the biofilters from WTP A and WTP C, while little humics were removed by the other biofilters. LMW humics/acids were verified to be highly degradable within biofilters (Huber, 2002; Elhadidy, 2015), which was consistent with the results found in this study that 24% and 50% removal was detected in WTP A and WTP C. Negative removal was detected in WTP B, which suggested the formation of it from the breakdown of other fractions. LMW neutrals are less biodegradable than LMW acids/neutrals (Elhadidy, 2015), consequently, less removal was detected through the WTP A and WTP C biofilters. Previous studies didn't have consistent observations for the removal of low molecular weight fractions within biofilters.

Elhadidy (2015) suggested the high variability in removal of LMW neutrals and humics/acids is because of the difficult integration by the software analysis. Chen et al. (2016) also found very low percent removal of these fractions. However, Pharand et al. (2015) found 31% removal of LMW acids during biofiltration. Consequently, removal of building blocks, LMW humics/acids and LMW neutrals can not provide information about the biofilters' performance.

ATP was measured for each type of full-scale biofilter media, which could indicate the level of biomass and indirectly the biological activities on the media. In the batch test method, a sub-sample of the collected biofilter media was sterilized by autoclaving in order to provide a non-biological control for this experiment. These samples were also analyzed for ATP. Results are shown in Figure 4.11.

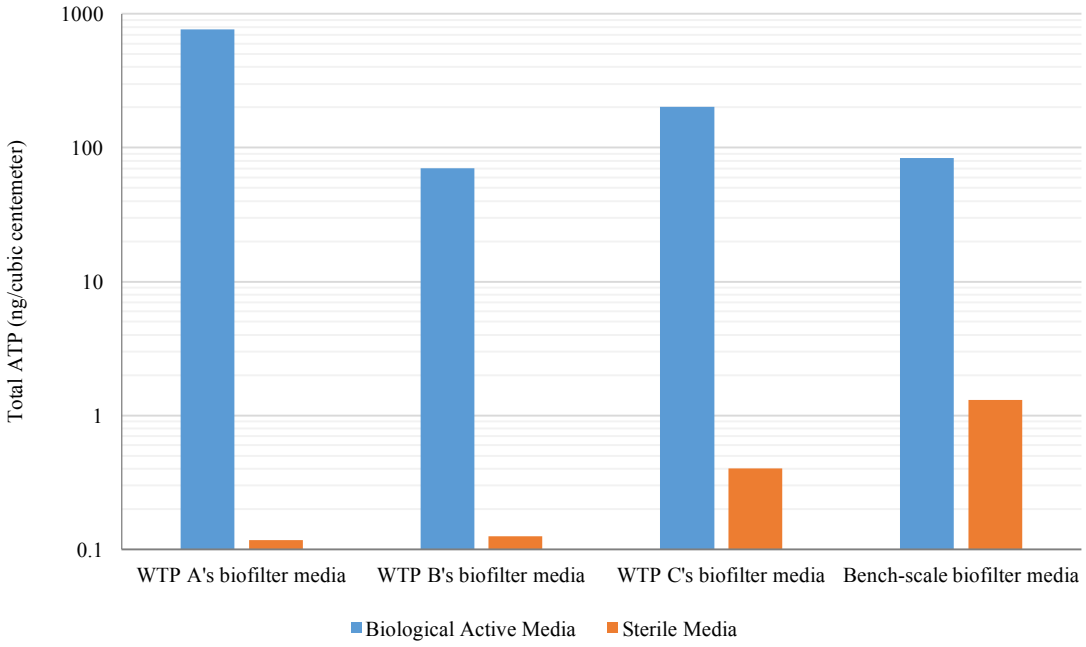

Figure 4.11. ATP tested for each type of media tested in this experiment.

As expected, results showed that the biofilter media had much greater ATP values than the sterilized media. The sterilized media had very low values of 0.1 ng/cm^3 to 2 ng/cm^3. ATP values of biofilter media varied from different plants. WTP A's biofilter media had the highest level of ATP that was nearly 800 ng/cm^3, which was in a reasonable range, referenced in Pharand et al. (2013). WTP C biofilters had less ATP that was 200 ng/cm^3. WTP B's biofilters and Elgin WTP's bench-scale biofilters had 70 and 84 ng/cm^3.

Pharand et al. (2013) compared published data on biofilters' ATP, values and found a range from 10^2 to 10^3 ng/cm^3 was typical in acclimated biofilters. Consequently, WTP A and WTP C has ATP levels in the expected range. However, ATP values on media from WTP B and the bench-scale biofilter were lower. The reason for greater ATP values measured in WTP A and WTP C biofilters was probably due to better condition for bacterial growth. Before the water entering the biofilters, ozone was added to the water, which wasn't applied at the other two water treatment plants (WTP B and Elgin Area WTP). Ozonation was demonstrated to enhance two to three times the ATP value in biofilters (Pharand et al., 2014). Ozone can make the natural organic matters more biodegradable (e.g. Yavich et al., 2004). Bacteria easily take advantage of the ozonated biodegradable organic matters to grow, and at the same time, ozone also increases the organic matter removal in biofilters. On the other hand, WTP A and WTP C used river water as the source water, which had higher organic matter concentration than lake water so that the bacteria may receive more nutrients in river water than in lake water. Comparatively, ultrafiltration can also decrease the amount of organic matters such as biopolymers (Tashi, 2016) entering the WTP B's biofilter, which has a negative impact on bacterial growth in biofilters, and results in lower level of ATP values. Ultrafiltration may also decrease the number of bacteria reaching the downstream biofilter.

ATP values were compared with biopolymer removal in Figure 4.12. ATP was shown related to the removal of biopolymers, since the WTP A's biofilters had the greatest ATP and the greatest biopolymers removal, while the WTP B had the lowest ATP and the lowest biopolymers removal.

ATP concentration on media surface and DOC removal for various biofilters were compared by Pharrand et al. (2014), and no direct relation was found among the studied biofilters. Similarly, Elhadidy (2015) found that the performance (organic matter removal) of biofilters related to the amount of ATP concentration detected per cell instead of the bulk ATP values. However, some studies have also found that DOC removal was enhanced by an increased ATP value (Seger & Rothman, 1996; Lauderdale et al., 2012). This experiment compared four different biofilters (full-scales and bench-scales), and ATP values showed a positive relation to the removal of organic matters, especially for the removal of biopolymers.

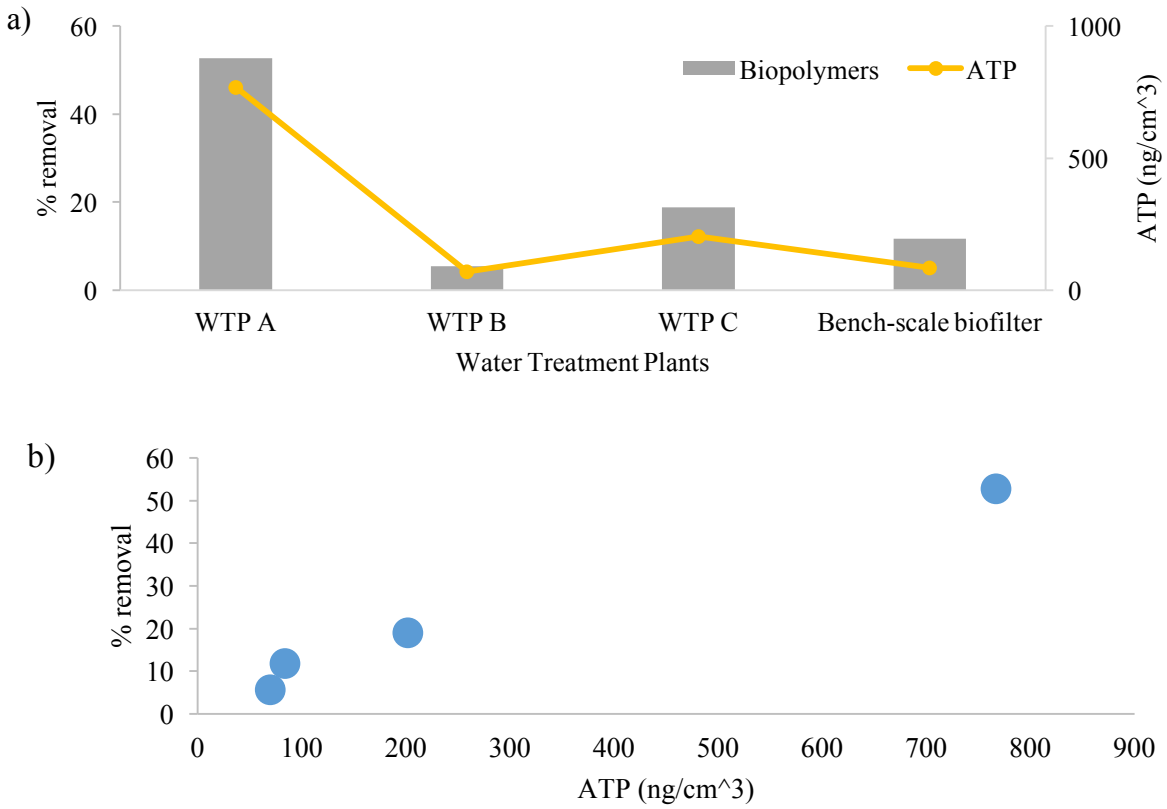

Figure 4.12. Biopolymers percent removal through the 3 full-scale biofilters and the bench-scale biofilter. ATP values of biofilter media from each biofilter were also shown in this figure. Figure a) shows the percent removal of media samples from each plant, and b) shows the relationship of percent removal and ATP values.

4.2.2 Batch testing of biofilter media for microcystin-LR removal

For each type of media tested in this experiment, the procedure was similar. Media from the biofilters was added to bottles containing different types of water, including biofilter influent water, biofilter effluent water and spring water with and without acetate. Different water types were tested to determine if nutrient concentration would affect biodegradation of MC-LR. Previous studies have shown the presence of nutrients might have different impacts on the biodegradation of MC-LR (Eleuterio & Batista, 2010; Li et al., 2011; Li et al., 2012; Li et al., 2014; Morón-López et al., 2017). Effects of NOM on the biodegradation of MC-LR was examined by Eleuterio & Batista (2010) who showed that TOC was the first to be biodegraded by MC-LR degradable bacterial communities, and MC-LR started to be biodegraded after

TOC. Glucose was found to inhibit the biodegradation of MC-LR at a concentration more than 100 mg/L, while no effects were observed with using low concentration of glucose. Acetate at 30 mg/L was also found to be a strong competitor to MC-LR degradation (Eleuterio & Batista, 2010). Consequently, the current research used the four different types of water with various water qualities to investigate the influence of NOM and additional carbon sources. Also, the microbial communities in the individual biofilters were acclimated to their respective feed waters. Biofilter feed water and effluent water contained different contents of NOM because effluent water was treated by the respective biofilter. Spring water had no NOM in contrast to the NOM-rich biofilter feed and effluent water. Acetate at 1 mg/L was added to spring water to examine the effects of an easily biodegradable carbon source on MC-LR biodegradation. The media from the full-scale plants was tested immediately after it was collected. Media from the bench-scale biofilter (BF1) was taken directly from the glass columns after the second biofiltration column testing. Influent water samples were taken from the intake water tank, and effluent water was taken from the effluent sample port before MC-LR was spiked into columns so that there was no MC-LR detected in the water samples. Biofilter media and sterile (autoclaved) control media from each location were tested in duplicate for MC-LR removal over a 3-day period.

Results from WTP A (Figure 4.13) shows MC-LR in the sterilized biofilter media bottle did not change very much in the 3 days after MC-LR was added, while biofilter media with biological activity caused the MC-LR concentration to decrease to 3 μg/L by day 1, and below 1 μg/L by the second day. As a result, media with biofilms caused a decrease in MC-LR concentration due to biodegradation. A similar pattern of MC-LR removal happened in all four types of water tested. Thus, water quality including nutrient concentration was not an important factor affecting MC-LR degradation by biofilter media, at least during the time of these experiments.

Figure 4.13 shows that the initial concentrations of MC-LR at 0 h varied, and they were all lower than the targeting initial concentration (40 μg/L). These differences at time 0 were also found in other biofilter media. There wasn't a pattern found in these different types of water and different types of biofilter media, and the reason was unknown. However, a possible reason could be MC-LR attachment to particles in water

or on the biofilter media surface.

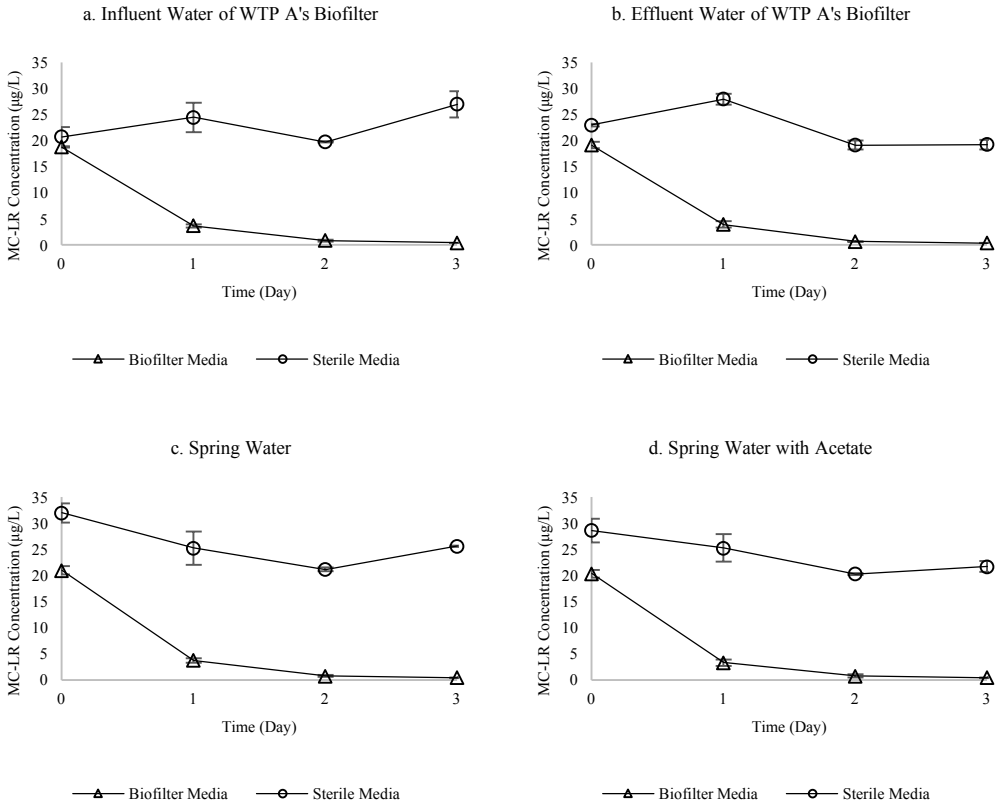

Figure 4.13. Concentration of MC-LR changes in shaker tests of WTP A's biofilter media. Biofilter influent water, biofilter effluent water, spring water and spring water with acetate were tested with biofilter media and sterile biofilter media. Data points show average values, and error bars represent standard deviations from duplicate bottle samples.

Research (Table 4.7) has studied the ability of bacterial communities in batch reactors for the biodegradation of MC-LR (Ho et al., 2010; Eleuterio & Batista, 2010; Li et al., 2012; Yang et al., 2014). The effectiveness and efficiency for bacterial degradation of MC-LR varied in different experiments. The fastest removal was noted by Yang et al. (2014), who used a specific bacterial culture to degrade MC-LR in a mineral salt medium that provided necessary nutrients for the bacterial growth. They found it took 36 hours for the bacteria to completely degrade MC-LR from an initial concentration of 11 mg/L. Evidence can also be found from Ho et al. (2010) that it took about three days for natural bacterial communities to degrade 20 µg/L MC-LR in treated wastewater. Eleuterio & Batista (2010) also suggested an enhanced

efficiency for MCLR biodegradation by a single bacterial species compared with natural bacterial communities.

As WTP A's biofilter media sterilized and with no effective ATP could not remove any MC-LR in any of the four types of water, this demonstrated that MC-LR removal was due to biodegradation and not adsorption to the anthracite media. Furthermore, it would also be necessary to do column testing to investigate the potential for WTP A's biofilter media to remove MC-LR by the full-scale biofilters, but this couldn't been done in this study because of a lack of time. An experiment like that can provide direct information on the potential of MC-LR to be removed by the full-scale biofilters. Additionally, WTP A's media also had the largest value of biological activity of the four media samples tested, which might indicate that an efficient ATP value could be an indicator of the biofilms' capacity for removing MC-LR. Hence, future work can look into the relationship between ATP values on biofilter media and MC-LR removal.

Table 4.7. Results of studies that tested the biodegradation of MC-LR in different water samples.

Reference	Source of inoculated bacterial communities	Initial MC-LR concentration	Maximum removal	Time when maximum removal reached	Water sources
Ho et al., 2010	Natural bacteria cultures within the tested water	20 µg/L	100%	3 days	Treated Wastewater
Eleuterio & Batista, 2010	Natural bacteria cultures within the water from cyanotoxins bloom lake	100 µg/L	80%	7 days	Bacterial culture enrichment medium
Eleuterio & Batista, 2010	Bacterial cultures from a full-scale biofiltration systems media	100µg/L	80%	6 days	Bacterial culture enrichment medium
Li et al., 2012	Biofilms within a biological water treatment tank	100µg/L	100%	7 days	Sterile distilled water
Yang et al., 2014	A specific bacterial culture isolated from sludge samples in the research lake	11 mg/L	100%	36 hours	Mineral salt medium
This study	Natural biofilms on a full-scale biofilter	40µg/L	99%	3 days	Biofilter influent water (treated)

The batch-test results for WTP B's biofilter media are shown in Figure 4.14. Figure 4.14 includes four figures which show four different types of water including influent and effluent water from WTP B's biofilter, and spring water and spring water with acetate. Coal-based F300 GAC manufactured by Calgon

Carbon was used as the biofilter media in this WTP.

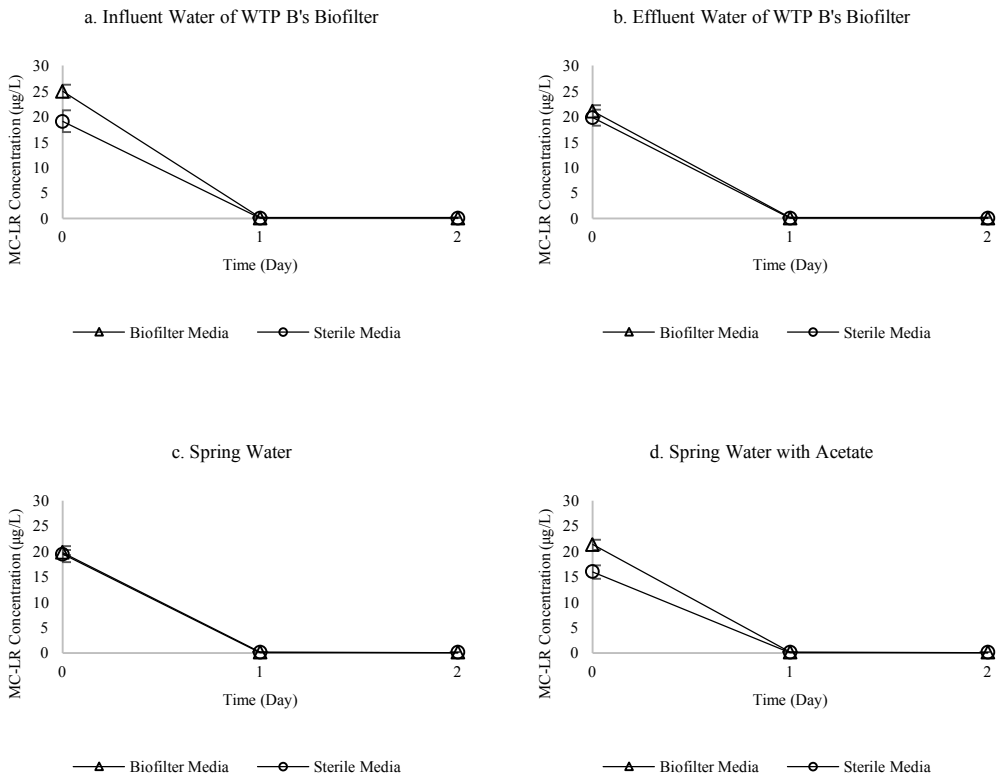

Figure 4.14. Concentration of MC-LR changes in shaker tests of WTP B's biofilter media. Biofilter influent water, biofilter effluent water, spring water and spring water with acetate were tested with biofilter media and sterile biofilter media. Data points show average values, and error bars represent standard deviations from duplicate bottle samples.

Results show that the MC-LR concentration decreased to lower than 0.5 µg/L by day one in every experimental bottle. A strong effect of media itself can be observed, as both the sterilized and non-sterilized media could remove MC-LR at a similar rate. Consequently, adsorption of MC-LR by the GAC media can be determined as the main mechanism of removal. Moreover, any potential biodegradation of MC-LR by the biofilms on the GAC could not be measured due to the fast adsorption. However, in continuous flow testing, adsorptive capacity could actually decrease or become exhausted, leaving a role for biodegradation.

Vlad (2015) found that pre-loaded GAC from WTP B had slightly higher capacity to adsorb anatoxin-A, another type of common-studied cyanotoxin, than virgin GAC, possibly due to the pre-loaded surface charge on GAC (Vlad, 2015). As a result, the GAC used in this experiment was not completely exhausted,

which could explain the main mechanism of GAC in removing MC-LR. Similar to WTP A, the concentration of MC-LR at time 0 h varied and was lower than the 40 ug/L target concentration added to each bottle. Although MC-LR samples were collected right after the MC-LR stock solutions were spiked, GAC might have resulted in rapid initial adsorption of the MC-LR.

A similar method was used for batch testing of MC-LR removal by WTP C biofilter media. As for the media from the other plants, biologically active biofilter media (GAC) and sterilized GAC was combined with four different types of water (WTP C's biofilter influent water, effluent water, spring water, and spring water with acetate). Results are shown in Figure 4.15. Since fast removal of MC-LR by GAC was observed in the earlier experiment for WTP B, an additional sampling time at 7 h was added. The GAC used in WTP C's full-scale biofilters was Filtrasorb® 816 (Calgon Carbon), and samples were collected on July 17, 2017. Spanjers (2017) tested the GAC from the same biofilter from this water treatment plant, and confirmed that the GAC was not exhausted, and could still adsorb natural organic matter.

The biofilter media (GAC) from WTP C was very effective in removing MC-LR due to its adsorptive capacity, similar to the results found with WTP B's biofilter media. In each treatment type, the MC-LR concentration was reduced to lower than 5 μg/L at 7 h (Figure 4.15). The concentration decreased to lower than 1 μg/L at 24 h, which was similar to results for WTP B. Also, there was no difference in results between sterilized GAC and non-sterilized GAC, which shows a strong adsorption of MC-LR by GAC media. As well, the four different types of water had similar removal rates, which indicated that water quality had no effect on removal during the time of this test. Again, MC-LR concentration at 0 h varied. There was faster removal of MC-LR by sterile compared with non-sterile GAC. It is possible that attached biofilms repressed the adsorption of MC-LR to the media.

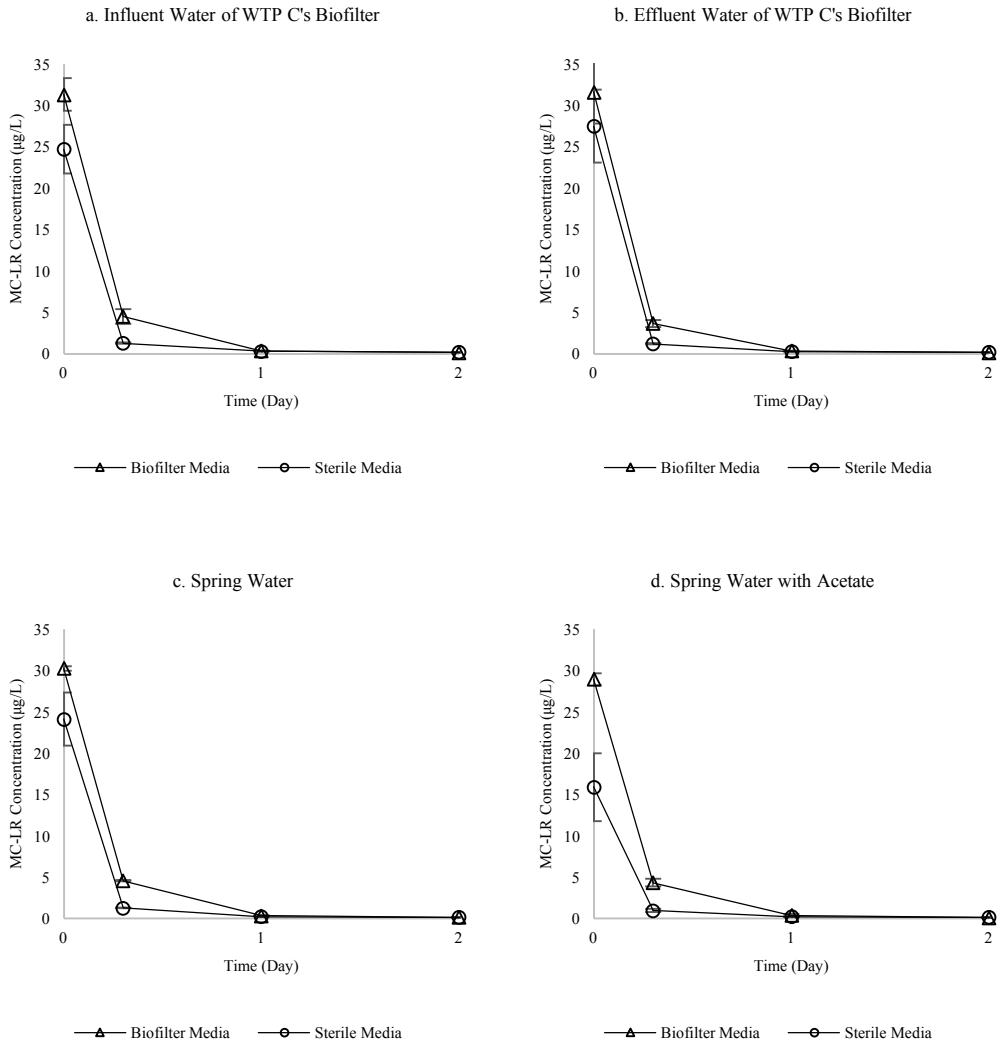

Figure 4.15. Concentration of MC-LR changes in shaker tests of WTP C's biofilter media. Biofilter influent water, biofilter effluent water, spring water and spring water with acetate were tested with biofilter media and sterile biofilter media. Data points show average values, and error bars represent standard deviations from duplicate bottle samples.

Results of bench-scale biofilter media that had been acclimated at the Elgin Area WTP for nine months are shown in Figure 4.16. The bench-scale biofilter media was taken from columns that were tested for MC-LR removal (Section 4.1). Biofilter media with biologically active biofilms could remove MC-LR to less than 5 µg/L after incubation for 24 h (Figure 4.16). MC-LR removal by the biologically active media incubated with biofilter effluent water was initially more rapid compared to the other three types of water (Figure 4.16b), but by day 3 removal was the same for all water types.

Due to a limited amount of media in the biofilter columns, only biofilter influent water was tested with sterilized biofilter media. Results (Figure 4.17) showed that sterile control media removed MC-LR under an unknown mechanism, possibly adsorption to anthracite particles. However, there were some differences in removal by sterile control media compared with biofilter media. Biofilter media decreased MC-LR concentration in a slower rate during the first day when compared to the results from the control filter media, possibly due to the biofilms blocking the adsorptive spaces on the media surface to adsorb MC-LR. It was also found in GAC adsorption to MC-LR that the adsorption could be hindered by active biofilms by either blockage of adsorptive surface on GAC and adsorption of biofilms releasing proteins (Wang et al., 2007; Drogui et al., 2012). However, at days 2 and 3 the MC-LR concentration in bottles containing biofilter media were lower than those of the sterile controls. The average MC-LR concentration was 0.26 μg/L at the third day in bottles containing biofilter media, while in those containing sterile control media it was 1.09 μg/L, although the absolute differences were small, statistically. T-test was done, and significant differences (95% significance level) were found when data in the sterile media and biofilter media were compared. The differences on the removal rate and amount of removal were possibly caused by the following processes: at the beginning when the biofilter media contacting with MC-LR, the adsorption probably played the main role on removing of MC-LR, and at the second and third day, MC-LR was removed through biodegradation so that more MC-LR were taken up by the media, and a lower concentration of MC-LR was detected than the sterile anthracite.

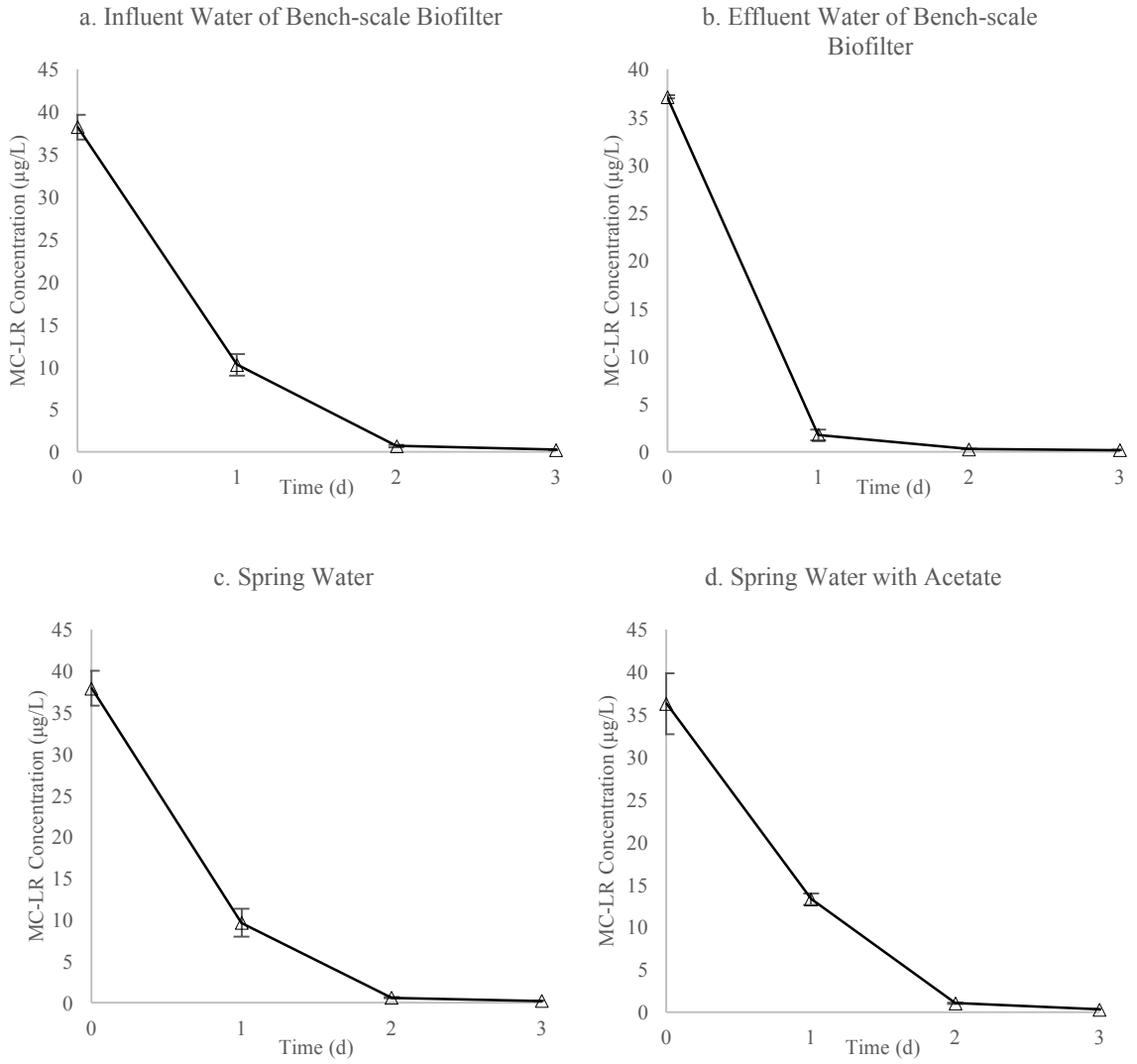

Figure 4.16. Concentration of MC-LR changes in shaker tests of bench-scale biofilter media. Biofilter influent water, biofilter effluent water, spring water and spring water with acetate were tested with biofilter media and sterile biofilter media. Data points show average values, and error bars represent standard deviations from duplicate bottle samples.

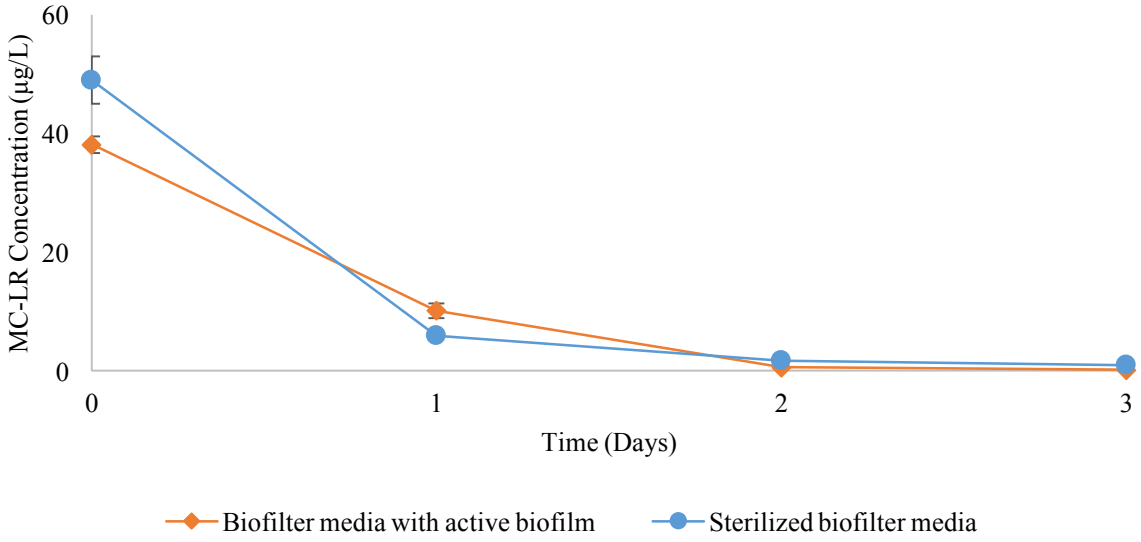

Figure 4.17. MC-LR concentration measured in biofilter influent water with biofilter media with and without active biofilm. Data points show average values, and error bars represent standard deviations from duplicate bottle samples.

In the batch testing for MC-LR removal by biofilter media from WTP A, anthracite without biologically active biofilms did not remove MC-LR. However, in the experiment testing MC-LR removal by anthracite from the pilot-columns, there was an unknown mechanism responsible for removing MC-LR by anthracite that was non-biologically active. Drogui et al. (2012) found a similar result and showed that an anthracite filter could reduce the MC-LR concentration as effectively as a GAC filter. A possible reason might be adsorption of MC-LR to the anthracite. Since the sterilized biofilter media (anthracite) was found to be able to remove MC-LR in the experiment for bench-scale biofilters, additional batch tests were done to explore more about the mechanisms for removing MC-LR. Autoclaved media taken from the control column (section 4.1) which had been in contact with natural lake water for less than a day (8 hours) was tested, together with new anthracite media that had been washed with ultrapure water 20 times before using. Bottles containing influent water used for the bench-columns but without media was also tested as a control. MC-LR concentration changes are shown in Figure 4.18, and MC-LR results were plotted on a log-scale, which made the differences clearer. Results show that the influent water itself without media did not show

a decrease in MC-LR concentration. However, new anthracite could remove MC-LR to less than 0.1 µg/L in 12 hours, which was the fastest among the three types of non-biologically active media. Comparatively, the sterilized biofilter media that was acclimated to natural water for 9 months has the lowest removal rate (Figure 4.18) and could only decrease the concentration of MC-LR to about 1 µg/L after 3 days. The sterilized filter media from the control column (exposed to raw lake water for only 8 h) had MC-LR removal that was in between removal of the new and the media acclimated for 9 months, and it could reduce the MC-LR concentration to about 1 µg/L in 1h and 0.2 µg/L in three days.

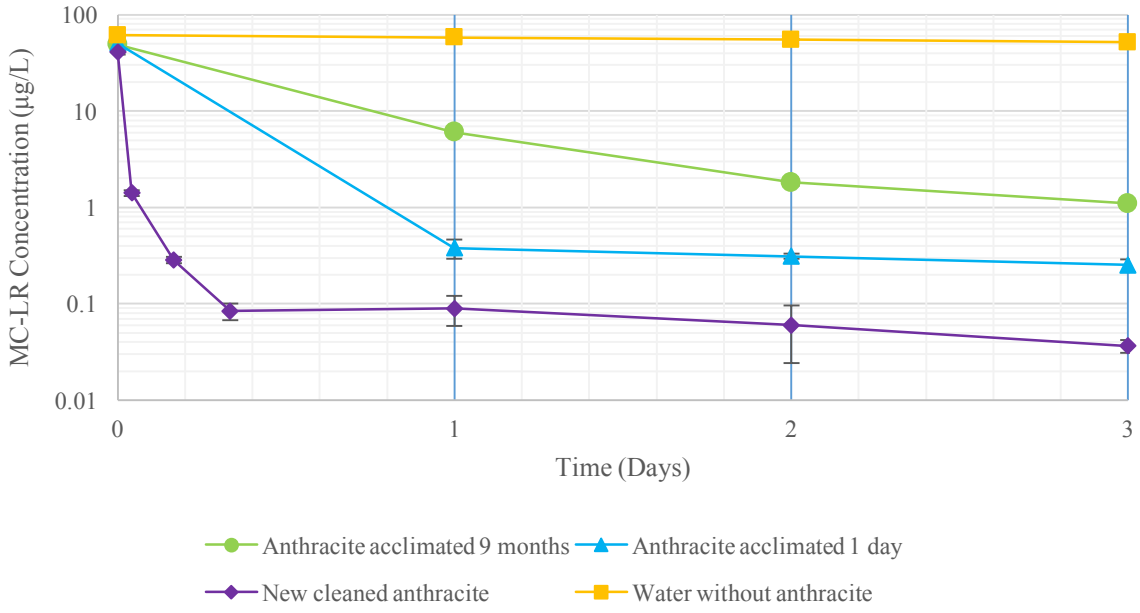

Figure 4.18. MC-LR concentration changes tested in biofilter influent water only, or combined with anthracite, including new virgin media, and sterile (non-biological) media from columns that had been exposed to natural lake water for 1 day or 9 months. Data points show average values and error bars represent standard deviations from duplicate bottle samples.

As a result, MC-LR removal by non-biological anthracite media was related to the time the anthracite was in contact with natural water. A potential reason for this is that the natural organic matter in lake water might have pre-loaded the anthracite. Pre-loading of organic matter might take up the adsorptive spaces on

the anthracite surface, and reduce the adsorptive spaces for MC-LR. However, no studies are found to demonstrate the mechanism of anthracite's adsorption to MC-LR. The fast removal for MC-LR by new anthracite could explain the removal of MC-LR at the beginning of the first biofiltration tests (Figure 4.6). However, after 1-h contact with natural water, the biofilters' capacity to adsorb MC-LR was hindered by NOM so that no further removal was found after 1 h (Figure 4.6). Another explanation is that the very limited adsorption capacity of anthracite was quickly used up. The adsorption was not observed in the testing of sterile biofilter media of WTP A, which possibly was due to the anthracite that had been used in WTP A's biofilters for a long time and had caused the anthracite to be exhausted.

4.2.3 Reaction rates

The reaction rate of cyanotoxin biodegradation has been demonstrated to be a pseudo first-order reaction, for compounds including MC-LR, cylindrospermopsin and geosmin (Ho et al., 2012a). To determine if MC-LR removal rates measured in the current study were pseudo-first-order reaction, MC-LR concentrations were simulated by the pseudo-first-order model using the following equation:

$$\ln C = \ln C_0 - kt \quad (1)$$

where t (days) is the reaction time, C (μg/L) is the concentration at t day, and C_0 (μg/L) is the initial concentration at time 0 day. k (day^{-1}) is the pseudo first-order rate constant.

Transform equation (1) to a concentration-based form results in the following equation:

$$C = C_0 e^{-kt} \quad (2)$$

The method has been determined to be effective in modelling the degradation of cyanobacterial metabolites in various studies (Ho et al., 2007; Zhou et al., 2011; Ho et al., 2012a). Non-linear regression with the use of least sum of squares was done to fit the pseudo-first-order reaction model to the data observed in this experiment, and to determine the reaction rate k (day^{-1}). The modeling was completed using Solver in Microsoft®.

The pseudo first-order model equation was matched to the MC-LR concentrations measured in the batch testing of biofilter media from WTP A, B, C, and bench-scale columns. As is shown in Figure 4.19, the pseudo-first-order kinetics could predict the reaction kinetics detected in the four different types of water. Pseudo-first-order reaction rates (k), the sum of squared errors of prediction (SSE) and half-lives in each type of water were calculated (Table 4.8). However, only small differences can be observed when results from different water types were compared, which demonstrated that the NOM didn't affect the biodegradation of MC-LR in this experiment. The smallest rate constant was 1.6 day^{-1} using the influent water with biologically active biofilter media, and spring water with acetate combining with biofilter media had the highest rate constant (1.8 day^{-1}) for removing MC-LR, which suggested that MC-LR was biodegraded slowest (10.5-hour half-life) in the biofilter effluent water, and fastest (9.3-hour half-life) in spring water with acetate. However in practice, this is a relatively small difference. Reaction in spring water with acetate had slightly higher SSE than other water types, which indicated that a higher variability of model prediction happened in the kinetics of reaction with spring water with acetate.

The same regression method was also applied to the observed data points when sterilized biofilter media and four different types of water was tested. The regression plots are shown in Appendix I, and results are summarized in Table 4.8. The pseudo-first-order reaction kinetics did not predict the data points of sterilized media testing since the sum of squared errors were great. However, the regression data from the 4 water types provided comparable results. The rate constants in the sterilized media testing did not exceed 0.1 day^{-1}, which was much lower than the rate constants found in non-sterilized biofilter media (1.6 day^{-1} to 1.8 day^{-1}). Half life times were also higher than the non-sterilized biofilter media testing, and a minimum of 149.3 h found in the spring water with acetate. Consequently, with the four types of water, sterilized media could not effectively remove MC-LR.

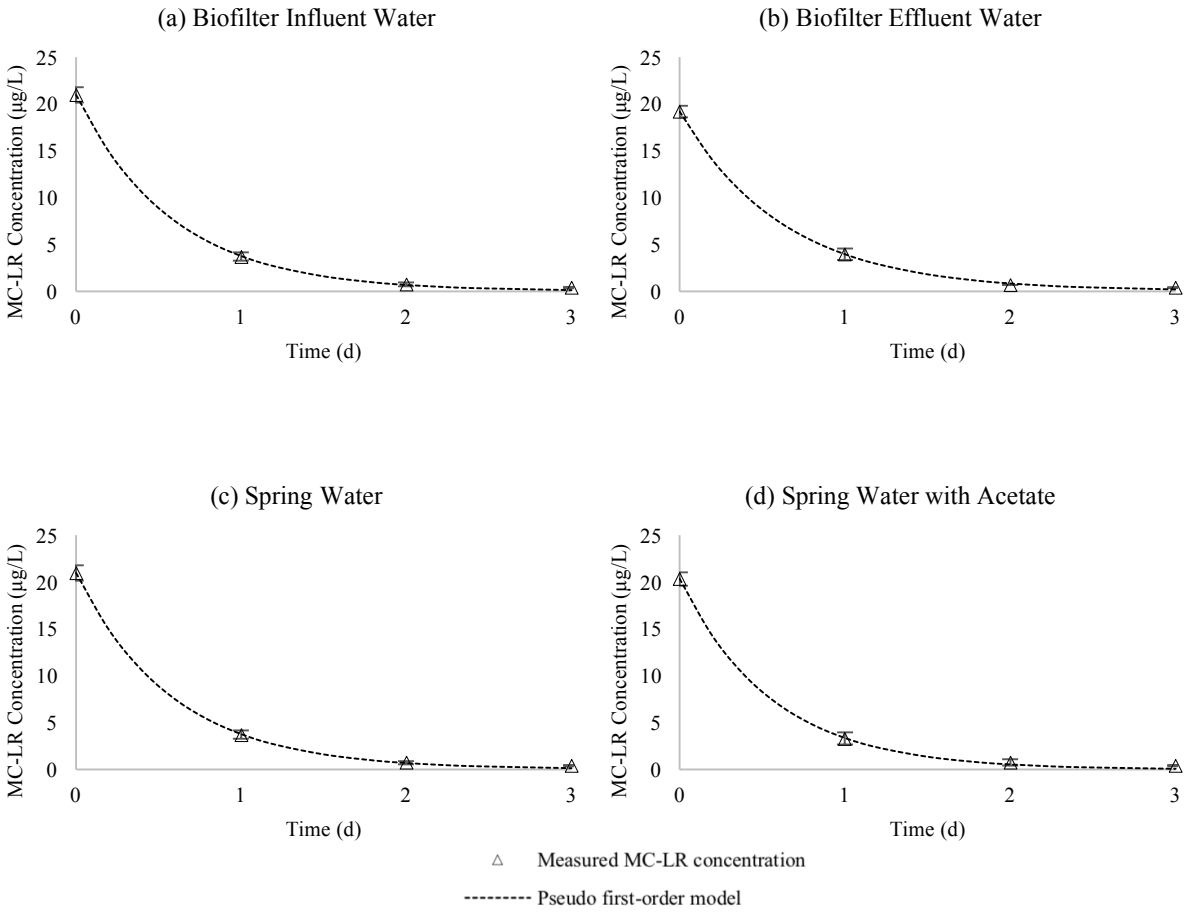

Figure 4.19. Removal of MCLR by biofilter media from WTP A showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values

Table 4.8 Parameters from nonlinear regressions of MC-LR concentration detected in the testing of WTP A biofilter media using pseudo-first-order reaction kinetics. Sterilized WTP A biofilter media regression results are also included.

	Biofilter media (767 ng/cm^3 ATP)				Sterilized biofilter media (0 ng/cm^3 ATP)			
	k (day^{-1})	k (h^{-1})	SSE	Half time (h)	k (day^{-1})	k (h^{-1})	SSE	Half time (h)
Influent water	1.7	0.072	0.09	9.7	0.09	0.004	30.44	184.8
Effluent water	1.6	0.066	0.07	10.5	0.08	0.003	32.51	207.9
Spring water	1.7	0.072	0.09	9.7	0.10	0.004	30.44	170.3
Spring water with acetate	1.8	0.075	0.14	9.3	0.1	0.005	7.68	149.3

The pseudo first-order model was also applied to MC-LR removal data from the bench-scale biofilter media (Figure 4.20). Results (Table 4.9) show that the pseudo-first-order model can fit the reaction kinetics using the non-linear least square method. Reaction rate constants, SSE, and half-life were calculated similarly to the modeling of the WTP A data. For the bench-scale media, it was noted that samples incubated with biofilter effluent water had much different data than the other three. The rate constant observed in effluent water testing was 3.0 day^{-1}, while values were 1.4, 1.5 and 1.2 day^{-1} in influent water, spring water, and spring water with acetate, respectively. Consequently, the half life of MC-LR in the effluent water was also as low at 5.5 h, which was much shorter than in the other types of water (8.3 to 14.3 h). The slow removal rate and long half life time could also explain the reason that no removal of MC-LR was found in the two biofiltration tests (Section 4.1). Effluent water had less organic fractions than influent water because effluent was treated by the bench-scale biofilters. As a result, the possible reason for the higher rate constant was the lower concentration of NOM. However, spring water and spring water with acetate had even less NOM than both influent water and effluent water, but a similar rate of removal as the influent water. Therefore, it is possible that the high removal at 1 h in the effluent water was an outlier, and the experiement would have to be repeated and tested together with sterilized filter media to confirm this result.

The same regression method was also applied to the sterilized bench-scale media testing with biofilter influent water (recall that there was insufficient media available to test all water types). The regression is shown in Figure 4.20 (e), and the results are included in Table 4.9. The rate constant was 2.0 day^{-1} and half life time was 8.3 h, which suggested a higher removal rate and shorter half life than the MC-LR removal found in the testing of non-sterilized biofilter media (1.4 day^{-1} and 11.9 h). The comparison suggests that MC-LR adsorption was hindered by the active biofilms on the media surface.

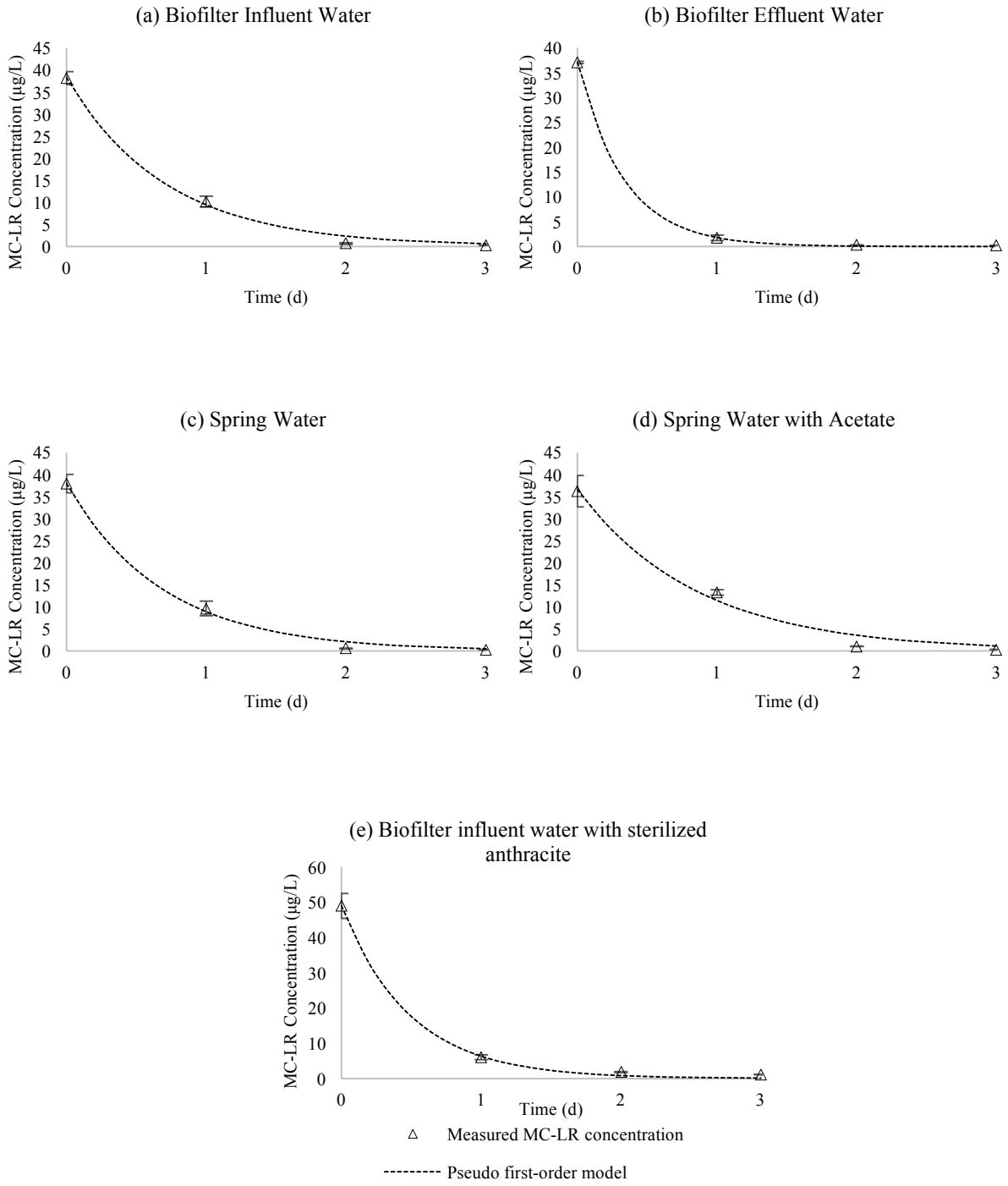

Figure 4.20. Removal of MCLR by biofilter media from the bench-scale biofilter showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). e) showed the pseudo-first-order model on the batch testing of influent water with sterilized bench-scale biofilter media. Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.

Table 4.9. Parameters from nonlinear regressions of MC-LR concentration detected in the testing of bench-scale biofilter media using pseudo-first-order reaction kinetics.

Parameters	Media types	k (day^{-1})	k (h^{-1})	SSE	Half life time (h)
Influent water	Acclimated anthracite	1.4	0.058	3.36	11.9
Influent water	Sterilized acclimated anthracite	2.0	0.083	2.07	8.3
Effluent water	Acclimated anthracite	3.0	0.126	0.09	5.5
Spring water	Acclimated anthracite	1.5	0.063	2.81	11.1
Spring water with acetate	Acclimated anthracite	1.2	0.048	10.57	14.3

MC-LR adsorption to GAC was also found in this experiment. The ability of activated carbon adsorptive to MC-LR has been demonstrated to be effectively simulated by the first-order model (Ho & McKay, 1999; Chennette, 2017). It was also applied in this experiment to investigate if the reactions matched the kinetics, which is shown in Figure 4.21 and Figure 4.22. Results in Figure 4.22 suggested that the adsorption of MC-LR to biofilter media from WTP B and WTP C could be predicted by the pseudo-first-order kinetics, which was also demonstrated by the low values of SSE. Reaction rates and SSE are shown in Table 4.10 (WTP B) and Table 4.11 (WTP C). Although the fit was also good in Figure 4.21, the essentially complete removal at the first data point (1 day) means that it is not really possible to distinguish how other reaction orders might fit. Reaction rate constants were observed between 5.0 to 5.4 day^{-1} in the testing of WTP B's biofilter media and 6.3 to 7.2 day^{-1} in the testing of WTP C's biofilter media. WTP B's biofilter media had slightly slower removal than WTP C's biofilter media, which was possibly due to GAC properties and other factors such as feed water type and service life. Different types of water used in the batch tests did not show much variability for media from either plant, which indicated that the GAC adsorption was not affected by the water quality changes in this experiment.

Regressions of data points observed in the testing of sterilized GAC from WTP B and WTP C biofilter media were shown in Figure I.2 and Figure I.3 in Appendix I. Results were summarized in Table 4.10 (WTP B) and Table 4.11 (WTP C). The pseudo-first-order kinetics could well predict the removal of MC-LR with sterilized GAC. Rate constants range from 5.5 day^{-1} to 5.7 day^{-1} in the WTP B biofilter media testing, which was slightly higher than they were observed in the non-sterilized GAC. In the testing of WTP C biofilter media, rate constants range from 9.3 day^{-1} to 10.5 day^{-1}, which was much higher than the non-sterilized biofilter media (6.3 to 7.2 day^{-1}). Consequently, the sterilized GAC could have better performance in adsorbing MC-LR, and the MC-LR adsorption may have been hindered by biofilms attached on the GAC. Comparatively, GAC with and without bacterial colonization was examined by Drogui et al. (2012) to investigate the adsorption of MC-LR in bench-scale filters. Results of their study showed that the GAC colonized by bacteria were less effective in removing MC-LR with an average residue of 2 μg/L in the effluent, compared to the GAC without bacteria that could completely remove MC-LR within the filters (initial concentration of 12 μg/L). A possible reason found by Drogui et al. (2012) was that released proteins by biofilms competed with MC-LR adsorption. In addition, the biofilms on the GAC could block the MC-LR transporting to the GAC surface, and reduce the adsorptive rate (Wang et al., 2007).

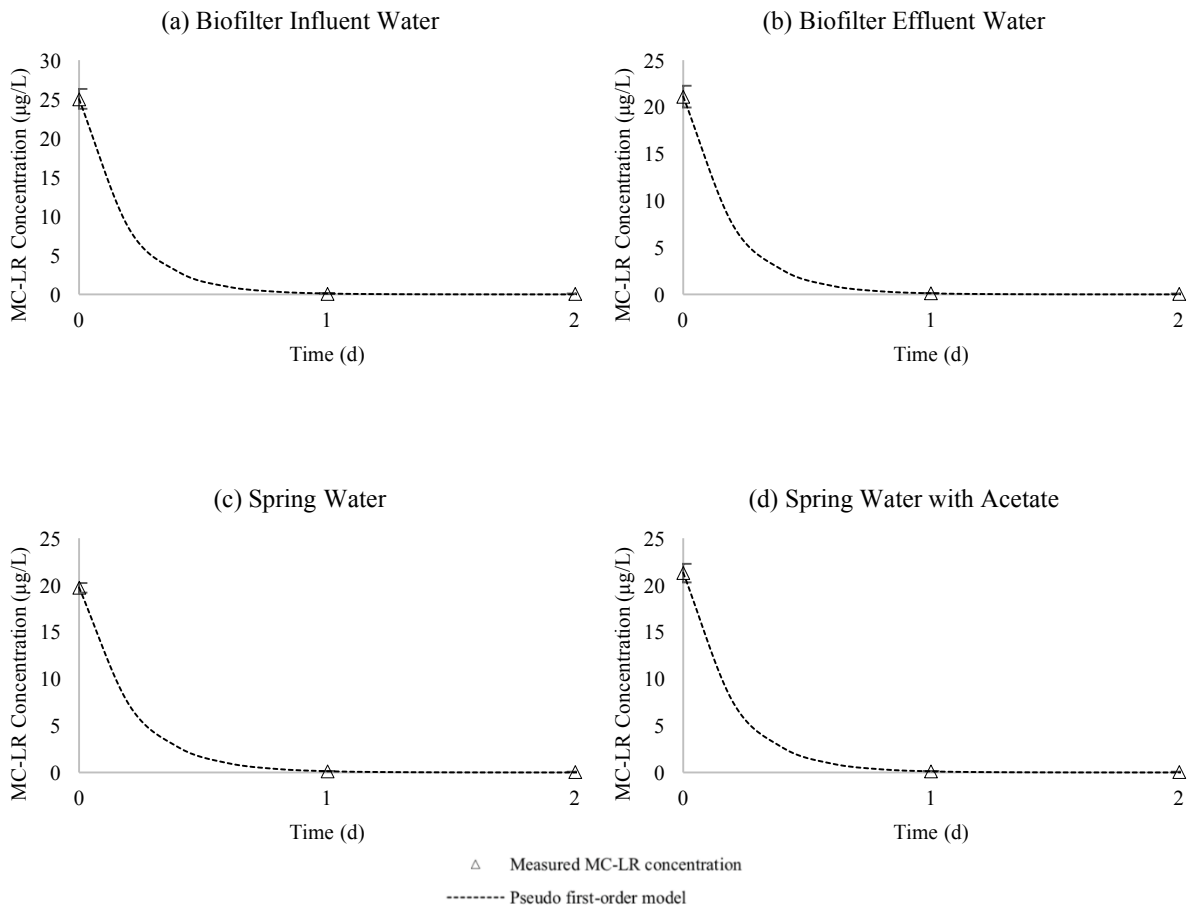

Figure 4.21. Removal of MCLR by biofilter media from the WTP B showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.

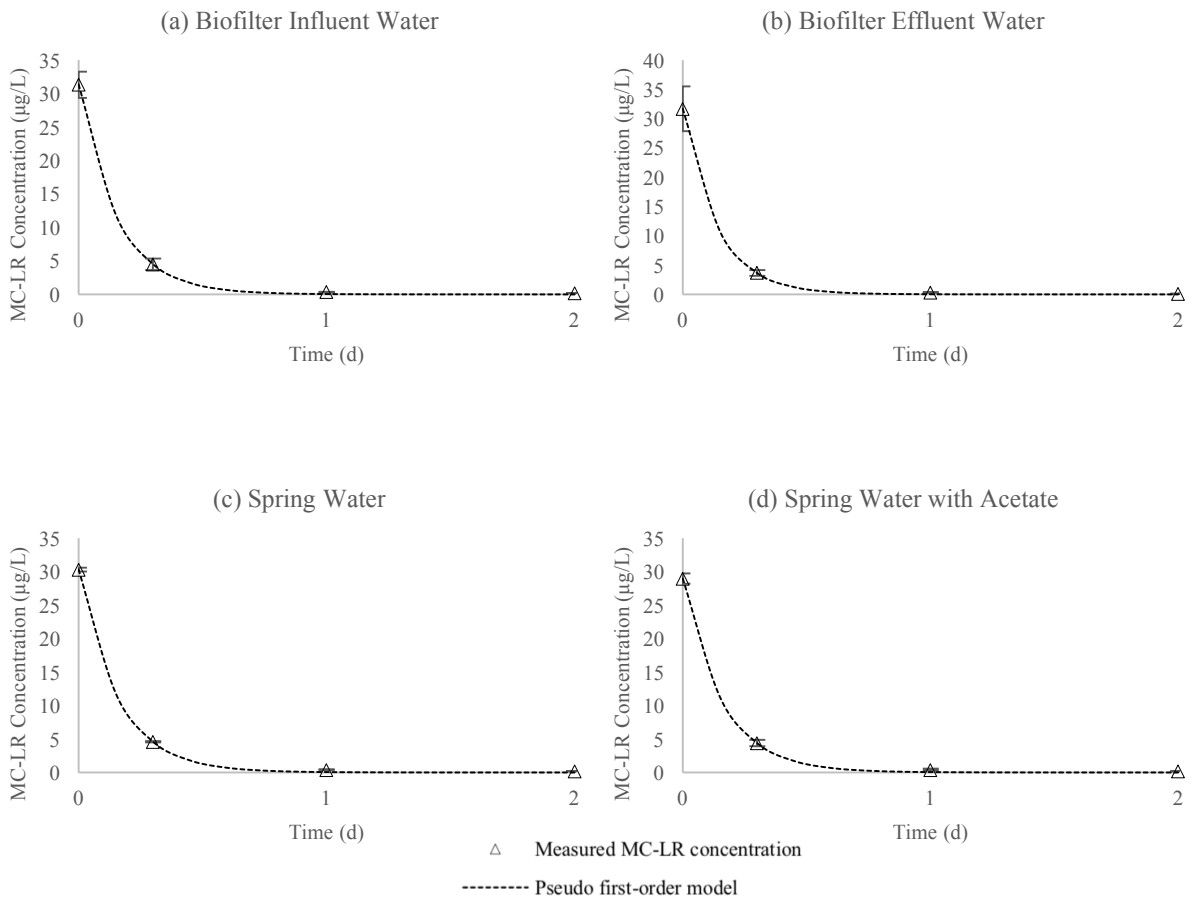

Figure 4.22. Removal of MCLR by biofilter media from the WTP C showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.

Table 4.10. Parameters from nonlinear regressions of MC-LR concentration detected in the testing of WTP B (Figure 4.21) biofilter media using pseudo-first-order reaction kinetics.

	WTP B's biofilter media (70 ng/cm^3 ATP)				Sterilized WTP B's biofilter media (0 ng/cm^3 ATP)			
Parameters	k (day^{-1})	k (h^{-1})	SSE	Half time (h)	k (day^{-1})	k (h^{-1})	SSE	Half time (h)
Influent water	5.4	0.225	0.01	3.1	5.7	0.236	0.003	2.9
Effluent water	5.2	0.218	0.01	3.2	5.7	0.238	0.004	2.9
Spring water	5.0	0.207	0.01	3.3	5.7	0.238	0.004	2.9
Spring water with acetate	5.2	0.217	0.01	3.2	5.5	0.228	0.003	3.0

Table 4.11. Parameters from nonlinear regressions of MC-LR concentration detected in the testing of WTP C sterilized biofilter media (Figure 4.22) using pseudo-first-order reaction kinetics.

	WTP C's biofilter media (202 ng/cm^3 ATP)				Sterilized WTP C's biofilter media (1 ng/cm^3 ATP)			
Parameters	k (day^{-1})	k (h^{-1})	SSE	Half time (h)	k (day^{-1})	k (h^{-1})	SSE	Half time (h)
Influent water	6.5	0.269	0.10	2.6	9.9	0.414	0.12	1.7
Effluent water	7.2	0.300	0.12	2.3	10.5	0.438	0.10	1.6
Spring water	6.3	0.262	0.14	2.6	9.7	0.405	0.08	1.7
Spring water with acetate	6.3	0.262	0.15	2.6	9.3	0.388	0.09	1.8

4.2.4. Summary of MC-LR removal by biofilter media

Table 4.12 summarizes the reaction rates observed in the current experiment for the 4 different types of biofilter media mixed with biofilter influent water and spiked with MC-LR and compared them with values from the literature. However, these studies only investigated the MC-LR biodegradation in natural water instead of using filter media. As is shown in Table 4.12, bench-scale biofilter media (anthracite) and WTP A biofilter media had much lower reaction rate constant than GAC from the other two water treatment plants biofilters, and as a result, the half-life times were also shorter with GAC than with anthracite, which was expected because of the strong adsorptive capacity from GAC, while no or little adsorption happened on anthracite media. Also, the adsorption by GAC covered the effects of biodegradation so that it was difficult to quantify the rate of biodegradation observed on these media.

Although the bench-scale biofilter media showed some adsorption in either biofiltration column testing and biodegradation batch testing, it did not show a greater reaction rate constant and longer half-life time than the anthracite from WTP A biofilter that did not adsorb MC-LR. Total biomasses measured as ATP on WTP A biofilter media (767 ng/cm^3) was detected much more than the bench-scale biofilter media (84 ng/cm^3), which possibly cause the higher rate of biodegradation of MC-LR.

Table 4.12. Results of the pseudo first-order model for the four types of biofilter media tested. Results from three other studies were also included to compare with this study.

Media	Media types	Water types	Pseudo-first-order reaction rate constant	Half-life time (d)	Source
Bench-scale biofilter media	Acclimated anthracite	Biofilter feed water	1.4	0.50	This study
Bench-scale biofilter media	Sterile acclimated anthracite	Biofilter feed water	2.0	0.35	This study
WTP A biofilter media	Acclimated anthracite	Biofilter feed water	1.7	0.40	This study
WTP B biofilter media	Acclimated GAC	Biofilter feed water	5.4	0.13	This study
WTP C biofilter media	Acclimated GAC	Biofilter feed water	6.5	0.11	This study
No media		Reservoir Water		3 to 4	Cousins et al. (1996)
No media		Wastewater	0.12		Ho et al. (2010)
No media		Lake water	0.03 to 0.31	2.2 to 17	Ho et al. (2012a)

Results of MC-LR biodegradation in media from WTP A were compared with other studies that used the same kinetic model. Ho et al. (2010) conducted an experiment using activated sludge effluent water to test the biological organisms in water to degrade MC-LR in a batch reactor. 20 μg/L MC-LR were spiked into the reactor, and pseudo-first-order decay rate was around 0.12 day^{-1}, which was one order of magnitude less than the results obtained in this experiment. Another experiment also conducted by Ho et al. (2012a) measured MC-LR biodegradation rate by natural organisms collected on a dam wall in a lake, and they found that the biodegradation had rate constants between 0.03 to 0.31 day^{-1}. 3-4 days half-life time was observed by Cousins et al. (1996), when they tested MC-LR biodegradation by natural microbial communities in reservoir water with an initial MC-LR concentration of 10 μg/L. Consequently, biodegradation of MC-LR in natural water and treated wastewater found in these three studies were much slower than the biodegradation rates detected on the anthracite media. The differences might be an indicator

of the positive effects from the media itself added into the reactor in this experiment, as biofilter media could be a carrier for bacterial cultures that attach as biofilm, so that they could have higher biodegradation activities and degrade more MC-LR than the bacterial culture living in water in their experiments.

For the WTP A biofilter media testing, the removal rate (presented as pseudo-first-order reaction rate constant) of MC-LR was similar in each type of water tested as shown in Table 4.6. However, the four types of water tested in the experiment varied, and influent water of the full-scale biofilter had the highest DOC (Table 4.13). Spring water had the least DOC among the four types of water because it mainly consisted of mineral salts. Acetate was added to the spring water to provide an additional carbon source and to directly compare the effects of readily biodegradable carbon on MCLR removal. Consequently, total DOC in the tested water ranged from 0.1 mg/L to 3.6 mg/L, and DOC did not affect the biodegradation of MC-LR found using the WTP A biofilter media.

Table 4.13. DOC contents of four types of water and estimated duration for biofilter media reducing MC-LR concentration to below 1µg/L. DOC contents are included to examine the direct relationship between DOC contents in water and the removing efficiency.

Water types	Biofilter Influent water	Biofilter Effluent water	Spring water	Spring water with Acetate
DOC content (mg/L)	3.6	3.0	1.3	0.1
Pseudo-first-order reaction rate constant (day^{-1})	1.7	1.6	1.7	1.8

In contrast, studies have found that water quality could be a significant factor for MC-LR biodegradation (Surono et al., 2008; Eleuterio & Batista, 2010; Li et al., 2012). For example, the addition of an energy source could help with the bacterial cell maintenance and survival (Surono et al., 2008). They found that, in the presence of 1 % glucose, MC-LR biodegrading bacteria could reach the most removal for MC-LR in mineral water (130mM sodium chloride, 10 mM sodium phosphate) (Surono et al.2008). However, Li et al., (2012) found that the addition of phosphate and glucose to natural lake water could suppress the biodegradation of MC-LR, from 7-days for complete removal (without nutrients) to 10-days

for complete removal with nutrients. The authors reasoned that by adding nutrients, this increased the communities of non-biodegrading bacteria so that they competed with MC-LR biodegrading bacteria and reduced their population. Eleuterio & Batista (2010) used acetate as the additional carbon source for testing the effects of water quality on the removal for MC-LR, and found that when acetate was added to the medium for bacterial enrichment, acetate significantly reduced the removal rate. However, the current experiment also used acetate as the additional carbon source in spring water, and found that it did not show any competing relationship with MC-LR. This suggests the explanation provided by Li et al (2012) may be correct, and that the acetate did not directly compete with MC-LR as a carbon source, but instead the added nutrients stimulated the growth of competing bacterial populations in water. The lack of response of acetate added to spring water is interesting both in the current study and in the study by Li et al (2012). It is possible that spring water lacked nutrients or other conditions that were not optimal for growth of the competing bacterial populations. Elhadidy (2016) found that bacteria isolated from the same river water used as the source at WTP A could not grow using inorganic minimal media or natural spring water. In future studies, it would be interesting test the effect of nutrients added to river water to determine the effect on MC-LR removal.

NOM concentration in the water samples has also been shown to have significant effects on GAC adsorption of MC-LR, since NOM can be competitive to MC-LR, and may reduce removal when NOM concentration is high (Huang et al., 2007). Removal rate of MC-LR was indicated by the pseudo-first-order reaction rate constant for the testing of WTP B and WTP C biofilter media (Table 4.13). As is summarized in Table 4.14, DOC content varied in each testing, and testing of WTP B biofilter media used water having a range of 0.1 mg/L to 3.3 mg/L, while testing of WTP C biofilter media used water having a range of 0.1 mg/L to 4.2 mg/L. However, few differences were found when comparing the rate constant in each type of water in both water treatment plants' biofilter media, which suggested that the DOC content didn't affect the MC-LR adsorption. A possible reason was the extensive adsorptive capacity from the GAC that could have the ability to quickly remove a large amount of both NOM and MC-LR so that no competition was found.

Table 4.14. MC-LR concentration at 0 h in the testing of biofilter media from WTP B and WTP C. DOC was also shown for each type of water from each plant to show relations to the concentration of MC-LR.

		Pseudo-first-order reaction rate constant (day^{-1}) from the testing using WTP B biofilter media			Pseudo-first-order reaction rate constant (day^{-1})) from the testing using WTP C biofilter media	
	DOC Content (mg/L)	Acclimated media	Sterilized meida	DOC Content (mg/L)	Acclimated media	Sterilized meida
Biofilter's influent water	3.3	5.4	6.5	4.2	5.7	9.9
Biofilter's effluent water	3.2	5.2	7.2	3.3	5.7	10.5
Spring water with acetate	1.3	5.0	6.3	1.3	5.7	9.7
Spring water without acetate	0.1	5.2	6.3	0.1	5.5	9.3

Chapter 5 Conclusions and Recommendations

5.1 Conclusions

The goal of this proejct was to determine if biofilters in drinking water treatment plants could remove MC-LR. To achieve the goal, two experiments were done in this study. In the first experiment, three bench-scale biofilters set-up in the Elgin Area Water Treatment Plant, Ontario, Canada, were acclimated. Acclimation of the biofilter media was done in order to build biofilms on the media surface using the natural water. During the 9-month acclimation, biofilters were taken into the University lab twice to run with natural water spiked with MC-LR for 6 hours. The first biofiltration column testing was done in March 2017 after 4 Month of acclimation at low temperature, while another one was completed in August 2017, which had varied acclimating water temperature that might affect the biofilms and biodegradation processes on the biofilter media. Based on the results observed in the bench-scale biofiltration column testing, the following conclusions can be drawn:

- Bench-scale biologically active filters acclimated with raw water from the Elgin Water Treatment Plant could not effectively eliminate MC-LR in drinking water in the first 6 hours after the MC-LR was added to the source water.
- EBCTs of 9 and 18 min were tested in the present study, and both EBCTs did not show MC-LR removal, which shows that longer EBCT was not a significant factor to affect the biofilters ability to remove MC-LR.
- A longer biofilter acclimation time (4 months versus 9 months) increased the biological activity (ATP values) on the biofilter media. However, the increased ATP values did not correspond with increased MC-LR removal by the biofilters during the short period of testing.

Few studies have provided evidence of the ability of drinking water biofilters to remove MC-LR either in full-scale or bench-scale. However, this study provided data to show that biofilters not previously

exposed to cyanotoxins could not effectively handle the sudden occurrence of cyanotoxins brought about by cyanobacterial blooms. It is likely that the time needed for building up the ability to remove MC-LR will take longer than 6 hours. Ho et al. (2006) have demonstrated the significance of time that allowed biological filter media to be pre-exposed to MC-LR before it developed the ability to remove MC-LR. Therefore, some alternative treatment methods to eliminate MC-LR are necessary at least initially to ensure drinking water safety when cyanobacteria/cyantoxins are suddenly detected in source water.

Since there was no removal observed in the column testing, batch testing was conducted to have a better understanding of the biodegradation processes of MC-LR on biofilter media. Specifically, biofilter media samples were taken from three full-scale water treatment plants (WTP A, B and C) in Ontario and the bench-scale biofilters tested in the biofiltration column testing. Twenty grams of media were mixed with 100mL of four different types of water spiked with 40 μg/L MC-LR. Tested water included biofilters' influent water, effluent water, spring water, and spring water with acetate. LC-OCD was used to measure each type of water, and showed the varied content of dissolved organic components that might be able to affect the efficacy of the biodegradation of MC-LR.

Based on the results observed in the biofilter media batch testing, the following conclusions can be drawn:

- Different types of biofilter media showed various results in terms of MC-LR removal, and summary results of removing MC-LR through the four types of media are shown in Table 5.1.
- Anthracite from WTP A biofilters with attached biologically active biofilms effectively removed MC-LR possibly through biodegradation. There was no MC-LR removal observed in sterilized media, therefore the biofilter media demonstrated removal of MC-LR by biodegradation. However, the removal rate of MC-LR found in the WTP A biofilter media was slow (1.7 day^{-1} as pseudo-first-order reaction rate).
- GAC from WTP B biofilter and GAC from WTP C biofilter removed MC-LR very fast through adsorption (5.4 day^{-1} and 6.5 day^{-1} as pseudo-first-order reaction rate), which indicates that GAC may have a better performance of removing MC-LR in full-scale biofilters than anthracite media

because of the high rate of adsorption.

Table 5.1. Summary of the results from the shaker tests for different types of media from four water treatment plants' biofilters.

Biofilter location	Scale	Media type	Biofilter media removing MC-LR	Sterilized media removing MC-LR	Water quality affecting the removal	Pseudo-first-order reaction rate constant of biofilter media removing MC-LR
WTP A	Full-scale	Anthracite	Yes	No	No	1.7 day^{-1}
WTP B	Full-scale	GAC	Yes	Yes	No	5.4 day^{-1}
WTP C	Full-scale	GAC	Yes	Yes	No	6.5 day^{-1}
Bench-scale biofilter	Bench-scale	Anthracite	Yes	Yes	No	1.4 day^{-1}

- Because of the strong adsorptive capacity of GAC in removing MC-LR, the potential for MC-LR biodegradation by biofilms could not be measured.
- Anthracite from the bench-scale biofilters acclimated in the Elgin Area WTP could remove MC-LR by adsorption. A slightly lower removal rate by non-sterile compared with sterile media shows that biodegradation potentially occurred, however the removal rate was very slow (1.7 day^{-1} as pseudo-first-order reaction rate). This slow removal rate could be the main reason why no removal through the bench-scale biofiltration columns was detected during the short time of testing.
- GAC adsorption had a much higher removal rate than the anthracite's biodegradation or adsorption based on the quantitative comparison of the pseudo-first-order reaction rate.
- Water quality, as measured by LC-OCD, turbidity, pH was not found to be an influencing factor affecting the removal of MC-LR in the shaker test in this experiment.
- Anthracite was found to have adsorptive capacity to MC-LR. Brand-new anthracite was found to have very fast adsorption for MC-LR in the shaker test, and the removal was hindered by the

duration that it had been used in biofilters.

A few previous studies have demonstrated that MC-LR can be biodegraded by bacterial communities (Ho et al., 2010; Eleuterio & Batista, 2010; Li et al., 2012; Yang et al., 2014). Instead of testing isolated bacterial cultures, this study showed that microorganisms associated with biofilm on biofilter media could biodegrade MC-LR. Of the four different types of biofilter media tested, WTP A biofilter media was the only one where biodegradation was the only mechanism to remove MC-LR, which indicates that there is a possibility and feasibility of using biofilters to remove MC-LR in full-scale drinking water treatment plants.

5.2 Recommendations for future work

From the conclusions of this research, several recommendations for future work are:

- WTP A biofilter media showed potential for MC-LR removal through biodegradation. Future work could focus on the mechanisms and influencing factors for biodegradation from the perspective of microbiology. Biofilms could be isolated from the media surface to identify the MC-LR-biodegrading bacterial organisms.
- WTP A biofilter media is recommended to be extracted from the full-scale biofilter and tested in bench-scale biofilter columns to determine if the biofilter media can remove MC-LR when water is flowing through the column.
- In order to further evaluate the ability of the bench-scale biofilters to remove MC-LR, the biofilters could be acclimated using water spiked with MC-LR, since media with a longer contact-time to MC-LR in the bottle testing could remove MC-LR. The positive effects of pre-loading MC-LR on low-flow biofilters have been studied by Ho and his co-workers (2006).
- Additional investigations are recommended to evaluate the availability and efficacy for inoculating identified MC-LR-biodegrading bacteria to the biofilter media.
- Additional investigations and comparisons with other commonly used biofilter media that does not adsorb MC-LR are needed, since the various media types might be a significant influencing factor for the performance of biofilters to remove MC-LR.

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Appendix A. Sample MC-LR calibration curve

Table A.1. MC-LR calibration standards as measured by LC-MS/MS

MC-LR Concentration (µg/L)	Internal Standard (IS) Peak Area	MC-LR Peak Area	MC-LR Peak Area /IS Peak Area
0.1	8,879	1,214	0.14
0.25	9,150	3,029	0.33
0.5	9,669	5,703	0.59
1	9,337	11,276	1.21
10	8,614	102,505	11.90
20	8,630	235,334	27.27
40	9,082	470,839	51.84

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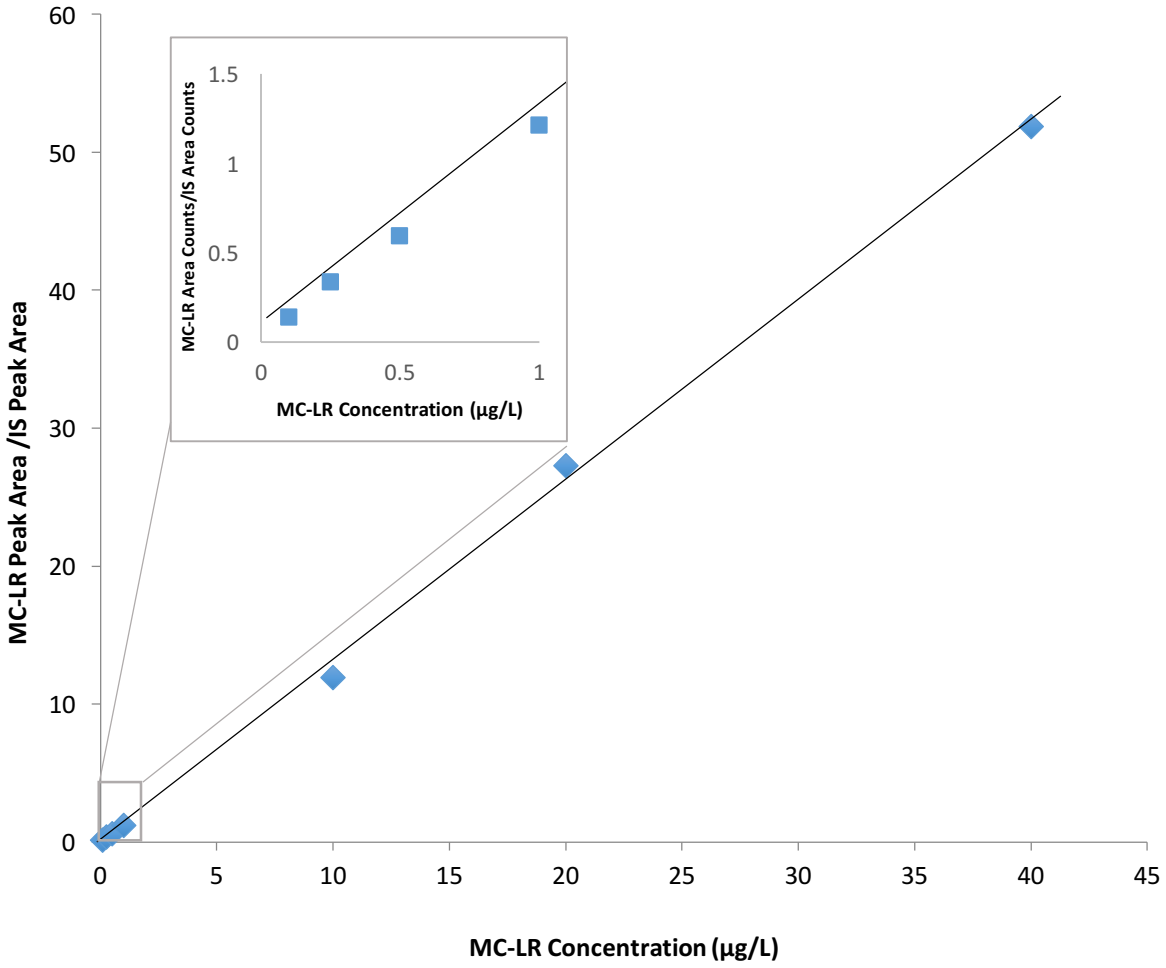

Figure A.1. Calibration curve of MC-LR detection on LC-MS/MS. R^2 (coefficient of determination) for the regression line is 0.999.

Appendix B. Formula used for calculation of ATP values on biofilter media

Calculate ATP values on media sample A

$$\text{Total ATP } \left(\frac{\text{ng}}{cm^3}\right) = \frac{Media\ sample\ A\ RLU\ value\ (RLU)}{Standard\ RLU\ value\ (RLU)} \times \frac{50000\ pg\ ATP}{Dry\ media\ sample\ A\ weight} \times \text{Media density } \left(\frac{\text{g}}{cm^3}\right) \times \frac{1}{1000}\left(\frac{pg}{ng}\right)$$

Appendix C. Diagrams of Pilot-Scale Biofilter Column Set-up

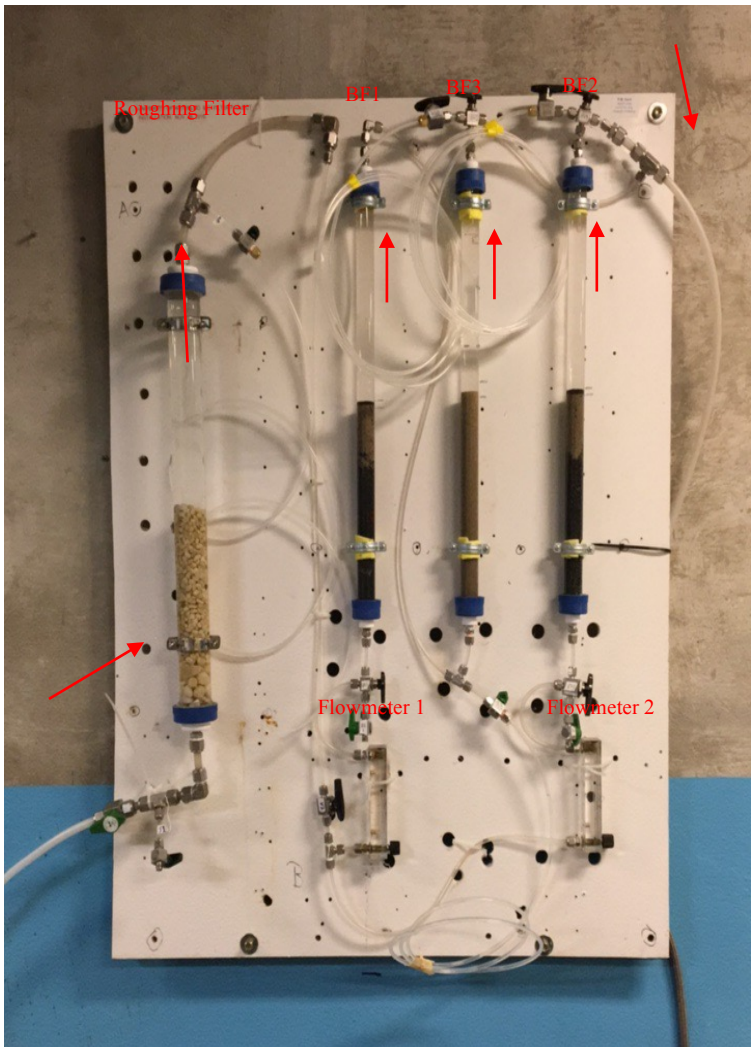

Figure C.1. Pilot-scale filter column set-up mounted at the Elgin Area Water Treatment Plant. Red arrows indicate the flow direction. BF1, BF2 and BF3 are biofilters.

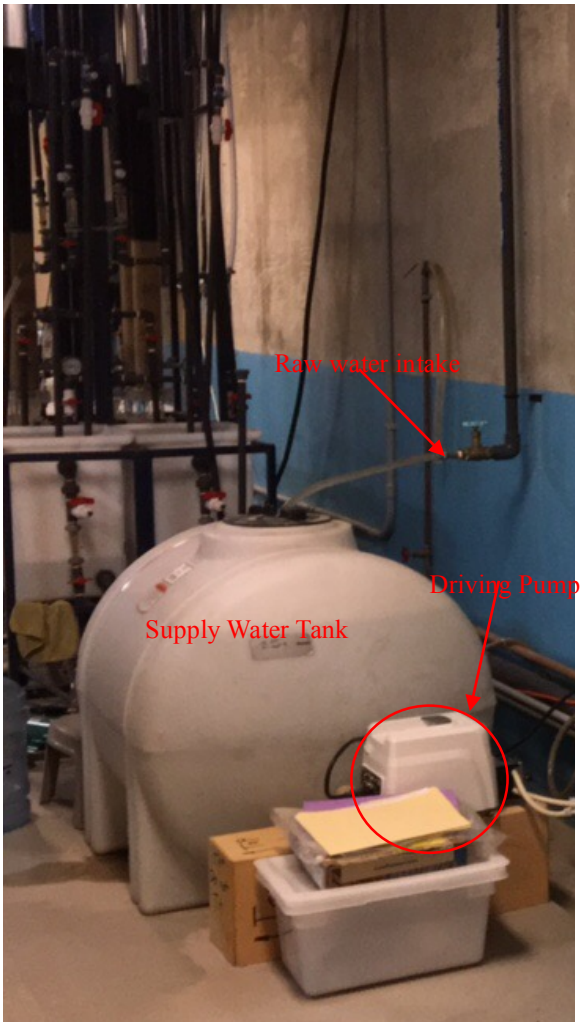

Figure C.2. Water tank used to feed the pilot-scale filter columns at the Elgin plant. The tank continuously received a supply of raw water from the full-scale plant. A peristaltic pump was used to transfer water from the tank to the columns.

Appendix D. Biofilter Column testing setup used for testing MC-LR removal at the University of Waterloo

(a) (b)

Figure C.1. Column testing setup at the University of Waterloo laboratory. Three biofilters (a) and two control filters (b) were mounted on a metal frame. Red arrows indicate the flow direction.

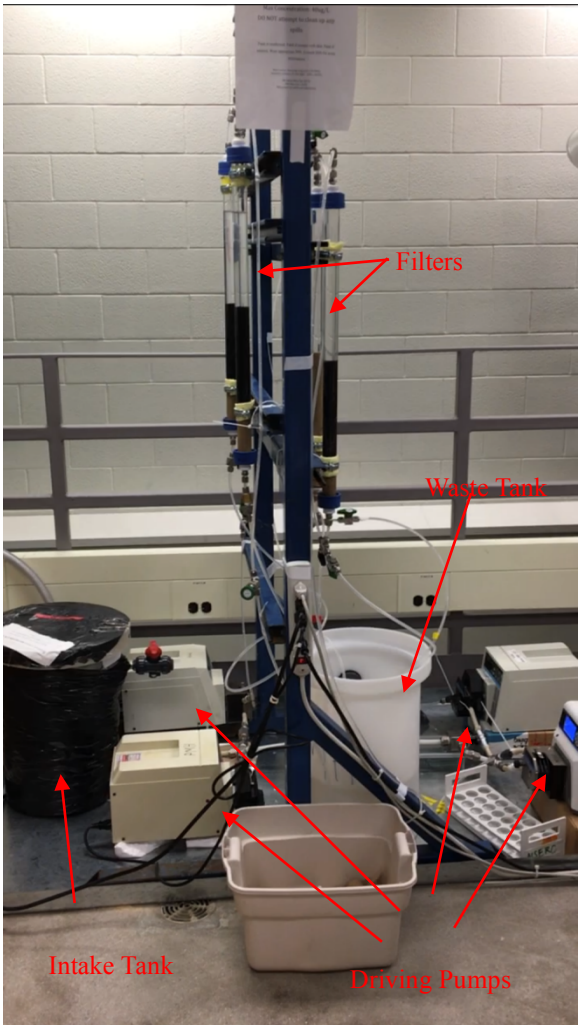

Figure D.2. Full-view of the column setup.

Figure D.3. Feed water intake tank used in the experiment containing raw water from the Elgin and spiked with MCLR. It was covered with black plastic to preventing light.

Appendix E. Spring water and spring water with acetate Composition

Spring water and spring water with acetate used in the shaker testing was made by adding a mineral salts solution to commercial Evian Natural Spring Water (Cedex, France). The Evian Natural Spring Water composition (Table A.1) was provided on the original package of the water bottle. Additional carbon (as acetate), nitrogen (as ammonia) and phosphorus (as hydrogen phosphate) were added as follows. 1 mL stock solution of mineral salts solution (0.171 g/L K_2HPO_4 & 0.767 g/L NH_4Cl) were added to the Evian Natural Spring Water result in final concentrations as shown in Table A.2. 1mL stock solution of mineral salts solution and 250 µL stock solution of carbon source (0.400 g/L sodium acetate) was added into the Evian Natural Spring Water to get the spring water with 1 mg/L acetate as carbon. The spring water was autoclaved (121℃, 15 min) after the mineral solution was added. It was noted that acetate, ammonia, and phosphate were calculated. pH was measured. For all other parameters, the values are those provided as the original packs.

Table E.1. The composition of spring water used to prepare the spring water.

Mineral	Concentration (mg/L)
Ca	81
Mg	27
Na	6.7
K	1
HCO3	360
SO4	13
Cl	9
NO3 as N	0.88
SiO2	15
F	0.1
Mn	0.001
Ni	0.001
Ba	0.11
Uranium	0.002

Table A.2. The final concentration of additional mineral salts in the spring water.

Mineral	Spring Water	Spring Water with Acetate
	Concentration (mg/L)	Concentration (mg/L)
Acetate as C	0	1.0
NH_4 as N	0.2	0.2
HPO_4 as P	0.03	0.03
Ca	81	81
Mg	27	27
Na	6.7	6.7
K	1	1
HCO3	360	360
SO4	13	13
Cl	9	9
NO3 as N	0.88	0.88
SiO2	15	15
F	0.1	0.1
Mn	0.001	0.001
Ni	0.001	0.001
Ba	0.11	0.11
Uranium	0.002	0.002
pH	7.38	7.45

Appendix F. Organic matters content analyzed by LC-OCD

Table F. 1. Organic matter data analyzed by LC-OCD in the influent and effluent of bench-scale biofilters that were acclimated at the Elgin Area Water Treatment Plant. Water samples were taken every month from raw water feed and effluent from BF1, BF2, BF3. All the concentrations are shown as µg-C/L.

Time	Water sample	DOC	Biopolymers	Humics	Building blocks	LMW neutrals	LMW acids
Jan, 2017	RAW	2138	369	1234	296	204	59
	BF1	2100	317	1263	252	221	84
	BF2	2074	347	1234	228	220	81
	BF3	2237	353	1269	258	262	104
Feb, 2017	Raw	2099	265	1462	106	267	39
	BF1	2090	292	1251	259	237	46
	BF2	1993	229	1244	276	264	58
	BF3	1977	258	1231	290	261	47
Mar, 2017	RAW	2084	317	1144	276	159	51
	BF1	1941	362	1182	232	174	49
	BF2	2046	323	1128	304	238	54
	BF3	2184	323	1148	269	188	56
Apr, 2017	RAW	1945	303	1176	289	181	56
	BF1	1952	346	1218	246	177	53
	BF2	1878	324	1172	288	168	52
	BF3	1932	342	1147	306	167	54
May, 2017	RAW	2068	256	1267	219	235	64
	BF1	1550	230	988	181	125	42
	BF2	1466	218	962	167	123	33
	BF3	1426	202	987	198	112	34
Jun, 2017	RAW	2720	283	1092	529	285	212
	BF1	2323	280	1253	326	274	143
	BF2	2012	277	1160	309	262	130
	BF3	2294	272	1329	344	281	125

Jul, 2017	RAW	1924	253	1145	221	247	68
	BF1	1511	206	988	227	161	51
	BF2	1466	142	958	217	151	51
	BF3	1463	182	992	188	135	44
Aug, 2017	RAW	1870	393	1158	169	204	64
	BF1	1821	334	1117	188	188	68
	BF2	1754	321	1105	144	179	68
	BF3	1695	325	1125	127	187	69

Appendix G. MC-LR concentration measured in the first biofiltration column testing

Table G. 1. MC-LR concentration detected in the influent water.

Time	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
(h)	Rep.1	Rep.2	Rep.3		
0	29.76	30.69	30.05	0.48	30.17
1	30.14	27.47	28.09	1.40	28.57
2	28.97	28.47	27.37	0.82	28.27
3	30.42	30.70	29.17	0.81	30.10
4	29.07	30.84	29.65	0.90	29.85
5	30.76	30.00	34.22	2.25	31.66
6	35.87	36.31	38.23	1.26	36.80

Table G. 2. MC-LR concentration detected in the effluent water of BF1.

Time	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
(h)	Rep.1	Rep.2	Rep.3		
0	5.51	1.27	1.88	2.29	2.89
1	27.31	27.88	20.29	4.22	25.16
2	29.39	26.30	28.56	1.60	28.08
3	30.88	28.06	31.62	1.88	30.19
4	30.36	30.96	30.27	0.38	30.53
5	30.93	32.22	31.68	0.65	31.61
6	33.42	30.88	35.42	2.28	33.24

Table G. 3. MC-LR concentration detected in the effluent water of BF2.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
0	0.17	0.20	0.17	0.02	0.18
1	27.61	27.33	28.93	0.85	27.96
2	27.36	26.92	28.43	0.78	27.57
3	28.47	30.21	28.40	1.03	29.03
4	30.05	31.40	31.28	0.75	30.91
5	31.82	31.90	30.38	0.86	31.37
6	36.43	36.43	33.77	1.54	35.54

Table G. 4. MC-LR concentration detected in the effluent water of BF3.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
0	19.81	22.25	20.77	1.23	20.94
1	27.74	26.90	28.15	0.64	27.59
2	28.37	26.52	26.67	1.03	27.19
3	30.02	29.79	31.01	0.65	30.27
4	32.06	31.59	30.94	0.56	31.53
5	29.21	31.64	31.06	1.27	30.64
6	32.78	38.17	35.85	2.70	35.60

Table G. 5. MC-LR concentration detected in the effluent water of CF1.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
0	0.59	0.42	0.52	0.08	0.51
1	16.04	17.32	16.31	0.68	16.56
2	24.67	25.68	25.09	0.51	25.15
3	28.21	31.43	30.41	1.64	30.02
4	30.72	30.12	29.99	0.39	30.27
5	29.83	28.19	29.68	0.90	29.24
6	35.52	32.30	34.16	1.61	34.00

Table G. 6. MC-LR concentration detected in the effluent water of CF2.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
0	1.36	1.29	1.55	0.14	1.40
1	20.44	21.33	22.63	1.10	21.47
2	26.49	25.58	25.41	0.58	25.83
3	31.75	31.65	30.08	0.93	31.16
4	29.79	28.21	31.45	2.29	29.82
5	29.78	31.57	29.35	1.18	30.23
6	33.20	30.93	34.15	1.65	32.76

Appendix H. MC-LR concentration measured in the Second biofiltration column testing

Table H. 1. MC-LR concentration detected in the influent water.

Time	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
(h)	Rep.1	Rep.2	Rep.3		
2	39.19	37.13	38.44	1.05	38.25
3	35.69	35.73	40.04	2.50	37.15
4	32.74	39.67	33.15	3.89	35.19
6	33.27	36.88	33.23	2.10	34.46

Table H. 2. MC-LR concentration detected in the effluent water of BF1.

Time	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
(h)	Rep.1	Rep.2	Rep.3		
2	35.21	40.85	36.23	3.00	37.43
3	39.97	34.91	34.65	3.00	36.51
4	32.44	33.45	32.00	0.74	32.63
6	35.35	32.77	33.29	1.36	33.80

Table H. 3. MC-LR concentration detected in the effluent water of BF2.

Time	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
(h)	Rep.1	Rep.2	Rep.3		
2	39.38	34.64	36.69	2.37	36.90
3	37.24	37.19	37.67	0.27	37.37
4	36.08	36.67	38.23	1.11	37.00
6	32.92	31.85	32.32	0.53	32.36

Table H. 4. MC-LR concentration detected in the effluent water of BF3.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
2	38.07	40.07	38.77	0.83	38.97
3	34.95	35.34	33.87	0.62	34.72
4	34.52	31.22	33.96	1.44	33.23
6	30.31	30.16	30.77	0.26	30.41

Table H. 5. MC-LR concentration detected in the effluent water of CF1.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
2	35.61	34.39	38.76	2.26	36.25
3	37.67	39.06	33.64	2.81	36.79
4	32.17	29.35	31.75	1.52	31.09
6	34.43	35.50	32.25	1.66	34.06

Table H. 6. MC-LR concentration detected in the effluent water of CF2.

Time (h)	MC-LR Concentration (µg/L)			Standard Deviation	Average MC-LR concentration (µg/L)
	Rep.1	Rep.2	Rep.3		
2	36.97	33.94	33.70	1.82	34.87
3	34.54	33.92	39.53	3.08	36.00
4	37.76	34.88	31.34	3.21	34.66
6	31.79	31.21	31.73	0.32	31.58

Appendix I. Pseudo-first-order reaction model regression to sterilized media of WTP A, B and C

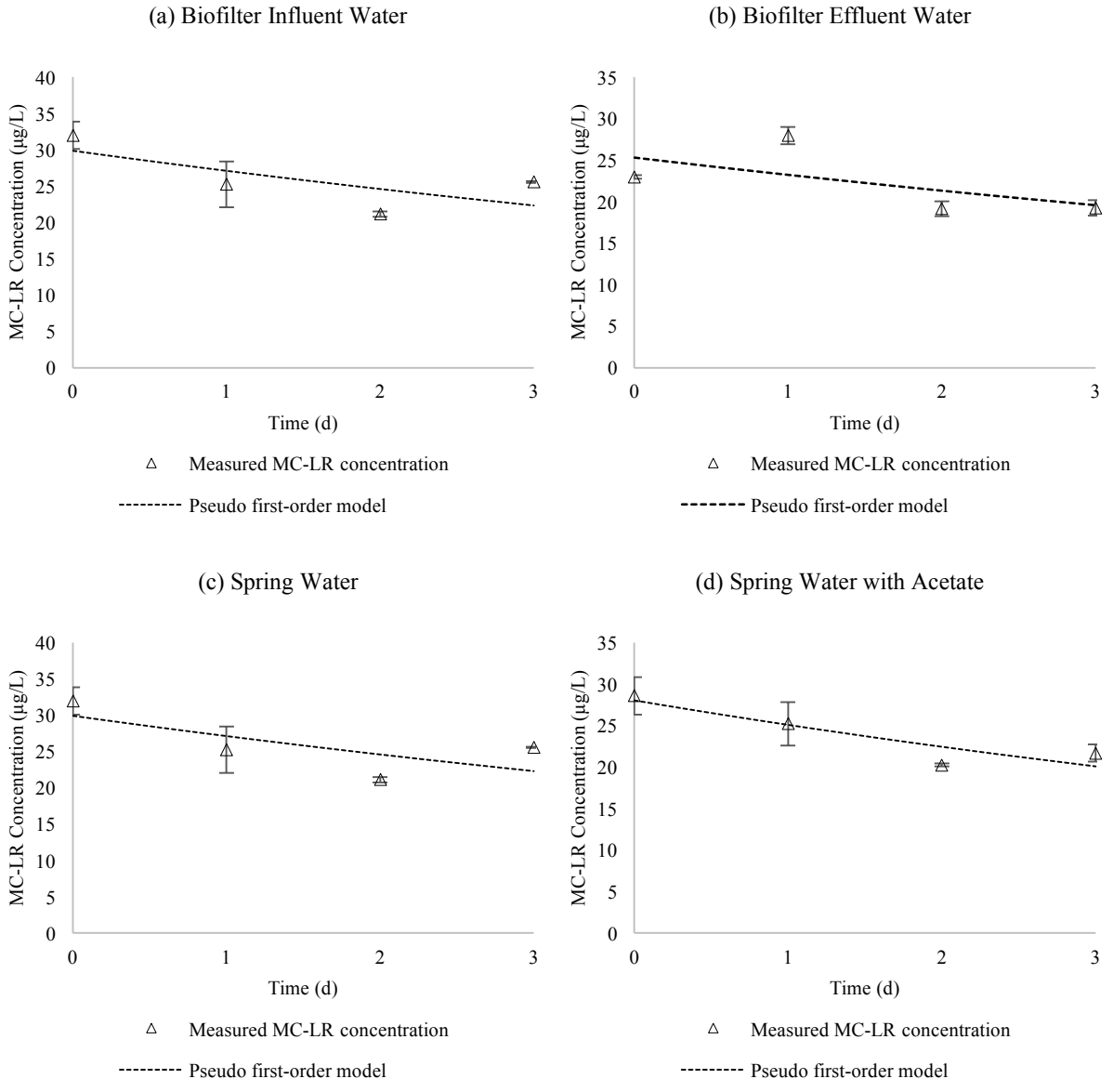

Figure I.1. Removal of MCLR by sterilized biofilter media from WTP A showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.

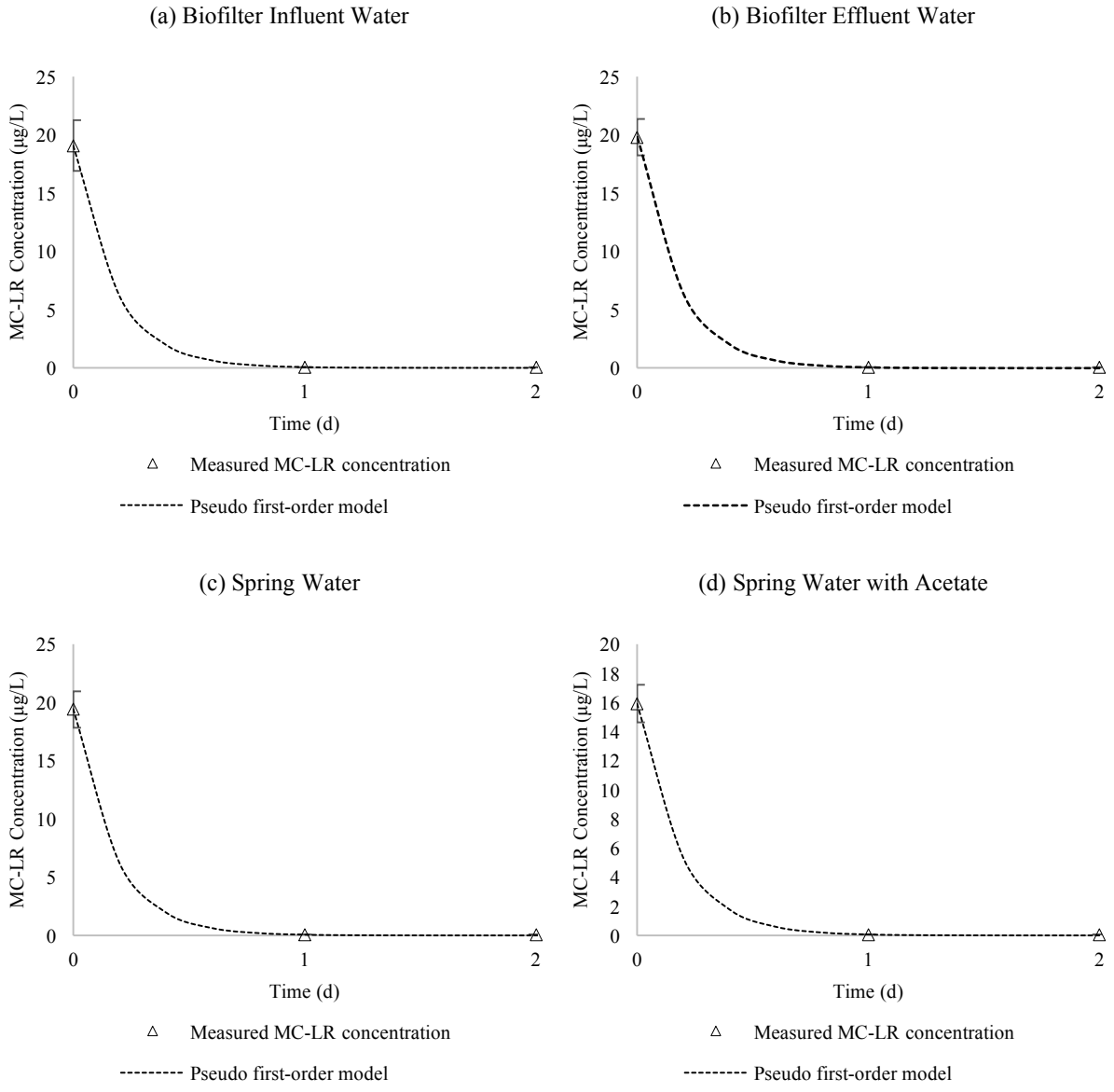

Figure I.2. Removal of MC-LR by sterilized biofilter media from the WTP B showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.

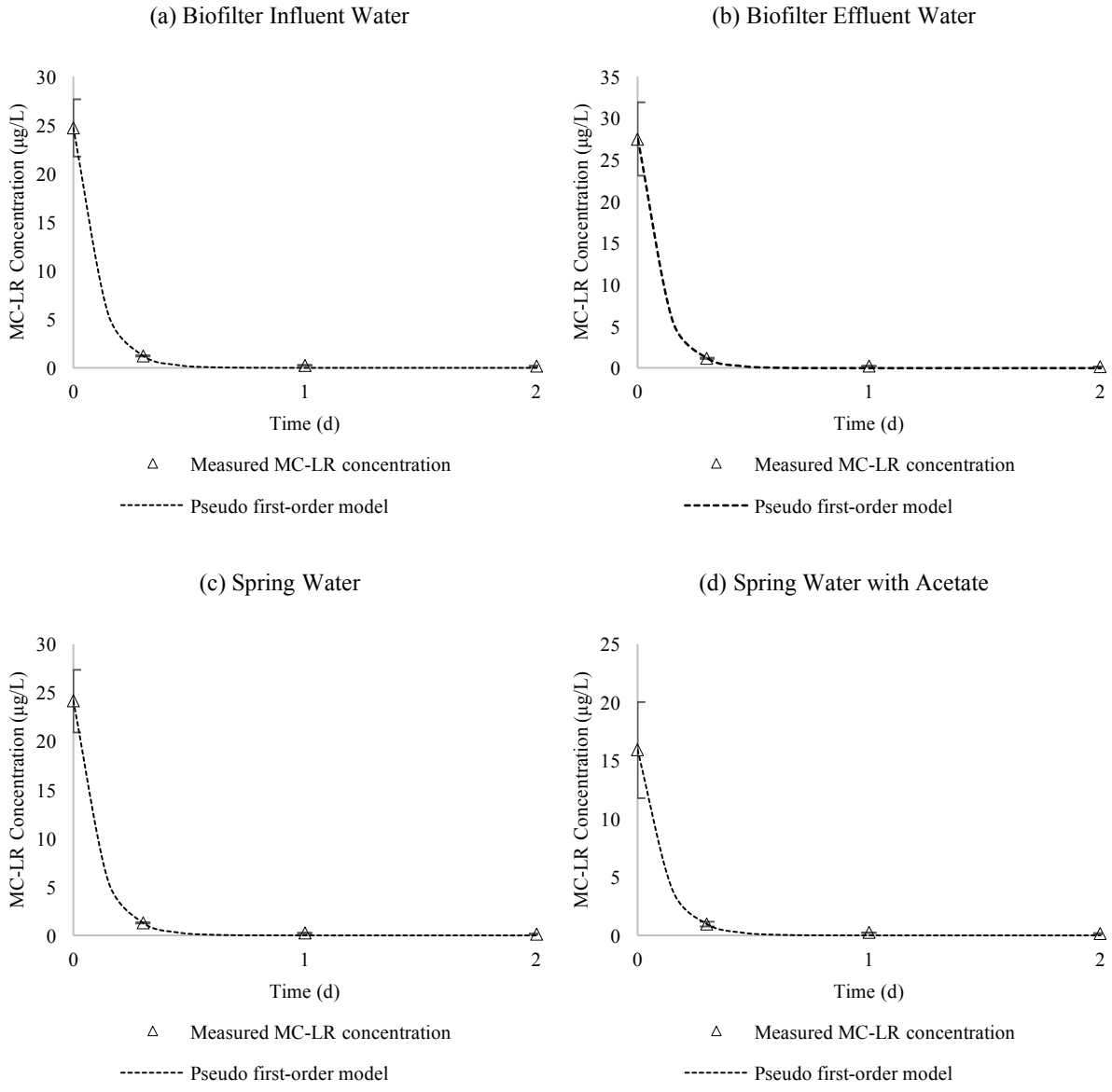

Figure I.3. Removal of MCLR by biofilter media from the WTP C showing measured MC-LR concentrations and associated pseudo first-order biodegradation model data. Four different types of water were tested including biofilter influent water (a), biofilter effluent water (b), spring water (c), and spring water with acetate (d). Error bars represent the standard deviation of samples from duplicate bottles, and data points show the average values.