Biofiltration Polishing of Ozone Treated Secondary Municipal Wastewater Treatment Plant Effluent

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Biofiltration Polishing of Ozone Treated Secondary Municipal Wastewater Treatment

Plant Effluent

by

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January 7, 2013
DECLARATION OF ORIGINALITY

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ABSTRACT

Ozone has been shown to be very effective in the transformation of several CECs that escape the wastewater treatment process, but there is concern whether toxic transformation products are formed. Two parallel biofilter columns with granular activated carbon (GAC) and filter sand following a pilot scale ozone unit to treat secondary municipal wastewater treatment plant effluent were studied. The biologically activated carbon (BAC) biofilter outperformed the sand biofilter in terms of DOC, DO and UV\textsubscript{254} removal. In addition, GAC supported more biological activity than sand media. Genotoxicity results show reduced wastewater genotoxicity following ozonation and further reduction following BAC and sand biofiltration. However, bacterial re-growth did occur in both biofilters following ozonation and needs to be taken into consideration when implementing ozone-biofiltration units.
DEDICATION

To my parents. Without their support, I would not have been able to continue my education.
ACKNOWLEDGEMENTS

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I am thankful to Saileshkumar Singh for teaching me how to run the ozone pilot unit, for his help and suggestions to design the biofilter pilot units and for guidance in the laboratory. I am also thankful to Dr. Kerry McPhedran for his help and suggestions while performing toxicity testing, solid phase extractions and for helping to edit my thesis. Furthermore, thanks to Dr. Elizabeth Fidalgo da Silva for her effort and suggestions with the E-SCREEN. Unfortunately the E-SCREEN did not materialize, but I am very thankful for getting the chance to work with Dr. Fidalgo da Silva.

I am thankful to our lab technicians, Bill Middleton and Matt St. Louis. Bill Middleton provided support and suggestions while performing laboratory analysis. Matt St. Louis assisted in fabricating the biofilter pilot units.

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LIST OF ABBREVIATIONS

AOD  Applied ozone dose
AOP  Advanced oxidation process
ATP  Adenosine tri-phosphate
BAF  Biologically active filter
BOD  Biological oxygen demand
CECs  Chemicals of emerging concern
CFU  Colony-forming units
COD  Chemical oxygen demand
CDPH  California Department of Public Health
DAF  Dissolved air flotation
DBP  Disinfection by-product
DO  Dissolved oxygen
DOC  Dissolved organic carbon
DOM  Dissolved organic matter
DW  Dry weight
ECA  Environmental Compliance Approval
E. coli  Escherichia coli
EDCs  Endocrine disrupting compounds
EfOM  Effluent organic matter
GAC  Granular activated carbon
HPC  Heterotrophic plate count
HRT  Hydraulic retention time
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>IJC</td>
<td>International Joint Commission</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>Lpm</td>
<td>Litre per minute</td>
</tr>
<tr>
<td>LRPCP</td>
<td>Little River Pollution Control Plant</td>
</tr>
<tr>
<td>MAC</td>
<td>Maximum acceptable concentration</td>
</tr>
<tr>
<td>MCL</td>
<td>Maximum contaminant level</td>
</tr>
<tr>
<td>MDL</td>
<td>Method detection limit</td>
</tr>
<tr>
<td>mgd</td>
<td>Million gallons per day</td>
</tr>
<tr>
<td>MF</td>
<td>Microfiltration</td>
</tr>
<tr>
<td>MOE</td>
<td>Ministry of the Environment</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>MWWE</td>
<td>Municipal wastewater effluent</td>
</tr>
<tr>
<td>MWWTP</td>
<td>Municipal wastewater treatment plant</td>
</tr>
<tr>
<td>NDMA</td>
<td>N-Nitrosodimethylamine</td>
</tr>
<tr>
<td>NF</td>
<td>Nanofiltration</td>
</tr>
<tr>
<td>ng/L</td>
<td>Nanogram per liter</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural organic matter</td>
</tr>
<tr>
<td>NPOC</td>
<td>Non-purgeable organic carbon</td>
</tr>
<tr>
<td>O₃</td>
<td>Ozone</td>
</tr>
<tr>
<td>OM</td>
<td>Organic matter</td>
</tr>
<tr>
<td>OMOE</td>
<td>Ontario Ministry of the Environment</td>
</tr>
<tr>
<td>ONPG</td>
<td>o-nitrophenyl-β-D-galactopyranoside</td>
</tr>
<tr>
<td>PAC</td>
<td>Powdered activated carbon</td>
</tr>
</tbody>
</table>
PCPs  Personal care products
PhACs  Pharmaceutically active compounds
POC   Purgeable organic carbon
PPCPs Pharmaceutical and personal care products
RO    Reverse osmosis
SOP   Standard operating procedure
SPE   Solid phase extraction
SUVA  Specific UV absorbance
TOC   Total organic carbon
TOD   Transferred ozone dose
UF    Ultrafiltration
USEPA United States Environmental Protection Agency
UV    Ultraviolet radiation disinfection
UVA   UV absorbance
UV\textsubscript{254} Ultraviolet absorbance at 254nm wavelength
WW    Wet weight
WWT   Wastewater treatment
WWTP  Wastewater treatment plant
CHAPTER 1
1.0 INTRODUCTION

1.1 Background

Water is an ever increasing commodity of importance. Two billion people in 48 countries are expected to lack an ample supply of fresh water by 2050 according the United Nations (U. N. Water, 2009). Geographic locations affected by droughts and that have few fresh water resources are investing in technologies to be able to reuse water (e.g., Australia, southern USA). Fortunately, the Great Lakes contain one fifth of the world’s fresh surface water supply so water reuse is not a concern along the Canada – USA border (Manninen, 2012). However, care needs to be taken to keep the Great Lakes and other fresh water bodies clean so that the water enjoyed by this generation can be enjoyed in future generations. A significant source of pollution discharge to the Great Lakes and other water bodies in the vicinity of urban areas are from municipal wastewater treatment plants (MWWTP). It is important for MWWTPs to discharge clean effluent into these water bodies since they are used as a source of drinking water and used for recreational activities (e.g., swimming at beaches).

MWWTPs utilize processes that are grouped into different stages: preliminary, primary, secondary and tertiary (advanced) wastewater treatment (Tchobanoglous et al., 2003). Preliminary treatment removes big objects and grit that could damage equipment. Primary treatment uses clarifiers to settle most of the remaining solids from wastewater. Secondary treatment uses biodegradation to remove organic matter. Tertiary treatment includes any of the following processes: disinfection, filtration, nutrient removal, advanced oxidation processes or adsorption. Roughly 99% of Ontario utilities and 89% of USA utilities that discharge treated wastewater into the Great Lakes use at least secondary treatment (IJC, 2011). Secondary
treatment is now common. Tertiary treatment is more common for communities that have water shortages and need to reuse water. In Ontario, only 14% of facilities that discharge into the Great Lakes have tertiary treatment (IJC, 2011).

Within the past 15 years compounds called “chemicals of emerging concern” (CEC) have been studied. CECs are usually unregulated and mainly include pharmaceutically active compounds (PhACs), personal care products (PCPs), antibiotics, hormones and endocrine disrupting compounds (EDCs). Metabolized and unmetabolized CECs are disposed into shower drains, toilets and sinks becoming wastewater. Wastewater flows through sewers eventually reaching MWWTPs for treatment. Many CECs are present in wastewater in ng/L levels and are only partially removed or not removed at all in wastewater treatment plants around the world (Ternes, 1998; Miao et al., 2004; Paxeus, 2004; Clara et al., 2005; Nakada et al., 2006). Therefore, these potentially harmful compounds are passing through wastewater treatment plants and being discharged into surrounding water bodies.

Removing CECs from wastewater treatment plant effluent is desirable to further improve the quality of treated wastewater. The current knowledge base on many of these CECs in low concentrations (ng/L and µg/L levels) does not give any indication if they pose risks to human health. However, studies have shown that chronic exposure to EDCs even at low concentrations can cause health effects on aquatic organisms, as well as reptiles, mammals and birds (Kidd et al., 2007; Tyler and Jobling, 2008; Lange et al., 2009; Bloetscher and Plummer, 2011). The concern is whether these CECs can potentially cause adverse health effects on humans or other wildlife from low level (eg. ng/L) long-term exposure.

The possibility for human or wildlife health effects has encouraged research into removing these CECs from water and wastewater. To improve removal of emerging
contaminants, upgrading wastewater treatment plants by providing tertiary treatment has been investigated in recent years. Upgrading wastewater treatment plants by providing ozonation has been studied to remove CECs because of ozone’s high oxidation potential. Ozone has shown to be very effective in oxidizing many CECs from wastewater treatment plants (Ternes et al., 2003; Huber et al., 2005; Snyder et al., 2006; Hollender et al., 2009; Reungoat et al., 2010; Gerrity et al., 2011; Lee et al., 2012; Singh, 2012). At Little River Pollution Control Plant in Ontario, Canada the effect of ozonation on secondary treated municipal wastewater was investigated for disinfection and the transformation of several CECs (Singh, 2012). A transferred ozone dose of 0.72 mg O$_3$/mg DOC was sufficient to consistently achieve the Ontario Ministry of Environment (MOE) disinfection limit, while transforming the majority of the detected CECs by over 80%.

However, ozonation does not completely mineralize CECs and other organic matter (OM) to carbon dioxide and water. The majority of these compounds are oxidized into more biodegradable ozone transformation products (TPs). The major uncertainty with ozone is the production of unidentified TPs that are more toxic than their parent compounds (Joss et al., 2008; Hollender et al., 2009). Biofiltration in ozonated water treatment has been studied for removal of biodegradable OM to prevent bacterial re-growth in the distribution system (Lechevallier et al., 1992; Krasner et al., 1993; Rittmann et al., 2002; Emelko et al., 2006). More recently it has been suggested that biofilters after ozone treatment of wastewater may be effective in removing possible toxic TPs (Joss et al., 2008; Hollender et al., 2009; Stalter et al., 2010).

Results from only a few studies on biofilters in combination with ozonation for municipal wastewater treatment are available and the results are promising. However, these studies are from different parts of the world and there are several differences in the level of treatment provided before ozonation, biofilter media used and process operations (Hollender et al., 2009;
Gerrity et al., 2011; Reungoat et al., 2011; Stalter et al., 2011; Lee et al., 2012). The efficacy of ozone-biofiltration is dependent upon the preceding process train as well as the wastewater matrix. Therefore, the performance of ozone-biofiltration in these studies is expected to vary not only due to these differences, but also due to possible differences in wastewater characteristics in different regions. Different methods of process assessments have been used and mechanisms responsible for the performance of biofilters with adsorptive and non-adsorptive media are not fully understood (Reungoat et al., 2011). Furthermore, despite the importance of meeting disinfection guidelines in Canada and the USA, the potential for bacterial re-growth in biofiltration units has only been investigated in a water reuse application (Gerrity et al., 2011). Given the encouraging results, there is a need for further evaluation of ozone-biofiltration as tertiary municipal wastewater treatment since its performance is affected by the preceding process train as well as differences in wastewater matrices in various regions of the world.

The efficacy of ozonation in achieving both disinfection and CEC oxidation for secondary treated municipal wastewater effluent (MWWE) was recently demonstrated by Singh (2012). However, no studies on the use of biofiltration to further oxidize the ozone TPs formed have been conducted in Canada.

1.2 Overall Objective

The main objective of the current study was to examine the effectiveness of biofiltration in reducing wastewater genotoxicity and improve the quality of ozone treated secondary municipal wastewater effluent in Canada.

The study was conducted at Little River Pollution Control Plant (LRPCP) in Windsor, Ontario, Canada using the ozonation pilot unit and transferred ozone dose established from a previous study (Singh, 2012). Two parallel pilot scale biofilters with sand and granular activated
carbon (GAC) as filter media were constructed. This provides a direct comparison between the adsorptive GAC media and the non-adsorptive sand media using the same ozonated wastewater. Experiments were divided into two phases: maturation phase and steady state performance phase.

1.3 Specific Objectives

The specific objective of the biofilter maturation phase was as follows:

- Determine the length of time both biologically activated carbon and sand biofilters require to establish a steady state DO, UV$_{254}$ and DOC removal

The specific objectives of the biofilter performance phase were as follows:

- Examine if ozone reduces the genotoxicity of wastewater
- Compare the effectiveness of two types of biofilters for removal of OM and genotoxicity
  - Biologically activated carbon (BAC) – biofilter with GAC (adsorbing media)
  - Sand biofilter (non-adsorbing media)
- Compare whether GAC or sand media support more biological activity
- Examine if bacterial re-growth in the biofilters raises the $E.~coli$ count above the disinfection limits established by the Ontario MOE
- Monitor traditional filter performance parameters: turbidity, suspended solids, and head loss. Examine if biofilters in wastewater treatment incur any operational problems, while providing acceptable head loss and filter run times.

1.4 Organization of Thesis

This thesis is organized into five chapters. Chapter 1 contains the introduction, overall objective and specific objectives of this study. Chapter 2 consists of literature review related to the concerns with municipal wastewater discharge, process units for removing toxic compounds,
the role of ozone and biofilters in wastewater treatment, biofilter performance monitoring parameters, previous studies and methods to measure toxicity, bacterial re-growth and viable biological activity measurements. Chapter 3 includes details of the experimental setup and methods used. The results and discussion are included in Chapter 4. Chapter 5 discusses the conclusions of this study and recommendation for future research.
CHAPTER 2
2.0 REVIEW OF LITERATURE

2.1 Concerns with Municipal Wastewater Discharge

The focus on environmental pollutant studies has shifted from ‘traditional pollutants’, such as PCBs, dioxins, and DDTs to ‘chemicals of emerging concern’ over the past two decades (CEC) (Ternes, 2007). The International Joint Commission (IJC) defined CECs as new compounds that have gained entry into the environment or those that have been recently characterized due to increases in concentrations in the environment or improvements in analytical techniques (IJC, 2011). The shift to monitoring CECs has been fuelled by two trends. First, improved analytical equipment now allows for organic compounds to be detected in ng/L levels (Ternes, 2007). Secondly, health effects from exposure to endocrine disrupting compounds on wildlife have attracted public concern (Colborn et al., 1993; Ternes, 2007).

CECs include three main groups: pharmaceuticals and personal care products (PPCPs), and endocrine disrupting compounds (EDCs). Many CECs are present below mg/L range and are only partially removed or not removed at all in conventional municipal wastewater treatment plants (MWWTP) around the world (Ternes, 1998; Miao et al., 2004; Paxeus, 2004; Clara et al., 2005; Nakada et al., 2006). CECs that are easily biodegradable and have high sorption characteristics are removed best (Ternes et al., 2004). Most CECs that pass through MWWTPs are commonly polar (cannot adsorb to settling suspended solids) and poorly biodegradable (Ternes et al., 2004; Ternes, 2007). MWWTPs are only designed to reduce solids, nutrients, dissolved biodegradable organic matter and pathogens. Therefore, many of these CECs are not removed and are being discharged into surrounding water bodies by MWWTPs.
Despite the widespread presence of CECs in the environment, there is little evidence they pose a risk to human health as an environmental contaminant (Gerrity and Snyder, 2011). However, studies on EDCs with low chronic ng/L levels show they cause feminization and other adverse health effects on aquatic organisms (Kidd et al., 2007; Tyler and Jobling, 2008; Lange et al., 2009). These studies have been conducted with the same concentrations (ng/L, µg/L levels) found in conventional municipal wastewater effluents (MWWE). Studies of varying EDC concentrations with other animals have shown that EDC chronic exposure has caused adverse health effects in reptile, amphibian, mammal and bird populations as well (Snyder et al., 2003; Bloetscher and Plummer, 2011). Several PPCPs have been reported as environmental contaminants, but little toxicological data exist on possible adverse health effects from trace levels of PPCPs (Snyder et al., 2003). Regardless, there is an ever-increasing amount of evidence to suggest that at the very least CECs may impact animals in surrounding ecosystems despite very low concentrations.

The concern is the potential unknown health-effects these chemicals may have on humans and more likely other animals in the surrounding environment from low concentration (eg. ng/L) long-term exposure. As of now, few emerging contaminants have regulations in Canada and the United States (IJC, 2011). Therefore, MWWTPs are not designed to remove CECs. The possibility for human or wildlife health effects has promoted research into removing these CECs from water and wastewater. Researchers have argued the precautionary principle should be applied to reduce human and wildlife CEC exposure until further toxicological data exist (Joss et al., 2008; Reungoat et al., 2011). To improve removal of emerging contaminants, upgrading wastewater treatment plants by providing tertiary treatment has been examined.
2.2 Treatment Process Units for CEC & Toxicity Removal

Ozone-biofiltration, advanced oxidation (UV/H\textsubscript{2}O\textsubscript{2}, O\textsubscript{3}/H\textsubscript{2}O\textsubscript{2}), membrane filtration, and activated carbon are technologies being researched to reduce the load of CECs and all other unidentified toxic compounds to the natural environment. Research studies on technologies other than ozone that have been shown to effectively remove CECs on mostly water and some wastewater matrices are: advanced oxidation processes (AOP) (Huber et al., 2003; Ternes et al., 2003; Sundaram et al., 2009; Gerrity et al., 2011; Katsoyiannis et al., 2011; Martijn and Kruithof, 2012), membrane filtration (Kimura et al., 2004; Snyder et al., 2007; Yoon et al., 2007; Lee et al., 2012), and adsorption (Ternes et al., 2002; Snyder et al., 2007; Yu et al., 2008). These studies are shown in Table 2-1.
Table 2-1: Technologies other than ozone that have been shown to effectively remove CECs

<table>
<thead>
<tr>
<th>Author</th>
<th>Technology</th>
<th>Process Unit</th>
<th>Matrix</th>
<th>Country Source</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ternes et al.</td>
<td>Adsorption</td>
<td>GAC</td>
<td>Water</td>
<td>Germany</td>
<td>2002</td>
</tr>
<tr>
<td>Yu et al.</td>
<td>Adsorption</td>
<td>GAC</td>
<td>Water</td>
<td>Ontario, Canada</td>
<td>2008</td>
</tr>
<tr>
<td>Huber et al.</td>
<td>AOP</td>
<td>O₃/H₂O₂</td>
<td>Water</td>
<td>Switzerland,</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>France, Finland</td>
<td></td>
</tr>
<tr>
<td>Ternes et al.</td>
<td>AOP</td>
<td>O₃/H₂O₂, O₃/UV</td>
<td>Wastewater</td>
<td>Germany</td>
<td>2003</td>
</tr>
<tr>
<td>Sundaram et al.; Gerrity et al.</td>
<td>AOP</td>
<td>O₃/H₂O₂</td>
<td>Water Reclamation</td>
<td>Reno, Nevada</td>
<td>2009;</td>
</tr>
<tr>
<td>Katsoyiannis et al.</td>
<td>AOP</td>
<td>UV/H₂O₂, O₃/H₂O₂</td>
<td>Water, Wastewater</td>
<td>Switzerland,</td>
<td>2011</td>
</tr>
<tr>
<td>Martijn and Kruithof</td>
<td>AOP</td>
<td>UV/H₂O₂</td>
<td>Water</td>
<td>The Netherlands</td>
<td>2012</td>
</tr>
<tr>
<td>Kimura et al.</td>
<td>Membrane</td>
<td>RO</td>
<td>Model Water</td>
<td>Japan</td>
<td>2004</td>
</tr>
<tr>
<td>Snyder et al.</td>
<td>Membrane</td>
<td>RO, NF, UF, MF</td>
<td>Water, Water Reclamation</td>
<td>USA</td>
<td>2007</td>
</tr>
<tr>
<td>Yoon et al.</td>
<td>Membrane</td>
<td>NF, UF</td>
<td>Water</td>
<td>Colorado, Nevada, New Jersey, USA</td>
<td>2007</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>Membrane</td>
<td>RO</td>
<td>Water Reclamation</td>
<td>New Mexico, USA</td>
<td>2012</td>
</tr>
</tbody>
</table>

Descriptions of the three categories of technologies other than ozone are given next. AOPs generate hydroxyl radicals to oxidize compounds that cannot be biodegraded or oxidized by conventional oxidants. AOPs differ from membrane filtration and activated carbon because organics are degraded rather than transferred to another phase (activated carbon) or concentrated for disposal (membrane filtration). Membrane filtration retains constituents less than the membrane pore size (as concentrate) and allows smaller constituents to pass (as permeate). Membrane filtration is a category made up of reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) (in order from smallest to largest pore size) (Tchobanoglous et al., 2003). In contrast, adsorption accumulates compounds from the liquid...
phase onto a solid surface; activated carbon is the most commonly used adsorbent (Tchobanoglous et al., 2003). Operational differences account for the variation in chemical consumption, energy use and operating costs among these three technologies and ozone.

MF and UF remove few CECs from wastewater and further advanced treatment is required for sensitive receiving waters (Snyder et al., 2007; Siegrist and Joss, 2012). NF and RO are capable of significantly reducing CEC concentrations (Snyder et al., 2007; Siegrist and Joss, 2012). However, NF and RO are not expected to compete with ozone-biofiltration in wastewater treatment for the following reasons. NF and RO must waste 20-25% of water to avoid scaling (concentrate), they require higher energy consumption, have higher capital costs and much more technical equipment (Joss et al., 2008; Lee et al., 2012). Concentrate waste contains high concentrations of toxic micro-pollutants that need to be treated further (eg. with ozone or activated carbon) (Siegrist and Joss, 2012). UV/H$_2$O$_2$ and O$_3$/H$_2$O$_2$ are other possible alternatives for CEC removal. O$_3$/H$_2$O$_2$ is generally used for bromate mitigation in high bromide waters. UV/H$_2$O$_2$ is limited by cost constraints from relatively high H$_2$O$_2$ consumption and the frequent requirement for pre-treatment (Gerrity and Snyder, 2011; Katsoyiannis et al., 2011).

In comparison, ozone and biofiltration have a simple setup, comparatively lower energy and chemical costs and 100% water recovery (when recycling backwash water) (Gerrity and Snyder, 2011; Lee et al., 2012). Adsorption with GAC removes CECs very well within the first few months, but with a higher organic matter loading in wastewater there is concern for its adsorption capacity to be exhausted quickly. GAC would need to be replaced or regenerated when exhausted, causing high operating costs. In contrast, biofilters allow microorganisms to colonize on media in a reactor to biodegrade organic matter. GAC in biofilters is not regenerated and media (sand, GAC or anthracite) need to be replaced far less frequently than columns
operated for adsorption. Consequently, ozone-biofiltration is becoming a progressively more popular technology to remove CECs and other unidentified toxic compounds in tertiary wastewater treatment and water reuse.

2.3 Ozone

2.3.1 Ozone Generation & Configuration

Ozone is generated from oxygen molecules. The electric discharge method is most commonly used for generating ozone in industrial applications. The electrical discharge splits an oxygen molecule into two oxygen radicals. The oxygen radical atom (O•) then combines with molecular oxygen (O₂) to form ozone (O₃). The concentration of ozone produced with air feed gas is 3–4% by weight and with oxygen feed gas is 10–13.5% by weight (Gottschalk et al., 2000). Ozone is one of the strongest disinfectants having a high oxidation potential. Ozone is also highly unstable, so it is produced by an ozone generator on site before use. Ozone produced on site is bubbled into a diffusion column and subsequent contact columns provide sufficient time for reaction.

2.3.2 Ozone Oxidation Mechanisms on Organic Matter (OM)

Ozone can oxidize and transform an organic molecule by two pathways: the direct or indirect pathway (Hoigne and Bader, 1976). In the direct pathway, ozone reacts directly with the organic compound to form product. In the indirect pathway, ozone breaks down to form hydroxyl radicals (OH•) which then react with the compound. Even though the oxidation potential of O₃ is 2.08 eV, that of OH• is 2.8 eV (Tchobanoglous et al., 2003). The direct reaction of ozone is more selective and slower in comparison to hydroxyl radicals (Hoigne and Bader, 1976; von Gunten, 2003). The oxidation pathway will depend on the concentrations and
reaction rate of ozone and hydroxyl radical with the organic molecule (von Gunten, 2003). Ozone and hydroxyl radicals can oxidize organic compounds and microbial constituents present in water or wastewater.

### 2.3.3 Role of Ozone in WWT

There were only 4 wastewater treatment plants using ozone for disinfection in 2010, compared to 201 water treatment plants using ozone as of 1997 in the USA (Rice, 1999; Oneby et al., 2010). Of the 4 ozone installations, all four were utilized only to meet disinfection requirements. In Canada, two MWWTPs were using ozone for disinfection in 1999 (Larocque, 1999) and the Montreal Urban Community WWTP is conducting pilot studies on ozone for disinfection.

Historically, the purpose of ozone in water and wastewater treatment has been for disinfection. Ozone disrupts the cell membrane of microorganisms rendering them inactive. However, ozone has another purpose that has more recently been studied as well as providing microorganism disinfection. Ozone has been shown to be effective in oxidizing a large number of CECs in wastewater into transformation by-products. Studies demonstrating ozone’s effectiveness in removing CECs from wastewater conducted in different countries around the world are listed in Table 2-2. Studies have demonstrated ozone can provide the dual purpose of both disinfection and CEC oxidation.
Table 2-2: Studies demonstrating ozone’s effectiveness in oxidizing CECs from wastewater

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ternes et al.</td>
<td>Germany</td>
<td>2003</td>
</tr>
<tr>
<td>Huber et al.</td>
<td>Switzerland</td>
<td>2005</td>
</tr>
<tr>
<td>Snyder et al.</td>
<td>Nevada, USA</td>
<td>2006</td>
</tr>
<tr>
<td>Hollender et al.</td>
<td>Switzerland</td>
<td>2009</td>
</tr>
<tr>
<td>Reungoat et al.</td>
<td>Australia</td>
<td>2010, 2011</td>
</tr>
<tr>
<td>Gerrity et al.</td>
<td>Nevada, USA</td>
<td>2011</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>New Mexico, USA</td>
<td>2012</td>
</tr>
<tr>
<td>Singh</td>
<td>Ontario, CA</td>
<td>2012</td>
</tr>
</tbody>
</table>

2.3.4 Ozone Transformation By-Products (TPs)

Ozonation does not completely mineralize CECs and other organic matter (OM) to carbon dioxide and water. Ozone’s reaction with dissolved organic carbon (DOC) is shown in Figure 2-1. Studies have shown DOC mineralization is < 10% with ozone doses typically used in practice (Rittmann et al., 2002; Reungoat et al., 2010). Consequently, > 90% of DOC still remains in the wastewater as oxidized transformation by-products (TP) (Figure 2-1). The major uncertainty with ozone is the production of unidentified TPs that may be more toxic than their parent compounds (Joss et al., 2008; Hollender et al., 2009).

![Figure 2-1: Ozone reaction with DOC](image)

When oxidation TPs do not have the same bioactive properties of the parent compound, mineralization may not be necessary because the TP cannot exert a biological effect. However, mineralization is very unlikely given the molecular structure of many of these CECs. Thus, there is a need to study these TPs for their potential adverse health effects they could cause on humans.
and animals. Currently, limited information is available on ozone TPs produced and whether they potentially cause adverse health effects. Initial studies indicate that bromate (von Gunten and Hoigne, 1994) and more recently N-Nitrosodimethylamine (NDMA) (Schmidt and Brauch, 2008) TPs are produced. NDMA and bromate are now recognized as probable human carcinogens by either the California Department of Public Health (CDPH), the United States Environmental Protection Agency (USEPA) or Health Canada (Health Canada, 1999; USEPA, 2001b; CDPH, 2006; Health Canada, 2011). Health Canada regulates both compounds. In the United States, bromate is regulated by the USEPA. Just like bromate, NDMA is most likely going to be regulated in the future in the USA according to CDPH (2006). These are just two ozone toxic TPs that are recognized. Further studies are needed to clarify the risk from the numerous number of other ozone TPs.

Ozone does not only just oxidize CECs in wastewater. Ozone is a powerful oxidant that also reacts with all other organic compounds present in wastewater. In general, ozone reacts with natural organic matter (NOM) to increase hydroxyl and carbonyl formation, increase polarity and degrade aromatics and double bonded compounds (Urfer et al., 1997). Organic ozone TPs are lower molecular weight and more biodegradable than their parent compounds (Urfer et al., 1997). Most biodegradable organics are removed in secondary treatment. Thus, there is a very small fraction of biodegradable OM remaining in secondary effluent. Post ozonation can convert refractory OM in secondary effluent into OM that is more biodegradable. Subsequent biological filter performance is considerably improved with pre-ozonation because the higher fraction of biodegradable OM provides more substrate for microbes to metabolize and grow on biological filter media (Urfer et al., 1997).
2.4 Biofiltration

2.4.1 Role of Biofilters in WWT

In the 1990s ozone treatment of water became more popular to deal with disinfectant-resistant pathogens and chlorine disinfection by-products (Emelko et al., 2006). Since ozone increases the fraction of biodegradable OM, some water utilities implementing ozone were having problems with bacterial re-growth in their distribution systems. As a result, studies comparing adsorptive media biofilters against other non-adsorbing media after ozonated water treatment were conducted for removal of biodegradable OM to prevent bacterial re-growth in the distribution system (Lechevallier et al., 1992; Krasner et al., 1993; Rittmann et al., 2002; Emelko et al., 2006). More recently it has been suggested that biofilters after ozone treatment of wastewater may be effective in removing possible toxic TPs (Joss et al., 2008; Hollender et al., 2009; Stalter et al., 2010). Consequently, biological filters in wastewater treatment have recently been recommended after ozone units to remove any potential toxic ozone TPs (Joss et al., 2008; Lee et al., 2012). As a secondary benefit, post-biofiltration has been shown to be effective in further reducing CEC concentrations (Gerrity et al., 2011; Reungoat et al., 2011). Few studies on both ozone and biofiltration for tertiary wastewater treatment have been conducted.

2.4.2 Biofilter Configuration

Biological filters are granular media filters where no disinfectant residual is present in the feed water. Biofilters act as a physical and biological treatment unit combined; removal of organic matter and particulates occur simultaneously. A biofilter is a cylindrical column reactor containing a granular media. A typical biofilter configuration is shown in Figure 2-2. Water is pumped through the inlet and flows downward through the media. The water exits at the bottom
of the column at the outlet. Microorganisms in the water colonize on the granular media forming a biofilm. This biofilm can metabolize biodegradable organic compounds present in the wastewater.

![Typical biofilter setup](image)

*Figure 2-2: Typical biofilter setup*

### 2.4.3 Biofiltration Mechanisms to Remove OM

#### 2.4.3.1 Biodegradable & Refractory OM

This description will help in understanding the removal mechanisms for biofilters with different media described below. OM is composed of both biodegradable organic matter (BOM) and refractory organic matter. Biodegradable organic matter removal is measured using BDOC (biodegradable dissolved organic carbon), assimilable organic carbon (AOC) or specific biodegradable organic molecules (eg. carboxylic acids). Refractory organic matter is not able to be biologically metabolized. Refractory organic matter is measured using non-biodegradable dissolved organic carbon (NBDOC). NBDOC is the difference between DOC and BDOC. An illustration is shown in Figure 2-3.
Biofiltration Mechanisms to Remove OM: Media Type

Biofilters differ by the type of media being used. The three most common media are anthracite, sand and granular activated carbon (GAC). GAC is more expensive than sand or anthracite. Sand is more common than anthracite because sand is cheaper (Table 2-3). Sand and anthracite are non-adsorbing media, whereas GAC is an adsorbing media (Urfer et al., 1997). Filters that regenerate or replace GAC media before biological colonization are termed GAC filters. When GAC filters become colonized with microorganisms, these filters are termed biologically activated carbon filters (BAC).

Table 2-3: Most common biofilter media general comparison

<table>
<thead>
<tr>
<th>Media</th>
<th>Cost</th>
<th>Adsorption</th>
<th>Biodegradation</th>
<th>Bio-regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>↓ $</td>
<td>x</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Anthracite</td>
<td>- $</td>
<td>x</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>GAC</td>
<td>↑ $</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

The removal mechanisms of OM in biofilters are dependent on the type of media used (eg. whether the media is adsorptive or non-adsorptive). BDOC is expected to be metabolized by the biofilm attached on both BAC and sand biofilters. However, sand has a negligible adsorptive capacity and is not expected to significantly remove refractory organic matter (NBDOC) (Urfer et al., 1997). NBDOC is only expected to be removed by BAC biofilters due to GAC’s ability to adsorb. However, only a small fraction of NBDOC can be removed once the
GAC is biologically activated (Servais et al., 1994). The main advantage of a BAC biofilter over other non-adsorbing media biofilters is they have the ability to perform bio-regeneration because of their adsorptive capacity. Bio-regeneration is the metabolism of biodegradable or slowly biodegradable OM adsorbed on GAC media by biomass attached to the media (Seredynska-Sobecka et al., 2006). Bio-regeneration allows for GAC adsorption sites to be reused.

Thus, sand biofilters have one OM removal mechanism and BAC biofilters have three OM removal mechanisms. Sand biofilters can only biodegrade OM (Table 2-3). BAC biofilters can remove OM through biodegradation, bio-regeneration or adsorption (Table 2-3). Figure 2-4 illustrates the organic matter removal mechanisms in a sand and BAC biofilter.

Figure 2-4: Types of organic matter expected to be removed in an adsorbing (BAC) and non-adsorbing (sand) media biofilter
2.4.4 Biofilter Backwashing

As water flows through a filter, solids accumulate in the void space of filter media and head loss gradually increases. Biofilters need to be backwashed when either the filter reaches the designed maximum available head loss or a turbidity breakthrough occurs (Viessman and Hammer, 2008). At this time the filter run ends and backwashing begins to clean the filter bed. Backwashing involves a low rate and high rate water wash cycle. Air scour or surface washing can be added to increase media collisions and abrasion during backwashing. The common methods of cleaning biofilters are water wash, air scour + water wash or surface wash + water wash. Water wash only backwashing sends a low rate water flow upwards, followed by a high rate water flow upwards to fluidize the bed and carry away solids in the bed void spaces. Air scouring occurs simultaneously with low rate backwashing to increase media collisions and abrasion. Surface washing uses nozzles hanging above the media to spray high-pressure water onto the top of the filter media to break up surface crust. After air scour or surface washing, high rate backwash fluidizes the bed and carries away solids in the bed void spaces.

Backwash rates (m/hr) need to be sufficient so that solids deposited in the bed are washed away, but not so high that a significant portion of the biofilm is removed too. Thus, in order to design a backwash protocol it is important to follow previous biofilter backwashing literature. Backwash conditions for air scour and non-air scour experiments on water treated biofilters are shown in Table 2-4. No biofilter backwash studies could be found using surface washing. Biofilter performance (TOC, BOM removal) was shown to be unaffected by air scour backwash conditions (Ahmad et al., 1998; Emelko et al., 2006). The same TOC and BOM removal occurred whether water backwash or both water and air scour backwash were used.
conducted. This demonstrates that biofilter performance was unaffected by air scour and that air scour as well as these velocities in Table 2-4 can be used in backwashing biofilters.

Table 2-4: Backwashing procedures for previous air scour and non-air scour biofilter studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Non- air scour</th>
<th>Air scour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmad et al., 1998</td>
<td>1. Low-rate wash: ~4 min at 12.5 m/hr</td>
<td>1. Low-rate wash ~4 min at 12.5 m/hr with simultaneous air scour at 0.9 m/min</td>
</tr>
<tr>
<td></td>
<td>2. High-rate wash: ~10 min at 25% bed expansion</td>
<td>2. High-rate wash: ~10 min at 25% bed expansion</td>
</tr>
<tr>
<td>Emelko et al., 2006</td>
<td>1. Low-rate wash: ~9 min at 10.7 m/hr</td>
<td>1. Air scour: 2 min at 0.9 m/min</td>
</tr>
<tr>
<td></td>
<td>2. High-rate wash: 11-12 min at 42.7 m/hr</td>
<td>2. Settling period: 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Low-rate wash: 9 min at 10.7 m/hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. High-rate wash: 11-12 min at 42.7 m/hr</td>
</tr>
</tbody>
</table>

2.5 Biofilter Performance Monitoring

Biofilter performance in wastewater treatment is measured mainly in terms of organic carbon reduction and toxin removal performance.

2.5.1 Total Organic Carbon (TOC)/Dissolved Organic Carbon (DOC)

Total organic carbon (TOC) is a measure of the bulk amount of organic matter. TOC has occasionally been reported in literature, but more often TOC has not been reported. Instead, dissolved organic carbon (DOC) has been reported. DOC is the dissolved fraction of TOC. Reduction in DOC measures the amount of organic matter biodegraded within a biofilter.
2.5.2 UV$_{254}$

DOC is not enough to measure performance alone. DOC is a bulk measure of organic matter. DOC does not give an indication of what fraction of organic matter is biodegradable or refractory. UV$_{254}$ measures changes in the biodegradable or refractory fraction of organic matter.

UV absorbing molecules absorb light directly proportional to their concentration. Aromatic compounds primarily absorb UV light at 254 nm and these compounds are poorly biodegradable (refractory). UV$_{254}$ gives an indication of the amount of refractory organic matter that can be biodegraded by a biofilter.

2.5.3 DO

DO is a measure of the amount of aerobic biological activity present within a biofilter. Anaerobic microorganisms are not expected to account for a significant portion of biodegradation in biofilters in comparison to aerobic microbes (Urfer and Huck, 2001). Thus, DO reduction across a biofilter indirectly measures the amount of organic matter being biodegraded.

2.5.4 Biomass/Bioactivity Measurements

It is difficult to determine what portion of organic matter is removed by adsorption or biodegradation in adsorbing media biofilters. Biomass measurements are useful to determine whether adsorbing media biofilters outperform non-adsorbing media biofilters because of adsorption or because they can support more biological activity. Biological measurements give an indication of the amount of biological activity or biomass a given biofilter media can support.
Viable biomass measurements will be elaborated on in Section 2.6: Measurement of Viable Biofilter Biomass.

2.5.5 Toxicity Bioassays

Although the above parameters measure biofilter performance for organic matter removal, they do not give an indication of whether toxic TPs or other toxic ozone refractory organics are being removed by a biofilter. Toxicity bioassays quantify relative to the influent the amount of toxic compounds biodegraded across a biofilter. Common bioassays to measure toxicity will be discussed in Section 2.8: Toxicity Bioassays.

2.5.6 Disinfection Indicator Microorganisms

Indicator microorganisms for fecal contamination are not a measure of biofilter performance per say. However, they need to be monitored because in the USA and Canada it is common to place a limit on these indicator microorganisms in MWWE. Common indicator disinfection microorganisms will be discussed in Section 2.7: Disinfection of Municipal Wastewater Effluent (MWWE).

2.5.7 Other Parameters

Total alkalinity, pH and temperature are commonly monitored in all wastewater treatment experiments. Turbidity, head loss and total suspended solids monitor filter performance.

2.6 Measurement of Viable Biofilter Biomass

2.6.1 History of Biofilter Biomass Measurements

Historically biomass or biological activity has not commonly been measured in biofilters. Biomass colonized onto biofilter media are responsible for removal of biodegradable OM formed
during ozonation. Organic parameter reductions indirectly assess biological activity in biofilters (eg. DOC, UV$_{254}$, DO).

The difference in performance of adsorptive (GAC) and non-adsorptive media (sand, anthracite) biofilters is not well understood. Studies have shown adsorptive media (BAC) biofilters outperforming non-adsorbing media biofilters after ozone treatment for removal of OM (Lechevallier et al., 1992; Krasner et al., 1993; Rittmann et al., 2002; Emelko et al., 2006; Reungoat et al., 2011). However, it is difficult to determine what portion of OM is removed by adsorption or biodegradation. The BAC biofilter outperformed non-adsorptive media biofilters in removing OM for one or both of the following two reasons:

- GAC provides a better support surface for biomass to colonize and grow
- GAC’s adsorptive capacity can adsorb OM onto macropores where it can be retained long enough to allow for slow biodegradation by the attached biofilm (bio-regeneration) (Seredynska-Sobecka et al., 2006)

Viable biomass measurements on filter media together with organic parameters are desirable to assess biofilter performance. Methods for accurate viable biomass determination on biofilter media have been developed: heterotrophic plate counts (Lechevallier et al., 1992), radiolabeled $^{14}$CO$_2$ respiration (Servais et al., 1991; Servais et al., 1994), phospholipid analysis (Wang et al., 1995; Emelko et al., 2006; Seredynska-Sobecka et al., 2006), biomass respiration potential (Urfer and Huck, 2001) and adenosine tri-phosphate (ATP) analysis (Magic-Knezev and van der Kooij, 2004). These methods have developed improvements over time.

Heterotrophic plate counts (HPC) count the amount of live heterotrophic bacteria from colonies formed on culture media. However, not all waterborne microorganisms are thought to be culturable heterotrophic bacteria; thus HPC counts do not represent all waterborne microorganisms (World Health Organization, 2003). The radio-labeled $^{14}$CO$_2$ method measures
the production of radio-labeled $^{14}$CO$_2$ by respiration of a saturated solution containing radio-labelled $^{14}$C-glucose. However, all laboratories may not have the analytical equipment required for analysis of radio-labelled compounds. Phospholipid analysis measures the amount of biomass by measuring the concentration of phospholipids from cell membranes. However, even though phospholipid analysis measures the amount of biomass it does not provide a measure of the amount of biological activity. A larger amount of biomass does not necessarily mean increased biological activity. Previous studies have shown the amount of phospholipid biomass does not correlate with biodegradable OM removal in biofilters (Wang et al., 1995; Emelko et al., 2006). Biomass respiration potential measures the amount of dissolved oxygen (DO) consumption per mass of biofilter media. DO consumption indirectly measures the amount of biological activity. DO measurements are problematic for GAC media because GAC naturally adsorbs DO; it would be unknown what fraction of DO consumption occurred from adsorption compared to biofilm cellular respiration. The most recent method first studied by Magic-Knezev and van der Kooij (2004) measures biological activity directly by measuring the ATP concentration on biofilter media. Magic-Knezev and van der Kooij (2004) used sonication to remove biomass from GAC media before analysis. Additional treatment steps are undesirable because they require more analytical equipment and provide more room for experimental error. Further research on ATP analysis developed a method to measure ATP directly on GAC media (Velten et al., 2007). It is assumed the same methodology would apply in measuring sand media for ATP.
2.6.2 **BacTiter-Glo™ ATP Microbial Cell Viability Assay**

ATP is the chief energy carrier of all cells. Cells maintain stores of ATP to drive endothermic cellular reactions, providing energy for growth and movement. ATP is created from cellular respiration of organic compounds in active microorganisms and therefore an appropriate indication of viable biological activity. Velten et al. (2007) developed a method using BacTiter-Glo™ proprietary reagent (Promega Corp.) to measure luminescence directly on biofilter media. BacTiter-Glo™ reagent mixed with biofilter media containing active biomass produces luminescence as shown in Figure 2-5. BacTiter-Glo™ reagent lysed bacterial cells and then luciferase oxidizes ATP in the presence of oxygen and magnesium to produce light. The amount of luminescence produced is proportional to the ATP concentration. Promega later adopted and published this method (Promega, 2007).

![Figure 2-5: BacTiter-Glo™ reaction with ATP (Promega, 2007)](image)

2.7 **Disinfection of Municipal Wastewater Effluent (MWWE)**

2.7.1 **Microbial Indicator Organisms**

MWWE is disinfected because many pathogens that cause human enteric diseases originate from excreted feces of sick people (Viessman and Hammer, 2008). Therefore, water
containing fecal contamination is potentially unsafe due to the possible presence of pathogens. Drinking water, surface water and wastewater are not tested directly for pathogenic microorganisms because either methods have not been developed or they are not reliable (Viessman and Hammer, 2008). The possible presence of pathogens is based on testing for an indicator organism. An ideal indicator organism is an organism that is present when pathogenic microorganisms are also present.

The most common indicator organisms for fecal contamination are total coliforms, fecal coliforms and E. coli. These two groups and one species of coliform bacteria can be measured easily and reliably. The presence of these indicator organisms confirm the water has been polluted with feces and thus may contain pathogens (Viessman and Hammer, 2008). The effectiveness of disinfection is determined by measuring one or all three of these indicator organisms.

2.7.2 MWWE Disinfection Requirements

MWWTPs in Canada and the United States are generally required to provide some level of disinfection. However, the level of disinfection differs by jurisdiction and sensitivity of the effluent’s receiving water body. Except when specifically exempted all municipal, institutional, and private communal sewage works discharging their effluent to surface waters are required to meet disinfection requirements and apply for ministry approval under Section 17 and 24 of the Ontario Water Resources Act (Ontario MOE, 2001). The Ontario Ministry of Environment (MOE) determines the disinfection requirement based upon the sensitivity of the receiving surface water on a case by case basis. The Ontario MOE may permit only seasonal disinfection requirements (disinfection only required in the summer when bacterial counts are highest). A
disinfection limit of 200 MPN *E. coli* organisms per 100 mL monthly geometric mean density applies to Ontario and is common in the United States and Canada (Minnow Environmental and Canadian Council of Ministers of the, 2005; Ontario MOE, 2008b).

### 2.7.3 Disinfection Concern with Ozone-Biofiltration

There is concern with the final treatment train process unit being biofiltration that wastewater disinfection requirements may not be met. Biofiltration units provide a place for microorganisms to colonize on granular media and form a biofilm. Consequently, biofilters provide protection for bacteria and other pathogenic organisms to re-grow. Pre-ozonation increases dissolved oxygen levels and the fraction of biodegradable organic carbon. Dissolved oxygen, available biodegradable organics and a shelter for growth provide an ideal environment for pathogenic re-growth.

Only Gerrity et al. (2011) studied the issue of bacterial re-growth in ozone-biofiltration in an indirect potable water reuse application. Bacterial re-growth occurred after ozonation in the biofilter by increasing as much as 1-log in fecal coliform (*E. coli* not measured). However, all three sampling events conducted measured fecal coliform counts < 100 MPN fecal coliform/100 mL. This would be acceptable to satisfy the Ontario MOE disinfection requirement.

Wastewater for indirect potable water reuse requires a higher level of treatment than MWWE since wastewater effluent is eventually recycled for potable drinking water. It is uncertain if Ontario MOE disinfection requirements could be met with higher total coliform, fecal coliform and *E. coli* counts present in MWWE.
2.8 Toxicity Bioassays

2.8.1 Biological Toxicity Tests & Chemical Analysis

Two analytical methods have been used to test for CEC removal: measuring concentrations (chemical analysis) or measuring toxicity with bioassays. The majority of studies have focused on using chemical analysis to measure the removal efficiencies of targeted CECs. Chemical analysis of a bounded set of compounds does not cover the potential health effects from the wide range of compounds that are present in municipal wastewater. Chemical analysis only determines the extent a CEC was removed and does not consider what ozone oxidation TPs were formed. Toxicity bioassays produce a response to toxic CECs, toxic TPs and unidentified toxic compounds. Although chemical analysis provides useful information to determine which known harmful compounds are present in MWWE, bioassays are desirable because they measure the additive, synergistic and antagonistic effects of the mixture of chemicals in wastewater.

2.8.2 Battery of Toxicity Tests Available

Many different toxicity tests for water and wastewater have been examined, but a combination of toxicity tests have not been agreed upon. Currently toxicity testing for regulatory purposes at LRPCP is based on two aquatic organisms: Rainbow Trout or *Daphnia Magna*. However, bioassay toxicity tests have become attractive choices to expensive, long-term animal studies because results can be obtained in a shorter period of time. Common bioassay toxicity tests that have been applied to water and wastewater treatment are summarized in Table 2-5. These toxicity tests expose toxicants to a biological agent to observe non-specific or specific modes of toxic action.
Table 2-5: Battery of toxicity tests most commonly applied to water and wastewater treatment

<table>
<thead>
<tr>
<th>Assay</th>
<th>Mode of toxic action</th>
<th>Measured by</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtox</td>
<td>Non-specific</td>
<td>- Reduction in luminescence of the naturally bioluminescent bacterium</td>
<td>ISO 11348, 1998</td>
</tr>
<tr>
<td>UMU-Chromo</td>
<td>Genotoxicity; DNA damage</td>
<td>- Induction of β-galactosidase enzyme as an indicator of DNA damage</td>
<td>ISO 13829, 2000</td>
</tr>
<tr>
<td>Modified Ames</td>
<td>Mutagenicity; DNA damage</td>
<td>- Reverse mutation of (his-) allows bacterium to grow in a histidine free medium</td>
<td>Maron and Ames, 1983</td>
</tr>
</tbody>
</table>

No single test can detect all toxic compounds, especially from an environmental sample where there are numerous chemicals present. It is desirable to use more than one bioassay because each bioassay assesses different toxicological endpoints with a different test organism (eg. E-SCREEN measures the presence of estrogen mimicking compounds with MCF-7 breast cancer cells). Secondly, it is important to put these bioassay results into context with human health effects. Many of these assays are performed in vitro or with bacterial cells as the test organism (Le Curieux et al., 1993). Toxicity tests in vivo with animals (whole organism assays) may provide results that are much more indicative of effects that would be observed in humans. Overall the above bioassays are difficult to extrapolate to humans, but they are helpful in determining the efficiency of different treatment units in removing toxic compounds.
2.8.3 Concentrating Samples: Solid Phase Extraction

MWWE samples need to be concentrated with solid phase extraction in order to elicit a response in the above bioassays (Escher et al., 2008). A method was developed to analyze for CECs in environmental samples involving four steps: filtration, solid phase extraction, evaporation and reconstitution. Solid phase extraction had been validated with different solid phase adsorbents at different pH’s to determine when CEC percent recoveries were highest (Escher et al., 2005; Escher et al., 2008). The procedure for solid phase extraction was described in Macova et al. (2010). After solid phase extraction, samples can be evaporated in a gentle stream of nitrogen and reconstituted. DMSO and ethanol are the common solvents used in reconstitution. Samples can be concentrated appropriately depending on the bioassay chosen for analysis and the location of sample collection.

2.8.4 Genotoxicity Testing: UMU-Chromo test

Genotoxic compounds are those capable of damaging DNA, often leading to mutations and cancer. Genotoxicity can be measured using the UMU-Chromo test. The UMU-Chromo test was developed by Oda et al. (1985) and was adjusted to a 96-well microplate by Reifferscheid et al. (1991). The UMU-Chromo test has been internationally recognized and standardized (ISO, 2000).

The UMU-Chromo test kit (umuC test) uses a genetically engineered bacterial strain that measures the response of a cell to DNA damage. A plasmid (pSK1002) containing a fused gene umuC-lacZ was fused into Salmonella typhimurium TA1535. The umuC test exposes Salmonella typhimurium to different concentrations of liquid samples in a 96-well microplate. Genotoxins cause genetic damage, which induce the umuC gene. Induction of the umuC gene is
proportional to the concentration of genotoxins. The *umuC* gene contains the lacZ gene, which induces the synthesis of β-galactosidase enzyme. β-galactosidase converts o-nitrophenyl-β-D-galactopyranoside (ONPG) into a yellow product and the activity of β-galactosidase is an indirect measure of DNA damage or genotoxicity. β-galactosidase activity can be assayed by measuring absorbance of yellow colour produced with a 96-well microplate reader.

2.9 Ozone-Biofiltration

2.9.1 Biofilter Maturation

No studies could be found on the maturation of non-adsorbing media biofilters. However, studies have been conducted on maturation of BAC biofilters treating drinking water with varying empty bed contact times (EBCT). Over time microbes colonize on biofilter media forming a biofilm by metabolizing DOC and nutrients. Steady state is reached when the microbial growth and removal rates (from backwash and death) are approximately equal. Biofilters operate at steady state for the duration of their lifetime.

Studies have been conducted on maturation of BAC biofilters. Servais et al. (1994) (EBCT 10 min) and Velten et al. (2011) (EBCT 15 min) reported approximately 3 months were required to reach steady state biomass. Griffini et al. (1999) reported 4 months to reach steady state (EBCT 20min). Seredynska-Sobecka et al. (2006) reported 8 months to reach steady state. However, a very low EBCT of 0.8 min was used (Seredynska-Sobecka et al., 2006). Such a low EBCT would make it more difficult for microbes to attach and colonize on biofilter media. This most likely is why such a long period of time was required to establish steady state. It is expected that approximately three to four months should be sufficient for microbial colonization in a biofilter treating drinking water to reach steady state. However, it is uncertain how long a
biofilter treating wastewater will take to mature; given wastewater has higher organic matter, nutrient and microbial content than drinking water.

2.9.2 O₃-Biofiltration in WWT

Few studies on both ozone and biofiltration for tertiary wastewater treatment have been conducted. Table 2-6 shows previous studies on both ozone and biofilter treatment of wastewater. These studies are from different parts of the world and there are several differences in process train configuration provided before ozonation, as well as biofilter media used. Reungoat et al. (2011) evaluated ozone-biofiltration from a water reclamation plant where conventional treated wastewater was being treated further for non-potable reuses. Lee et al. (2012) examined membrane bioreactor effluent into ozone-biofiltration pilot units to examine the transformation of CECs and removal of biodegradable organic carbon. The remaining studies have evaluated ozone-biofiltration following conventional activated sludge treatment (Hollender et al., 2009; Gerrity et al., 2011; Stalter et al., 2011).

However, toxicity evaluations across the treatment units have only been performed in a few studies (Gerrity et al., 2011; Stalter et al., 2011). Due to the variation of wastewater from different regions, there is uncertainty in the ozone TPs produced. Consequently, there is a need to further study wastewater from different geographic locations. There is also a desire to determine the mechanisms responsible for the performance of biofilters with different media (Reungoat et al., 2011). No studies have been conducted on parallel biofilters with different media used for tertiary treatment (fed with conventional MWWE). Studying biofilters in parallel provides insight into performance differences with secondary effluent. Furthermore, few studies have been conducted assessing the difference in the ability of biofilter media to support biofilm
activity. Filter media that have a grain structure which provides a more suitable environment for microorganisms to colonize would be expected to have better performance. Biofilm studies have been conducted to compare different biofilter media (Lechevallier et al., 1992; Wang et al., 1995; Emelko et al., 2006), but their methods suffer from proper measurement of biofilm activity.

Table 2-6: Wastewater process trains previously studied that used both ozone and biofiltration

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Process Train</th>
<th>Parameters Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hollender et al., 2009</td>
<td>Switzerland</td>
<td>activated sludge ($2^\circ$) → $O_3$ → sand biofilter</td>
<td>CECs, TPs</td>
</tr>
<tr>
<td>Reungoat et al., 2010;</td>
<td>Australia</td>
<td>$2^\circ$ effluent → pre-$O_3$ → coagulation-floculation-DAF → $O_3$ → BAC → sand biofilter</td>
<td>CECs, Variety of toxicity endpoints</td>
</tr>
<tr>
<td>Macova et al. 2010;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reungoat et al., 2011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stalter et. al., 2010;</td>
<td>Switzerland,</td>
<td>activated sludge ($2^\circ$) → $O_3$ → sand biofilter</td>
<td>Variety of toxicity endpoints</td>
</tr>
<tr>
<td>Stalter et. al., 2011</td>
<td>Germany</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gerrity et al., 2011</td>
<td>USA</td>
<td>activated sludge ($2^\circ$) → sand filter → $O_3/H_2O_2$ → BAC</td>
<td>CECs, Estrogenicity</td>
</tr>
<tr>
<td>Lee et al., 2012</td>
<td>USA</td>
<td>membrane bioreactor ($2^\circ$) → $O_3$ → anthracite biofilter</td>
<td>CECs, BDOC</td>
</tr>
</tbody>
</table>

Legend:
BDOC: Biodegradable dissolved organic carbon
CEC: Chemicals of emerging concern
DAF: Dissolved air flotation
TP: Transformation products

2.9.3 Comparing Adsorbing & Non-adsorbing Media Biofilters in WWT

Of the studies listed in Table 2-6, only one compared adsorbing and non-adsorbing media biofilters fed with wastewater (Reungoat et al., 2011). Reungoat et al. (2011) monitored, among other parameters, DOC and non-specific toxicity (Microtox) across an ozonation unit and two
parallel biofilter units in a water re-use application. Reungoat et al. (2011) operated biofilters with sand and GAC filter media. Sand represents a non-adsorbing media biofilter, whereas GAC media represents an adsorbing media biofilter. These biofilters were fed with ozonated wastewater and with non-ozonated wastewater. Only the ozonated wastewater comparison will be discussed.

Two sampling campaigns were conducted. In both sampling campaigns, the BAC removed more DOC than the sand biofilter. The BAC removed 54% and 53 ± 2%, whereas the sand biofilter removed 37% and 27 ± 7%. In terms of non-specific toxicity, the first sampling campaign showed the sand biofilter removed 65%, while the BAC removed 60% of non-specific toxicity relative to the influent of the ozonation unit. In the second sampling campaign, the sand biofilter removed 49 ± 3%, while the BAC removed 74 ± 10%. The BAC outperformed the sand biofilter in terms of DOC removal, but the non-specific toxicity results were conflicting. The first sampling campaign indicated the sand and BAC biofilter had comparable removals of toxic compounds. However, the second campaign indicated the BAC removed more toxic compounds than the sand biofilter. The purpose of biofiltration in wastewater treatment is to remove toxic compounds (either from possible toxic ozone TPs or toxins still present after ozonation); DOC removal is not a primary concern. Further research is needed to clarify which filter media can remove more toxins, or whether they have similar toxin removal performance.

It is important to note that Reungoat et al. (2011) studied wastewater fed through four more treatment units (pre-ozonation → coagulation → flocculation → DAFF) after conventional wastewater treatment in a water reclamation facility before being ozonated. The wastewater was being treated for non-potable reuse, which requires a higher level of treatment than MWWE. It
is uncertain if a sand and BAC biofilter would exhibit similar toxicity removal performance with conventional secondary effluent, which would be expected to have more toxins present.
CHAPTER 3
3.0 DESIGN & METHODOLOGY

3.1 Full Scale Process Flow

Pilot scale studies were conducted at Little River Pollution Control Plant (LRPCP) in Windsor, Ontario, Canada. LRPCP was first commissioned in 1966; since it has been upgraded to meet a demand of 73,000 m$^3$/d. The full scale wastewater treatment plant process train is illustrated in Figure 3-1.

![Figure 3-1: Full scale process train]

The LRPCP process train includes a grit removal chamber, chemically enhanced primary treatment, activated sludge biological treatment and seasonal (April – October) UV disinfection. LRPCP discharges effluent into Little River, which leads back into the Detroit River. During the months of April to October LRPCP is required to meet the Ontario MOE disinfection requirement of $< 200$ MPN $E. coli/100$ mL.
Effluent from the secondary sedimentation tanks (before disinfection) was fed into the pilot units (as shown in Figure 3-1). The pilot plant consists of a counter-current ozone unit and two parallel biofilter columns. The pilot plant process train is illustrated in Figure 3-2, along with sampling locations.

Figure 3-2: Pilot scale process train and sample identification numbers

3.2 Ozonation Pilot Unit

Figure 3-3 shows a picture of the ozone pilot unit previously constructed by Singh (2012). The counter-current ozone unit consisted of an ozone contactor, ozone monitor (Model 454M, Teledyne Instruments, San Diego, USA) and ozone generator (Lab 2B, Triogen, Glasgow, UK). The ozone contactor consisted of a dissolution column and four contact columns. Ozone was generated from a mixture of dry air and pure oxygen. The columns were constructed out of clear PVC and the fittings were ozone resistant stainless steel or Teflon. Ozone was bubbled from the bottom of the dissolution column (first column) through a coarse glass bubble diffuser. Secondary effluent was collected in a 300 L feed tank and conveyed to the top of the dissolution
column by a peristaltic pump. The wastewater flowed counter-current to the rising gas bubbles. Ozonated wastewater from the dissolution column entered at the bottom of the second column and flowed upwards. Likewise, the ozonated wastewater entered the third column from the top and fourth and fifth columns from the bottom. The dissolution chamber and four contact columns provided sufficient contact time to ensure ozonated effluent did not contain residual ozone (Singh, 2012).

![Figure 3-3: Picture of the ozone pilot unit (inset picture shows the dissolution column)](image)

Design parameters of the ozone pilot unit are shown in Table 3-1. The hydraulic retention time (HRT) in the dissolution chamber (column 1) and contact columns 2 – 4 was 1.7 minutes in each column. The HRT in contact column #5 was 10 minutes (#5 was larger in diameter to ensure any remaining residual ozone reacted completely).
Table 3-1: Design parameters of the ozone pilot unit (Singh, 2012)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column #</td>
<td>Column #1 – 4 Column #5</td>
</tr>
<tr>
<td>Column internal diameter</td>
<td>88.9 mm (3.5”) 215.9 mm (8.5”)</td>
</tr>
<tr>
<td>Column height (total)</td>
<td>1.8 m 1.8 m</td>
</tr>
<tr>
<td>Water height (average)</td>
<td>1.1 m 1.1 m</td>
</tr>
<tr>
<td>HRT @ design flow of 4 L/min</td>
<td>1.7 min 10.0 min</td>
</tr>
</tbody>
</table>

The ozone pilot unit operated at a wastewater flow of 4 L/min, HRT of 16.8 min and a transferred ozone dose (TOD) of approximately 3.5 mg/L (≈0.5 – 0.8 mg O₃/mg DOC). This ozone dose was chosen because it was the lowest dose that consistently met the LRPCP disinfection requirement of < 200 MPN E. coli/100 mL (Singh, 2012).

The ozone generator and analyzer were both pilot sized and not meant to be operated continuously. Therefore, the ozonation unit was operated twice per week (Monday and Thursday) and effluent was stored in a 1200 L capped tote. The tote was capped to reduce oxygen transfer between the atmosphere and the ozonated effluent.

3.3 Biofiltration Pilot Units

Sand and GAC media were selected in the current study to compare the more expensive GAC to the more economical sand media (shown in Table 3-3). Sand was selected over anthracite because sand is cheaper and more commonly used.

Table 3-2: Selected biofilter media general comparison

<table>
<thead>
<tr>
<th>Media</th>
<th>Cost</th>
<th>Adsorption</th>
<th>Biodegradation</th>
<th>Bio-regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>↓ $</td>
<td>x</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Anthracite</td>
<td>- $</td>
<td>x</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>GAC</td>
<td>↑ $</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Figure 3-4 shows a schematic and Figure 3-5 shows a picture of the biofilter pilot units. The columns were constructed out of clear PVC and the fittings were Teflon. Ozonated effluent was fed into each of the two parallel biofilters (diameter 3.5” (8.9 cm), height 1.8 m). The two biofilters contained granular activated carbon (GAC) (adsorptive media) and filter sand (non-adsorptive media). Both media were virgin at the beginning of operation. The GAC and sand media were ordered with the closest size specifications that a supplier would provide. The two media specifications are listed below:

- Calgon F-300 GAC, effective size = 0.8-1.0 mm, uniformity coefficient =2.1, bed depth = 65 cm
- CEI Filter Sand, 0.80-1.20 mm, effective size = 0.86 mm, uniformity coefficient = 1.19, bed depth = 65 cm
Design parameters for the biofilter pilot units are given in Table 3-4. The empty bed contact time (EBCT) in the biofilters was 40 min, with a hydraulic loading of 0.97 m/hr. Biofilters typically have an EBCT less than 30 min (Urfer et al., 1997). A lower EBCT was not chosen because of experimental issues with the pilot unit installation.

Table 3-3: Design parameters for BAC and sand biofilter pilot units

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BAC biofilter</th>
<th>Sand biofilter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic Loading</td>
<td>0.97 m/hr</td>
<td>0.97 m/hr</td>
</tr>
<tr>
<td>EBCT</td>
<td>40 min</td>
<td>40 min</td>
</tr>
<tr>
<td>Flow Rate (Q)</td>
<td>100 mL/min</td>
<td>100 mL/min</td>
</tr>
<tr>
<td>Media height</td>
<td>65 cm</td>
<td>65 cm</td>
</tr>
<tr>
<td>Clean bed head loss (h_L)</td>
<td>2 – 4 cm</td>
<td>2 – 4 cm</td>
</tr>
<tr>
<td>Water column height above media when Q = 0</td>
<td>10 cm</td>
<td>10 cm</td>
</tr>
<tr>
<td>Column internal diameter</td>
<td>8.9 cm (3.5”)</td>
<td>8.9 cm (3.5”)</td>
</tr>
<tr>
<td>Column height</td>
<td>1.8 m</td>
<td>1.8 m</td>
</tr>
</tbody>
</table>
3.3.1 Backwashing Procedure

Both biofilters were backwashed with biofilter effluent twice per week (Monday and Thursday). Table 3-4 details both biofilter’s backwash procedures. Backwashing protocol was chosen based on previous biofilter backwashing literature and Ontario MOE filter design guidelines (Ahmad et al., 1998; Emelko et al., 2006; Ontario MOE, 2008a). Backwashing procedure contained surface washing for 15 s, at 4 Lpm, 15 cm above the bed. After surface washing, the BAC biofilter followed with a 10 min low-rate wash at 10.7 m/hr (1.1 Lpm), then a high-rate water wash at approximately 25% bed expansion (41 m/hr or 4.3 Lpm). After surface washing, the sand biofilter backwashed at low-rate for 10 min at 10.7 m/hr (1.1 Lpm), then a high-rate water wash at approximately 25% bed expansion (50 m/hr or 5.2 Lpm). Surface washing was implemented to break up the crust that formed on the top couple centimetres of the media. Breaking up the crust prevented the formation of mud balls. Figure 3-6 shows a picture of the surface washing apparatus. The surface wash was constructed out of a nozzle, copper pipe and a wood stand. Surface washing was assumed to not affect biofilter performance analogous to air scour (Section 2.4.4: Biofilter Backwashing).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BAC biofilter</th>
<th>Sand biofilter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Rate</td>
<td>1. Surface wash</td>
<td>1. Surface wash</td>
</tr>
<tr>
<td></td>
<td>2. ~10 min at 10.7 m/hr</td>
<td>2. ~10 min at 10.7 m/hr</td>
</tr>
<tr>
<td>High Rate</td>
<td>3. ~10 min at 41 m/hr</td>
<td>3. ~10 min at 50 m/hr</td>
</tr>
</tbody>
</table>
3.3.2 Operation & Initiating Start-up

The BAC pilot biofilter began operation on October 25, 2011. The sand pilot biofilter was implemented on January 30, 2012 to compare the more economical sand media to the more expensive GAC media. Start-up was initiated by applying a ratio of 25:75 (secondary effluent:O\(_3\) effluent) for 4 – 6 weeks. A portion of secondary effluent (non-ozonated effluent) was fed into the biofilters because it was assumed that more live microorganisms in the influent would speed up microbial colonization on biofilter media in hopes the biofilters would reach biological steady state quicker. Previous water treatment experiments have also used non-ozonated water to reach a biological steady state quicker (Seredynska-Sobecka et al., 2006). Start-up was not initiated with one hundred percent secondary effluent because biofilter performance is considerably improved with ozonated effluent (Urfer et al., 1997). A higher portion of ozonated effluent was introduced into the biofilters because even when wastewater
was ozonated, not all microorganisms were destroyed. Therefore, start-up was initiated with a ratio of 25:75 (secondary effluent:O₃ effluent) so microbes (from secondary effluent) could metabolize the higher fraction of biodegradable substrate (from ozonated effluent) to begin colonizing on the media quicker.

After 4 – 6 weeks, ozonated effluent was fed into both biofilters for the duration of the study. The BAC and sand biofilters operated for 386 and 290 days respectively.

3.4 Sampling Procedure

DO, pH, temperature, head loss and flow rate were measured at LRPCP. All other parameters were analyzed from 1 L glass amber bottles at the University of Windsor. The sampling procedure is given in Appendix A.

3.5 Analysis Procedure

The list of parameters measured, their testing days and testing location are given in Table 3-5 for both biologically active filters (BAF) and the ozone unit.
Table 3-5: Parameters measured routinely, testing frequency and testing location

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>O₃ contactor</td>
</tr>
<tr>
<td>Ozone dose</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pH</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>Temperature</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>T-alkalinity</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>UV₂₅₄ (SUVA)</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>DOC</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>DO</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
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<td>Filtration</td>
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<td></td>
<td></td>
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<td>BAF</td>
</tr>
<tr>
<td>Head loss (h₁)</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>O₃ contactor &amp; BAF</td>
</tr>
<tr>
<td>Flow rate (Q_{water})</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>O₃ contactor &amp; BAF</td>
</tr>
<tr>
<td>Turbidity¹</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
<tr>
<td>SS¹</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BAF influent &amp; effluent</td>
</tr>
</tbody>
</table>

¹Measured until April 2012. Afterwards they were monitored periodically after 180 days since they were stable and to make more time available to perform other analytical work.

UV₂₅₄ and DOC were measured twice per week until biofilters reached steady state removals, as shown in Table 3-5. After steady state was reached DOC and UV₂₅₄ were monitored once per week, and pH, temperature, DO, head loss and flow rate were monitored twice per week. Furthermore, three samples were taken to measure genotoxicity, biological activity attached to biofilter media, and bacterial re-growth.

3.6 General Parameters

The general parameters monitored are described below.
3.6.1 Ozone Concentration

Ozone feed and vent gas concentrations were measured mechanically using Teledyne Instruments ozone gas analyzer, Model 454M. The ozone concentration in the feed and vent gas were used to calculate the transferred ozone dose (TOD). Feed gas air flow rate was measured with the ozone generator described above.

3.6.2 pH

The pH was measured with a Hach model HQ40d multimeter and PHC301 probe. The probe was calibrated at pH 4.00, 7.00 and 11.00 using Hach certified buffer solutions. Samples were taken in Wheaton biochemical oxygen demand (BOD) bottles and measured at LRPCP.

3.6.3 Temperature

Temperature was measured with a Hach model HQ40d multimeter with a PHC301 and LBOD101 probe. Samples were taken in Wheaton biochemical oxygen demand (BOD) bottles and measured at LRPCP. Both probes displayed the same temperature within ± 0.1 °C.

3.6.4 Total Alkalinity

Total alkalinity was measured following Standard Methods for Examination of Water and Wastewater 2320. Total Alkalinity is reported as mg/L CaCO₃.

3.7 Bulk Organic Parameters

The bulk organic parameters monitored are described below. DOC and UV₂₅₄ represent the organic matter (OM) content of the wastewater; DO reduction across a biofilter indirectly measured the OM content by quantifying the aerobic biological activity supported in a biofilter. Bulk organic parameters were monitored to give an indication when microbial colonization within the biofilters reached steady state.
3.7.1  **Dissolved Organic Carbon (DOC)**

Reagent & Material: 0.45 µm polyethersulfone filter, sulfuric acid  
Instrument: Shimadzu TOC-VCSH Total Organic Carbon Analyzer  
Procedure: Dissolved organic carbon (DOC) was measured following Standard Methods for Examination of Water and Wastewater (5310 B, Combustion-Infrared Method). Samples were collected approximately 24 hours after backwashing in glass amber bottles and stored at 4°C. They were analyzed within 48 hours of collection. Samples were filtered through a 0.45 µm polyethersulfone filter (Pall Supor-450, P/N 60173) and acidified to pH ≤ 2 with sulfuric acid (H₂SO₄). All 0.45 µm filters were pre-rinsed with of 500 mL of Milli-Q water to ensure no carbon was released from the filter into the filtrate. Since the concentration of carbon was < 10 mg/L, the non-purgeable organic carbon (NPOC) method from the TOC analyzer was chosen. NPOC is the non-volatile organic carbon within a water sample. The sum of the purgeable organic carbon (POC) and NPOC equals TOC. LRPCP secondary wastewater effluent was exposed to the atmosphere for 10 – 24 hours while it passed through the treatment train. Thus, it was assumed there would be negligible POC remaining as most would have likely evaporated to the surrounding environment already (POC ≈ 0). Therefore, NPOC was deemed an appropriate measurement of TOC. Steady state removals are reported as 95% confidence intervals in mg/L.

3.7.2  **Ultraviolet Absorbance at 254nm wavelength (UV<sub>254</sub>)**

Reagent & Material: 0.45 µm polyethersulfone filter  
Instrument: Varian Cary 50 Scan, UV-Visible Spectrophotometer  
Procedure: UV<sub>254</sub> was measured as per Standard Methods 5910-B. Samples were collected approximately 24 hours after backwashing. All samples were filtered through a 0.45 µm
polyethersulfone filter (Pall Supor-450, P/N 60173) and analyzed within 6 – 8 hours of sampling. Steady state removals are reported as 95% confidence intervals in cm$^{-1}$.

3.7.3 Dissolved Oxygen (DO)

Dissolved oxygen (DO) was measured with a Hach model HQ40d multimeter with a LBOD101 probe. Samples were taken in Wheaton biochemical oxygen demand (BOD) bottles and measured at LRPCP. Steady state removals are reported as 95% confidence intervals.

3.8 Filtration Parameters

Filtration has historically been restricted to water treatment because wastewater has higher suspended solid loadings, causing filter clogging. Total suspended solids (TSS), turbidity and head loss of the biofilters were monitored to ensure biofilters had no operational problems with wastewater (eg. filter clogging).

3.8.1 Head Loss ($h_L$)

The head loss is the difference between the water level when the column is not operating (no water flow) and the water level when the column is operating (water is flowing). Head loss was measured with a tape measure and reported in centimetres.

3.8.2 Water Flow Rate ($Q$)

Water flow rate was measured with a stopwatch, water collection container and a calibrated cylinder. Flow rate was calculated using the equation below.

\[
Q \ (mL/min) = \frac{Volume \ (mL)}{time \ (min)}
\]

3.8.3 Turbidity

Turbidity was measured with a 2100 AN Hach model turbidimeter following Standard Methods 2130-B. Turbidity is reported in nephelometric turbidity units (NTU).
3.8.4 Total Suspended Solids (TSS)

Total suspended solids (TSS) was measured according to Standard Methods 2540-D. Both TSS and turbidity were measured from September 2011 to April 2012. TSS is reported in mg/L.

3.9 Disinfection Parameters

Samples were taken at LRPCP on July 12, August 2 and August 6 2012. Multiple samples were taken over time to observe any possible variation in treatment. Total coliforms and \textit{E. coli} were the indicator microorganisms measured to determine ozone’s disinfection efficacy and potential microbial re-growth in both biofilters. \textit{E. coli} and total coliform enumeration was performed by the Colilert®-18 method (USEPA approved).

Reagent & Material: Colilert®-18, Quanti-Tray®-2000

Instrument: Quanti-Tray® sealer, UV lamp (365 nm)

Procedure: Samples were collected approximately 24 hours after backwashing in sterile bottles containing sodium thiosulphate. Samples expected of containing total coliform/\textit{E. coli} counts of > 2000 MPN/100mL were diluted with sterilized Milli-Q water. Colilert®-18 reagent was added to 100mL samples (or diluted sample) and then the samples were transferred to quantification trays (Quanti-Tray®-2000 by IDEXX). The trays were sealed mechanically and incubated at 37°C for 18 – 22 hrs. Sealed Quanti-Tray®-2000 trays have 49 big wells and 48 small wells. \textit{E. coli} enumeration was determined by counting fluorescent wells under 365 nm light. Yellow coloured wells were counted to determine the total coliform count. \textit{E. coli} and total coliform levels were then reported as the most probable number (MPN) per 100 mL.
3.10 Genotoxicity Testing

The UMU-Chromo test was chosen for the current study because the test can be easily performed, it has been widely researched and kits can be purchased containing reagents and lyophilized bacteria. The UMU-Chromo test is an in vitro bioassay that measures genotoxicity; genotoxic compounds are those capable of damaging DNA. Microtox, Modified Ames and E-SCREEN bioassays were attempted and ultimately were not successful for publication, financial or experimental issues.

3.10.1 Sampling & Pre-treatment

Samples were taken at LRPCP on June 4, June 19 and July 16 2012 approximately 24 hours after backwashing. Like the disinfection samples, multiple samples were taken over time to observe any possible variation. Four samples were taken: pre-ozone (S1), post-ozone (S2), effluent BAC biofilter (S3), and effluent sand biofilter (S4) (Figure 3-2, Table 3-6). Samples were collected in 1 L glass amber bottles and stored at 4˚C. Samples only need to be stored frozen (<-10˚C) if the solid phase extraction cannot be performed within 48 hours (USEPA, 2007).

Hydrochloric acid was added to each 1 L bottle to preserve the samples to a final concentration of 5 mM (Macova et al., 2010). Samples were stored in coolers on ice until they were transported to the University of Windsor and kept refrigerated (<6˚C) in the dark (USEPA, 2007). Milli-Q water was used as a procedural blank to determine if any contamination occurred from the extraction and to evaluate if the solvent used for reconstitution (DMSO) had any effect. Solid phase extraction was performed on samples the following day.
3.10.2 Solid Phase Extraction (SPE)

The procedure for solid phase extraction (SPE) was adapted from previous work (USEPA, 2007; Macova et al., 2010); the detailed procedure for the SPE can be found in Appendix B. SPE involved extracting the samples onto a sorbent, eluting the samples, evaporating the samples to dryness and then reconstituting them in dimethyl sulfoxide (DMSO). Pictures of the SPE setup and samples are shown in Figure 3-7 and Figure 3-8 respectively. In brief, samples were first filtered with Whatman 934-AH (1.5 µm) filters to prevent clogging in cartridges. Samples were then extracted using 1 g OASIS HLB sorbent in 20 mL cartridges (Waters, Mississauga). Cartridges were conditioned with 10 mL methanol and 20 mL of 5 mM HCl (flow rate is 1 drop/sec) (Macova et al., 2010). Samples were percolated through the cartridges under peristaltic pump (flow rate = 5–10 mL/min) (USEPA, 2007). Flow rate was measured with a stop watch and graduated cylinder. After loading the cartridges with sample, the cartridges were dried for 5 min under vacuum (USEPA, 2007). Any residual moisture was removed by gently dabbing the cartridges with Kimwipes. Cartridges were then eluted under gravity with 10 mL methanol, and then 10 mL hexane:acetone (1:1) (flow rate = 1 drop/2 sec). Following elution, eluates were stored at -20°C overnight. The next day the 20 mL eluates were evaporated to dryness in a gentle nitrogen stream and reconstituted in 1 mL DMSO. Extracts were then frozen (-20°C) and analyzed within 40 days (USEPA, 2007).
3.10.3 Concentration with SPE & Dilution in the Genotoxicity Test

Concentrating samples was necessary to ensure a response was exhibited with the UMU-Chromo test. The overall concentration factor (CF$^{\text{overall}}$) is a combination of the concentration factor from solid phase extraction (CF$^{\text{SPE}}$) and dilution factor (DF) in the UMU-Chromo test. Dilution was necessary to reduce the concentration of DMSO to less than 10% so as not to
exhibit any toxic effect from the solvent (ISO, 2000). Through trial and error the volumes of each sample were chosen. The more genotoxic the sample, the less volume that needed to be collected. The volumes of samples collected and concentration factors are shown in Table 3-6.

**Table 3-6: Volumes of samples taken for solid phase extraction, concentration factors and dilution factors**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Pre-O₃ (S1)</th>
<th>Post-O₃ (S2)</th>
<th>BAC biofilter (S3)</th>
<th>Sand biofilter (S4)</th>
<th>Blank (Milli-Q water) (S5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume extracted (mL)</td>
<td>2000</td>
<td>3000</td>
<td>3000</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>CF&lt;sub&gt;SPE&lt;/sub&gt;</td>
<td>2000</td>
<td>3000</td>
<td>3000</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>DF</td>
<td>240-30</td>
<td>144-18</td>
<td>144-18</td>
<td>144-18</td>
<td>144-18</td>
</tr>
<tr>
<td>CF&lt;sub&gt;overall&lt;/sub&gt;</td>
<td>66.7-8.3</td>
<td>166.7-20.8</td>
<td>166.7-20.8</td>
<td>166.7-20.8</td>
<td>166.7-20.8</td>
</tr>
</tbody>
</table>

Sample volume varied from 2000 – 3000 mL. All samples were concentrated to a volume of 1 mL; thus the samples have CF<sub>SPE</sub> = 2000 – 3000. An aliquot of the concentrated extracts were diluted in sterile water and UMU-Chromo test reagents in a 96-well microplate. The DF can be calculated using the equation below.

\[
DF = \frac{\text{total volume in bioassay}}{\text{volume of extract}}
\]

CF<sub>overall</sub> can then be calculated from CF<sub>SPE</sub> and DF using the equation below.

\[
CF_{\text{overall}} = \frac{CF_{\text{SPE}}}{DF}
\]

### 3.10.4 UMU-Chromo test

The UMU-Chromo test bioassay measures the amount of genotoxic compounds present within a sample. Kits were supplied by Environmental Bio-Detection Products Inc. (Mississauga, Ontario) and the bioassay was carried out according to the procedure provided
with the kit based on ISO (2000). Samples were tested without inclusion of liver supernatant fraction (S9).

Reagent & Material: UMU-Chromo test kit (Environmental Bio-Detection Products Inc.)

Instrument: VICTOR³ 1420 Multi Label Counter (PerkinElmer)

Procedure: The detailed procedure for the UMU-Chromo test can be found in Appendix C. In brief, lyophilized *Salmonella typhimurium* TA1535 [pSK1002] was reconstituted with growth media and incubated overnight at 37°C (16 – 18 hrs). The following morning, an aliquot of the extracts were initially diluted in sterile water. After the extracts were diluted in sterile water, 360 μL were added to the first three wells of the 96-well microplate. Samples were then 1:2 serially diluted to produce four separate concentration factors (CF\textsubscript{overall}) for each sample. The bacterial culture and reagents were added to the microplate and it was incubated for 2 hours at 37 ± 1°C. At the end of incubation, 30 μL of all wells were transferred to another microplate with pre-warmed fresh growth media. This microplate was incubated for 2 hours at 37 ± 1°C. Absorbance at 590 nm was measured to calculate the bacterial growth factor (G). The growth factor is a measure of how much bacterial growth occurred relative to the negative control (ISO, 2000). When finished incubating, 30 μL from all wells were added to a third microplate with pre-warmed o-nitrophenyl-β-D-galactopyranoside (ONPG) and a buffer. The third microplate was incubated for 30 minutes at 28 ± 1°C. Absorbance at 405 nm was measured to calculate β-galactosidase activity. β-galactosidase activity (U\textsubscript{b}) is a measure of the amount of substrate (ONPG) that was broken down to create a yellow colour as compared to the negative control (ISO, 2000). Genotoxicity was calculated in terms of an induction ratio (IR), defined as IR = U\textsubscript{b}/G.
3.10.4.1 Data Analysis

UMU Chromo test data were plotted as dose-response curves (dose=$CF_{overall}$, response=IR). A sample was considered genotoxic when a given $CF_{overall}$ induces an IR $\geq 1.5$ (ISO, 2000). It is more desirable to have a single value to compare each pilot unit along the treatment train, rather than an array of points making a dose-response curve. Thus, the genotoxicity of each sample was reported as the concentration factor required to elicit an IR = 1.5 ($CF_{IR=1.5}$) as per Macova et al. (2010). Macova et al. (2010) showed $CF_{overall}$ and IR exhibited a linear trend. The slope and y-intercept of each sample’s dose-response data were calculated using linear regression. $CF_{IR=1.5}$ could then be calculated from the equation below knowing the slope, y-intercept and using an IR=1.5.

$$IR = slope \times CF_{1.5} + \text{intercept}$$

$$CF_{1.5} = \frac{1.5 - \text{intercept}}{\text{slope}}$$

Genotoxicity was reported in terms of $CF_{IR=1.5}$ in order to allow comparison between each pilot unit for genotoxin removal. It is important to emphasize two concepts:

- $\uparrow CF_{IR=1.5}$ means the sample needed to be concentrated more to create a genotoxic effect
- Therefore, a higher $CF_{IR=1.5}$ indicates the sample is less genotoxic than a low $CF_{IR=1.5}$

($\uparrow CF_{IR=1.5} \alpha \downarrow$ genotoxic effect)

3.10.4.2 QA/QC

All results are expressed as the mean of triplicate analysis. For QA/QC a positive control (4-nitroquinoline-Noxide (4-NQO)), blank, solvent control and negative controls were tested. Solvent controls of 0.7% DMSO and 1.1% DMSO were chosen to reflect the solvent
concentrations of the pre-ozone sample and the remaining samples respectively. The test was considered valid if all criteria were met in Table 3-7.

Table 3-7: UMU Chromo Test QA/QC validation criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth of negative control</td>
<td>≥100% Environmental Bio-Detection Protocol</td>
</tr>
<tr>
<td>Growth of solvent control</td>
<td>≥50% Environmental Bio-Detection Protocol</td>
</tr>
<tr>
<td>Growth of Blank</td>
<td>≤10% Environmental Bio-Detection Protocol</td>
</tr>
<tr>
<td>IR of positive control</td>
<td>≥2 ISO (2000)</td>
</tr>
</tbody>
</table>

3.11 BacTiter-Glo™ ATP Microbial Cell Viability Assay

3.11.1 Sampling & Pre-treatment

Samples of biofilter media were taken at LRPCP on October 30, November 6 and November 13 2012 approximately 24 hours after backwashing. The procedure for sampling and ATP measurement was performed according to Velten et al. (2007). Sand and GAC samples were collected using a sampling spoon from the filter bed surface (upper 10 cm) and stored in sterile plastic bottles in filtrate at 4°C in darkness. Figure 3-9 shows a picture of the sampling spoon. The sampling spoon was constructed out of a wooden handle and plastic scooper. For pre-treatment, 5 g wet weight (WW) of media was rinsed three times with 100 mL phosphate buffer (3 mg/L KH₂PO₄ and 7 mg/L K₂HPO₄, pH 7).
3.11.2 Measurement of ATP

The BacTiter-Glo™ ATP Microbial Cell Viability Assay (Promega) was used to measure the amount of active biomass attached to the biofilter media (GAC and sand).

Reagent & Material: BacTiter-Glo™ ATP Microbial Cell Viability Assay (Promega Corp.), 10 mM ATP standard (Promega Corp.)

Instrument: Glomax 20/20 Luminometer (Promega Corp.)

Procedure: Measurement of ATP contained in cells attached to biofilter media was performed as follows:

- 200 mg media WW was weighed into an Eppendorf tube, and then 100 µL phosphate buffer was added and placed in a 30°C water bath
- Concurrently 300 µL BacTiter-Glo™ reagent was added to a second Eppendorf tube and placed in a 30°C water bath for 3min
- After 3min, the BacTiter-Glo™ reagent was transferred to the media sample
- After gently mixing for 5s, the mixture was placed in a 30°C water bath for 1.5min
• The Eppendorf tube was mixed gently every 30s
• After 1.5min, the tube was taken out of the bath and 200 µL supernatant was transferred into a unused Eppendorf tube
• 30s later the relative light units (RLU) were measured in a luminometer (Glomax 20/20, Promega)
• RLU was converted to an ATP concentration with a linear calibration curve using an ATP standard (Promega)
• All 200 mg WW samples were dried at 70°C for 24 hrs
• Dry weight (DW) was calculated by subtracting the measured mass from the mass of the same Eppendorf tube determined before use

The calibration curve was performed by first inactivating the microbial cells on the remaining biofilter media by suspending 5 g media WW in 5 mL phosphate buffer in a falcon tube for 21 hrs in a 60°C water bath. After inactivation, the media was washed five times with 15 mL phosphate buffer. Measurement of ATP for the calibration curve followed the same procedure as above for the samples. The only difference was instead of 100 µL phosphate buffer, 100 µL ATP standard solution was added in a concentration range of 0.125 – 4 µM (serially diluted 1:2). The calibration curve was determined using linear regression. A new calibration curve was created for each new BacTiter-Glo™ reagent and sample. Active biomass was reported in terms of (ng ATP)/(g DW).

3.12 Calculations

3.12.1 Applied Ozone Dose (AOD)

Applied ozone dose is the amount of ozone created from the generator and applied to the wastewater. The equation below calculates the applied ozone dose.

\[
AOD \left( \frac{mg}{L} \right) = Conc. O_3(\text{feed}) \times \frac{Q_{\text{feed gas}}}{Q_{\text{water}}}
\]
3.12.2 Transferred Ozone Dose (TOD)

Transferred ozone dose (TOD) was determined by taking the difference between the ozone generated in the feed gas and the vent gas. A mass balance yields the following equation to calculate TOD.

\[
\text{TOD (mg/L)} = (\text{Conc. } O_3\text{(feed)} - \text{Conc. } O_3\text{(vent)}) \times \frac{Q_{\text{feed gas}}}{Q_{\text{water}}}
\]

The feed gas and water flow rate were always set at 4 Lpm. Thus, TOD was calculated by taking the difference between the feed gas concentration and vent gas concentration of ozone.

3.12.3 Specific Ozone Dose

Specific ozone dose is the ratio of the mass of TOD and initial dissolved organic carbon. The equation below calculates the specific ozone dose.

\[
Z = \frac{\text{TOD}}{\text{DOC}_0}
\]

\[
\text{DOC}_0 = \text{initial dissolved organic carbon concentration (mg/L)}
\]

3.12.4 Specific UV absorbance (SUVA)

Specific UV absorbance (SUVA) is the ratio of UV absorption at 254 nm to dissolved organic carbon concentration. The formula to calculate SUVA is shown below.

\[
\text{SUVA (L/m·mg)} = \frac{U_{254}(cm^{-1})}{\text{DOC}_0(mg/L)} \times \frac{100 \; cm}{m}
\]

SUVA characterizes the organic matter content according to the amount of biodegradable and refractory organics present within the wastewater. According to Edzwald and Tobiason (1999):
• SUVA > 4 (L/mg·m) indicates the DOC is mostly hydrophobic with a high molecular weight (refractory)
• SUVA = 2 – 4 (L/mg·m) indicates the DOC is a mixture of hydrophobic and hydrophilic organic matter
• SUVA < 2 (L/mg·m) indicates the DOC is mostly hydrophilic with a low molecular weight (biodegradable)

A lower SUVA value indicates a higher fraction of biodegradable organic carbon within the wastewater.

3.13 Summary of Performance Parameters Monitored

Table 3-8 summarizes the parameters monitored to quantify the performance of the ozonation and biofiltration units.

Table 3-8: Summary of the parameters monitored to study ozone and biofilter performance

<table>
<thead>
<tr>
<th>Pilot Unit</th>
<th>Parameter</th>
<th>Method</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone</td>
<td>Genotoxicity (UMU-Chromo test)</td>
<td>ISO 13829, 2000</td>
<td>Environmental Bio-Detection Products Inc. (Mississauga, Ontario) provided kits</td>
</tr>
<tr>
<td></td>
<td>Disinfection</td>
<td>Standard Methods 9223-B</td>
<td>Colilert®-18 method by IDEXX</td>
</tr>
<tr>
<td>Bulk</td>
<td>DOC % removal</td>
<td>Standard Methods 5310-B</td>
<td>Shimadzu TOC-V&lt;sub&gt;CSH&lt;/sub&gt; analyzer</td>
</tr>
<tr>
<td>Organic</td>
<td>UV&lt;sub&gt;254&lt;/sub&gt; % removal</td>
<td>Standard Methods 5910-B</td>
<td>Varian Cary 50 Scan, UV-Visible Spectrophotometer</td>
</tr>
<tr>
<td>Parameters</td>
<td>DO % reduction</td>
<td>N/A</td>
<td>Hach HQ40d mulitimetre with a LBOD101 probe</td>
</tr>
<tr>
<td>Biofilter</td>
<td>Genotoxicity (UMU-Chromo test)</td>
<td>ISO 13829, 2000</td>
<td>Environmental Bio-Detection Products Inc. (Mississauga, Ontario) provided kits</td>
</tr>
<tr>
<td></td>
<td>Biological activity supported on media</td>
<td>Velten et al. (2007)</td>
<td>BacTiter-Glo™ Microbial Cell Viability Assay</td>
</tr>
<tr>
<td></td>
<td>Bacterial re-growth</td>
<td>Standard Methods 9223-B</td>
<td>Colilert®-18 method by IDEXX</td>
</tr>
</tbody>
</table>
CHAPTER 4

4.0 ANALYSIS OF RESULTS

Microorganisms were allowed to colonize on biofilter media before experiments were conducted on the performance of the ozone-biofiltration pilot unit. Without a steady state microbial growth the biofilters act as filters, removing suspended solids and turbidity only. This study was divided into two phases: maturation phase (Phase 1) and performance phase (Phase 2). In Phase 1, the biofilters were monitored as microorganisms begin colonizing on sand and GAC media. In Phase 2, the effectiveness of ozone and BAC versus sand biofiltration was determined by the removal of genotoxins and organic matter. Differences in biofilm activity across each biofilter were quantified with an ATP assay. Total coliform and *E. coli* were measured to assess potential bacterial re-growth in the biofilters. Visual observations during backwashing as well as total suspended solids (TSS), turbidity and head loss of the biofilters were measured to ensure biofilters had no operational problems with wastewater.
WATER QUALITY CHARACTERISTICS & OPERATIONAL ISSUES

4.1 Secondary Effluent Water Quality Characteristics

Table 4-1 summarizes the range of secondary water quality entering the ozone pilot unit from a number of samples collected throughout the study period. The general parameters monitored throughout the study period are shown in Appendix E; all other parameters are discussed below. These water quality parameters were within the same range previously reported at LRPCP (Singh, 2012).

Table 4-1: Range of secondary effluent water quality during the study period (September 2011 – November 2012)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>0.8 – 2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Total Suspended Solids</td>
<td>mg/L</td>
<td>3.6 – 12.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Water Temperature</td>
<td>ºC</td>
<td>12 – 26</td>
<td>19</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td>6.8 – 7.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>mg/L as CaCO₃</td>
<td>95 – 196</td>
<td>140</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>2.5 – 3.5</td>
<td>3</td>
</tr>
<tr>
<td>DOC</td>
<td>mg/L</td>
<td>4.5 – 7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>UV₂₅₄</td>
<td>cm⁻¹</td>
<td>0.07 – 0.13</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Legend:
DOC: Dissolved organic carbon
DO: Dissolved oxygen
UV₂₅₄: UV absorption at 254 nm

4.2 Monitoring Biofilter Operational Issues

Operational problems were determined visually during backwashing (eg. mud ball formation) or if a sudden spike in head loss, filter effluent turbidity or TSS occurred. The head loss in both filters was always < 30 cm at the end of every filter cycle, which is acceptable for design purposes (Ontario MOE, 2008a). As a result, no indications of operational issues were observed through head loss measurements. TSS and turbidity monitoring are shown in Figure
Figure 4-1 and Figure 4-2, respectively, for the first 180 days of operation. The figures show that although the influent TSS and turbidity varied, both biofilter effluent turbidity and TSS remained stable (no spikes). Consequently, TSS and turbidity did not give any indication of biofilter operational problems throughout the study. TSS and turbidity were only monitored periodically after 180 days since they were stable.

**Figure 4-1: Turbidity monitoring to observe operational problems**

**Figure 4-2: TSS monitoring to observe operational problems**
However, visual observations indicated an operational issue during backwashing on day 14 with the BAC biofilter (Figure 4-2). On day 14, mud balls could be seen being formed in the filter. Mud balls are formed when media grains get stuck together by sticky suspended solids. As filter run time increased, many solids that are sticky stuck to the top couple centimetres of filter media and formed a cake. This cake needed to be broken up manually during backwashing or else heavy mud balls formed and sunk deeper into the filter during high rate backwashing.

During the first two weeks the BAC biofilter was backwashed only with a water wash. In practice, surface washing or air scour are implemented to increase media abrasion during backwashing and prevent mud ball formation. Surface washing was implemented on day 16 to break up the crust that formed on the top couple centimetres of the media. Surface washing was chosen instead of air scour because it was more straightforward to implement. The surface wash was constructed with a commercially available spray nozzle at a flow rate of 4 Lpm, 15 cm above the bed. Breaking up the crust prevented the formation of mud balls, while maintaining the BAC biofilter’s performance for the remainder of the study. The sand biofilter was implemented on day 96 and included the same surface washing protocol as the BAC biofilter. Figure 4-1 and Figure 4-2 show that after implementation of the sand biofilter no spikes in turbidity or TSS occurred. No visible operational problems were encountered with either biofilter once the surface wash was implemented.

The first indication that an operational problem is occurring is to observe it visually, and if the problem is not fixed immediately it can be detected with spikes in turbidity, head loss or TSS. Mud ball formation was most likely not detected around day 14 with turbidity, head loss and TSS measurements with the BAC biofilter because every time the BAC biofilter was backwashed it was observed to see if the cake was broken up. Thus, mud ball formation was
noticed immediately. A surface wash was fabricated right away and on day 16 a surface washing protocol was implemented into the backwashing procedure. Had the surface wash not been constructed so quickly, more mud balls would have formed every time the filter was backwashed. Spikes in turbidity and TSS would have eventually occurred because particle removal cannot occur inside mud balls.
PHASE 1: MATURATION PHASE

The objective of the Maturation Phase was to determine the length of time the BAC and sand biofilters required to establish steady state by monitoring the trends of DO, UV$_{254}$ and DOC percent removal. DOC and UV$_{254}$ represent the organic matter (OM) content of the wastewater. DO reduction across a biofilter indirectly measures the OM content by quantifying the aerobic activity supported in both biofilters. The Maturation Phase is defined as the time period before steady state where DOC, UV$_{254}$ and DO percent removal are not constant. The Steady State Phase follows the Maturation phase and is defined as the time period in which there is no average change in DOC, UV$_{254}$ and DO over time.

Start-up was initiated with a volume ratio of 25:75 (secondary effluent:O$_3$ effluent) to provide more live microbes (from secondary effluent) to begin colonizing on the media quicker. After 4 – 6 weeks, ozonated effluent was fed into both biofilters for the duration of the study.

4.3 Comparison between BAC and Sand Biofilter Maturation Trends

Figure 4-3 and Table 4-2 closely show the maturation of both biofilters individually over the first 60 days of operation for DOC (a, b), UV$_{254}$ (c, d) and DO (e, f) removal. DOC and UV$_{254}$ will be discussed next; a discussion on DO will follow. Maturation is examined based on removals immediately observed (t = 2 days) and removals over 10–20 and 20–30 days to illustrate the trend exhibited by each parameter.

The data indicates that immediately after start-up the BAC biofilter exhibited significant DOC and UV$_{254}$ removal. Two days after start-up the BAC removed 69% of DOC and 82% UV$_{254}$. However, the sand biofilter did not significantly remove DOC and UV$_{254}$ right away. Two days after start-up the sand biofilter removed 1% of DOC and 2% UV$_{254}$. Likewise, the
BAC biofilter achieved higher OM removals during 10-20 days of operation. The BAC biofilter removed 59-68% DOC and 67-75% of UV$_{254}$, whereas the sand biofilter exhibited DOC removals of 7-10% and UV$_{254}$ removals of 2-3% from 10-20 days. Hence, the BAC biofilter removed approximately 52-58% more DOC and 65-72% more UV$_{254}$ over the first 10-20 days of operation.
Figure 4-3: Maturation of (a, c, e) sand and BAC (b, d, f) biofilters with respect to DOC (a, b), UV$_{254}$ (c, d) and DO (e, f)
Table 4-2: DOC, UV\textsubscript{254}, DO Percent Removal with respect to time

<table>
<thead>
<tr>
<th>Removal</th>
<th>DOC</th>
<th>UV\textsubscript{254}</th>
<th>DO</th>
<th>Removal</th>
<th>DOC</th>
<th>UV\textsubscript{254}</th>
<th>DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 2 days</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>t = 10 – 20 days</td>
<td>59</td>
<td>68</td>
<td>33</td>
</tr>
<tr>
<td>Removal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67</td>
<td>75</td>
<td>36</td>
</tr>
<tr>
<td>t = 20 – 30 days</td>
<td>11</td>
<td>16</td>
<td>4</td>
<td></td>
<td>52</td>
<td>54</td>
<td>42</td>
</tr>
<tr>
<td>Approx. day when steady state reached</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Removal Steady state</td>
<td></td>
<td>20 ± 2</td>
<td>11 ± 1</td>
<td>36 ± 2</td>
<td>41 ± 1</td>
<td>42 ± 2</td>
<td>62 ± 2</td>
</tr>
</tbody>
</table>

Legend:
Steady state % removals are calculated from all data obtained after 40 days and are displayed as 95% confidence intervals

As time progressed, the BAC biofilter’s DOC and UV\textsubscript{254} removals decreased, while the sand biofilter’s DOC and UV\textsubscript{254} removals increased to a steady state (Figure 4-3). Table 4-2 shows from 20-30 days the BAC removed 52-54% DOC and 59-67% UV\textsubscript{254}; these removals are less than from 10-20 days. In contrast, the sand biofilter exhibited 11-16% DOC and 4-6% UV\textsubscript{254} removal from 20-30 days; these removals are higher than those observed from 10-20 days. After 40 days of operation the BAC biofilter’s DOC and UV\textsubscript{254} removals no longer decreased (Figure 4-3). Likewise, the sand biofilter’s DOC and UV\textsubscript{254} removals no longer increased after 40 days, indicating these removals had reached a steady state value.

In terms of DO, Figure 4-3(e, f) show both biofilters gradually increased in DO reduction over time. Little DO reduction was observed in both biofilters immediately after start-up in
comparison to when a steady state DO reduction was established. Table 4-2 exhibits two days after start-up, the BAC and sand biofilter reduced only 11% and 5% DO respectively. However, during the first 30 days in the current study, DO reduction across both biofilters continuously increased. Biomass has also been shown to rapidly rise on biofilter media during the first 30 days of operation (Velten et al., 2007). This suggests that over time more aerobic microbial activity was occurring within both biofilters. Table 4-2 shows during 10-20 days the BAC biofilter had DO reductions between 33-36%; DO reduction increased further during 20-30 days (42-48%). Likewise, the sand biofilter between 10-20 days had DO reductions of 5-18%, which grew to 17-25% from 20-30 days. Figure 4-3 shows analogous to the DOC and UV$_{254}$ data, at approximately the 40th day of operation DO reduction across the biofilters no longer increased, indicating DO reduction across both biofilters had reached a steady state value.

To summarize the trends that occurred, DO reduction gradually increased for both biofilters over time until steady state. In contrast, DOC and UV$_{254}$ profiles exhibited opposite trends. Only the sand biofilter gradually increased in DOC and UV$_{254}$ percent removal, whereas the BAC biofilter steadily decreased in DOC and UV$_{254}$ percent removal.

4.4 DOC, DO & UV$_{254}$ Removal Mechanisms during Biofilter Maturation

Sand and GAC media have different maturation profiles because GAC can adsorb organic compounds onto GAC granules, while sand cannot. Sand biofilters initially do not remove any DOC, UV$_{254}$ or DO because no microbial colonization has taken place (Krasner et al., 1993). In contrast, BAC biofilters initially have high removals of OM (represented by DOC, UV$_{254}$) because OM is adsorbed effectively onto GAC granules (Simpson, 2008). Initially DO reduction is relatively low until microbes have colonized the GAC media (Pipe-Martin, 2010).
The process in which a GAC filter becomes biologically activated carbon (BAC) has been described previously as a three stage process (Simpson, 2008; Reungoat et al., 2012). These three phases are divided at 20 and 40 days because similar locations were chosen by Simpson (2008) (Figure 4-4). During phase one, little biodegradation is occurring in comparison to physical adsorption because biomass has not yet significantly colonized onto the GAC media (Simpson, 2008; Reungoat et al., 2012). Since little biodegradation has been observed to occur, this makes sense that Figure 4-4 shows DO reductions were relatively low in phase one in comparison to phase three. Furthermore, the first phase is expected to remove OM primarily by adsorption (Simpson, 2008; Reungoat et al., 2012); high DOC and NBDOC removal efficiencies have been reported previously. Initial removal of NBDOC has been reported to be as high as 50%, and over time dropped to less than 10% in phase three (Servais et al., 1994). Analogous results were obtained in the current study. High DOC and UV\textsubscript{254} removals were observed in phase one, suggesting adsorption was the primary mechanism for OM removal (Figure 4-4). In phase two a trade off occurs as physical adsorption decreases while at the same time biological degradation increases considerably (Simpson, 2008; Reungoat et al., 2012). In the present study, as Figure 4-4 shows, DOC and UV\textsubscript{254} removals gradually fell to near steady state proposing that less physical adsorption was occurring. At the same time, increases in DO reduction suggest there is an increase in aerobic bioactivity (Figure 4-4). In the third phase, biomass is established within the filter and most of the adsorption capacity of GAC is exhausted in comparison to the first phase (Simpson, 2008; Reungoat et al., 2012). Figure 4-4 shows steady state percent removals of DOC and UV\textsubscript{254} were much less than in phase one, suggesting physical adsorption had significantly reduced from phase one. A steady state reduction in DO suggested biodegradation and adsorption followed by biodegradation were removing OM in phase three.
If the GAC column was strictly operated for adsorption, phase one would be the expected performance. However, GAC would need to be continuously replaced or regenerated at the end of phase one causing high operating costs. The main benefit of biologically activating GAC is that adsorption can remove poorly biodegradable OM by adsorbing it to GAC macropores where it is retained long enough to allow for slow biodegradation by the attached biofilm (called bio-regeneration) (Seredynska-Sobecka et al., 2006). This allows the GAC to be continuously bi-regenerated.

To the best of my knowledge, the life of sand biofilters has not been described in previous literature. Similar to the maturation description by Simpson (2008) for a BAC biofilter, a pragmatic three phase maturation profile is constructed in Figure 4-5 knowing sand has poor adsorption ability. No significant OM removal occurred in phase one, suggesting a significant

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**Figure 4-4: Maturation of the BAC biofilter. Arrows indicate decreasing (↓) & increasing (↑).**
microbial community had not colonized onto the sand media. Low DO, DOC and UV$_{254}$ removals represent this phenomenon (Figure 4-5). In phase two, noteworthy DOC and UV$_{254}$ removals occurred while simultaneously DO reduction began to increase (Figure 4-5). This suggests that a significant amount of microbes began colonizing on the sand media approximately 20 days after start-up, metabolizing OM that passed through the filter. Steady state removal of DOC, UV$_{254}$ and DO were reached in phase three. Their steady state reduction suggested a steady state biofilm was present on the sand media that could biodegrade OM passing through the filter.

![Figure 4-5: Maturation of the sand biofilter](image)

Biomass measurements were not taken during the maturation phase. Consequently, no definitive statement can be made on the time it took both biofilters to reach a steady state in biomass colonization. However, as discussed, the above DOC, UV$_{254}$ and DO percent removals
suggest that a steady state biofilm was present on both filter media after approximately 40 days. In addition, previous studies have indicated biological steady state occurred at the same time DOC data became stable (Velten et al., 2011). This suggests, that at the very least, DOC data may be an appropriate surrogate to monitor when biological steady state is reached in a biofilter.

4.5 BAC Steady State Comparison to Other Studies

To my knowledge, no known sand maturation studies could be found in the literature. However, studies that monitored the maturation of a BAC biofilter are shown in Table 4-3. Previous studies have shown that the required time for a BAC biofilter to mature and reach steady state is approximately 75 – 120 days (Servais et al., 1994; Griffini et al., 1999; Velten et al., 2007; Velten et al., 2011). Seredynska-Sobecka et al. (2006) reported 240 days to reach steady state. However, a very low EBCT of 0.8 min was used. Such a low EBCT would make it more difficult for microbes to attach and colonize on biofilter media due to hydraulic shear stress. This most likely is why such a long period of time was required to establish steady state. A steady state removal of UV$_{254}$, DO and DOC occurred after approximately 40 days in the current study.

Table 4-3 evaluates the current study’s results versus previous studies. Each study monitored different organic or biological parameters to determine when steady state was reached. Three of the four studies monitored an organic parameter to give an indication of when a steady state biomass was established on GAC media. It is difficult to compare the current study to previous studies because no biomass measurements were taken. Assuming DOC, UV$_{254}$, and DO percent removals suggest that a steady state biofilm was present the data indicates that the current study reached steady state the quickest. However, all the other studies
in Table 4-3 treated drinking water. In comparison to drinking water, wastewater is expected to have higher organic matter, nutrient and microbial loading. Steady state DOC removal was reached quicker than previously reported (Griffini et al., 1999; Velten et al., 2011) presumably because the higher organic loading in wastewater can exhaust the GAC adsorption capacity quicker. Furthermore, for the first four weeks 25% of the feed was secondary effluent (non-ozonated), so a large number of viable microorganisms were present in the feed (as compared to the water treatment studies) that could colonize onto the filter media. Combined with more microorganisms, wastewater provides more nutrients and a higher organic matter loading that most likely allowed colonized microbes to prosper quicker than on those BAC biofilters treating drinking water.

<table>
<thead>
<tr>
<th>Parameter Monitored</th>
<th>EBCT (min)</th>
<th>DOC influent (mg/L)</th>
<th>Matrix</th>
<th>Time required to reach steady state (days)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC, UV_{254}, DO</td>
<td>40</td>
<td>5.5 ± 0.8</td>
<td>Wastewater</td>
<td>40</td>
<td>Current Study</td>
</tr>
<tr>
<td>DOC ATP</td>
<td>16.5</td>
<td>0.8-1.0</td>
<td>Water</td>
<td>75</td>
<td>Velten et al., 2007</td>
</tr>
<tr>
<td>DOC ATP</td>
<td>15.76</td>
<td>1.0-1.4</td>
<td>Water</td>
<td>90</td>
<td>Velten et al., 2011</td>
</tr>
<tr>
<td>BDOC, NBDOC radiolabeled (^{14})CO(_2) respiration</td>
<td>10</td>
<td>1.5-1.8</td>
<td>Water</td>
<td>90</td>
<td>Servais et al., 1994</td>
</tr>
<tr>
<td>DOC, aldehydes, ketoacids phospholipids (PO(_4))</td>
<td>20</td>
<td>1.69-2.21</td>
<td>Water</td>
<td>120</td>
<td>Griffini et al., 1999</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>7.76-11.62</td>
<td>Water</td>
<td>240</td>
<td>Seredynska-Sobecka et al., 2006</td>
</tr>
</tbody>
</table>
4.6 Significance

The results exhibit to the wastewater utilities the trends to expect and also a description of why these trends occur when monitoring the start-up of biofilters. DO is expected to gradually increase until steady state is reached in adsorbing and non-adsorbing media biofilters. DOC and UV$_{254}$ are expected to decrease in adsorbing media biofilters and increase in non-adsorbing media biofilters until steady state is reached. Instruments are readily available to wastewater utilities so the three parameters measured can be continuously monitored online to observe imbalances in performance and start-up maturation to steady state. Furthermore, this study suggests that approximately 40 days are required for both an adsorbing or non-adsorbing media biofilter treating wastewater to reach steady state removals for these three parameters. However, start-up was initiated with a ratio of 25:75 (secondary effluent:O$_3$ effluent) for 4 – 6 weeks. If 100% ozonated effluent was the feed it would likely take a longer amount of time to reach steady state.
PHASE 2: STEADY STATE PERFORMANCE PHASE

4.7 Long-term Monitoring

4.7.1 DOC Removal Profile

Figure 4-6 shows the DOC percent removal achieved by the sand and BAC biofilter over the study period. DOC to TOC ratio was always greater than 0.90. Longer term trends in DOC removal after 40 days of operation were not evident from the data.

Figure 4-6: Maturation of BAC and sand biofilters in terms of DOC removal. Steady state removals are reported as 95% confidence intervals.

4.7.2 UV$_{254}$ Removal Profile

Figure 4-7 shows the BAC and sand biofilter’s UV$_{254}$ removal over time. Similar to DOC, no long term trends were evident after 40 days of operation in terms of UV$_{254}$ removal.
Figure 4-7: Maturation of BAC and sand biofilters in terms of UV$_{254}$ removal. Steady state removals are reported as 95% confidence intervals.

4.7.3 DO Reduction Profile

Figure 4-8 shows the DO percent reduction achieved by the sand and BAC biofilter over the study period. Parallel to the DOC and UV$_{254}$ profiles, Figure 4-8 indicates that no long term trends were evident in DO reduction. However, DO reductions were more variable than DOC and UV$_{254}$ percent removals most likely because of the experimental setup. Ozonated effluent was stored in a tote over three to four days which allowed for DO to evaporate off into the atmosphere. Consequently, the influent DO to both biofilters dropped every day (from ~9 mg/L to ~4 mg/L) until the tote was refilled. Since the influent DO varied every day, DO reduction across a biofilter may have depended on the day it was measured.
Figure 4-8: Maturation of BAC and sand biofilters in terms of DO reduction. Steady state removals are reported as 95% confidence intervals.

4.8 Bulk Organic Removals from Biofilters at Steady State

The mean steady state removals across the BAC and sand biofilters are shown in Figure 4-9. As discussed above, both biofilters took approximately 40 days to reach steady state removals of the three parameters. Steady state removals were calculated from all samples taken after 40 days.
Figure 4-9: DOC, UV\textsubscript{254} and DO reduction across the BAC and sand biofilter at steady state. Error bars represent ± standard deviation from the mean.

The results indicate the BAC was able to remove more OM in terms of DOC removal (41 ± 1%) than the sand biofilter (20 ± 2%). Similar results were observed in UV\textsubscript{254} removal across the BAC (42 ± 2%) and the sand biofilter (11 ± 1%). In terms of DO reduction, the BAC (62 ± 2%) significantly outperformed the sand biofilter (36 ± 2%). The better performance of the BAC compared to the sand biofilter is in agreement with other studies comparing BAC and non-adsorbing media biofilters after ozonation for removal of OM (Lechevallier et al., 1992; Krasner et al., 1993; Rittmann et al., 2002; Emelko et al., 2006; Reungoat et al., 2011). Overall, the BAC biofilter outperformed the sand biofilter for all three bulk organic parameters.

The BAC biofilter can perform three processes: adsorption, bio-regeneration and biodegradation (Seredynska-Sobecka et al., 2006). Sand is a non-adsorptive media and presumably can only remove OM through biodegradation (Urfer et al., 1997). The BAC biofilter
outperformed the sand biofilter for DOC, UV$_{254}$ and DO removal for one or both of the following reasons:

- **Biological activity** – GAC provides a better support surface for active biomass to colonize and grow
- **Bio-regeneration** – biodegradable or slowly biodegradable OM can be adsorbed onto GAC macropores where they can be retained long enough to allow for slow biodegradation by the attached biofilm (bio-regeneration) (Seredynska-Sobecka et al., 2006)

In terms of biological activity, the DO data suggests the GAC media (62 ± 2%) supported more aerobic biological activity than sand media (36 ± 2%). This was reflected in DOC removal across the biofilters. However, DO measurements are problematic for GAC media because GAC naturally adsorbs DO. Consequently, the biological activity of both biofilter media will be examined in greater detail in Section 4.10: ATP Activity Supported on Media.

As for bio-regeneration, aromatic compounds primarily absorb UV light at 254 nm and these compounds are poorly biodegradable (refractory). The BAC (which can adsorb and biodegrade) removed significantly more refractory OM (measured with UV$_{254}$) than the sand biofilter (which can only biodegrade). This suggests that adsorption followed by biodegradation is present within the BAC. Adsorption can effectively increase the EBCT organic compounds remain in the biofilter by adsorbing OM, allowing attached biomass to metabolize these adsorbed organic compounds at a later time. Adsorption sites can then be reused after the biofilm metabolizes the adsorbed OM. Thus, the BAC biofilter’s biofilm can metabolize both OM passing through the filter and previously adsorbed OM (Seredynska-Sobecka et al., 2006). Sand biofilters can only remove OM passing through the filter.

Figure 4-9 indicates that not all UV$_{254}$ absorbing molecules are completely non-biodegradable. The data shows that some aromatic OM is able to be biodegraded because the
sand biofilter was able to remove 11 ± 1% of UV$_{254}$. However, the BAC biofilter most likely was able to remove more UV$_{254}$ (42± 2%) because it can perform bio-regeneration.

Table 4-4 shows the comparison of the current study’s biofilter performance to other studies on ozone treated wastewater. The table indicates variation in OM removal efficiency, as expected, due to differences in the MWWE characteristics. As shown in Table 4-4, the sand biofilter OM removals are similar to the other non-adsorbing media biofilter studies at comparable TOD. For BAC biofilters, Table 4-4 shows there is a bit more variation in OM removal efficiency.

The variation in DOC, UV$_{254}$ and DO removal efficiencies were attempted to be explained by two parameters commonly monitored: SUVA or EBCT. A lower SUVA value indicates a higher fraction of biodegradable organic carbon within the wastewater. Biofilters with lower SUVA would be expected to remove more OM since a larger fraction of the OM is biodegradable. Biofilter OM removal efficiencies from each study could not be explained by SUVA because the SUVA values were comparable (they overlapped). However, Table 4-4 indicates that biofilters with a longer EBCT had higher OM removal efficiencies. A longer EBCT allows for poorly biodegradable OM to be retained long enough to allow for slow biodegradation by the attached biofilm. This suggests that EBCT (up to 60 min) may be an important factor in removing OM in wastewater. However, there is a disagreement in the water treatment literature between whether EBCT affects OM removal (Urfer et al., 1997). Thus, further studies would need to be conducted in wastewater for confirmation. Other factors may also cause biofilter OM removal efficiencies to vary that were not monitored in any of these studies, such as nutrient concentrations (Li et al., 2010).
Table 4-4: OM removal by biofilters fed with ozonated wastewater

<table>
<thead>
<tr>
<th>Author</th>
<th>Biofilter Media</th>
<th>TOD (mg/L)</th>
<th>DOC pre-filter (mg/L)</th>
<th>SUVA ( \frac{L}{m \cdot mg} )</th>
<th>EBCT (min)</th>
<th>Removal post biofiltration %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DOC/TOC</td>
</tr>
<tr>
<td>Lee et al. (2012)(^1)</td>
<td>Anthracite</td>
<td>2</td>
<td>n.m.</td>
<td>2.2</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>1.5</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td>1.1</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Current study</td>
<td>Sand</td>
<td>3.5</td>
<td>5.2 ± 0.6</td>
<td>0.9 – 1.7</td>
<td>40</td>
<td>20 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Reungoat et al. (2011)(^2)</td>
<td>Sand</td>
<td>5</td>
<td>7.0 ± 0.1</td>
<td>n.m.</td>
<td>60</td>
<td>27 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Gerrity et al. (2011)(^3)</td>
<td>BAC</td>
<td>5 O(_3): 3.5 H(_2)O(_2)</td>
<td>7.2 – 7.4</td>
<td>1.0 – 1.1</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Current study</td>
<td>BAC</td>
<td>3.5</td>
<td>5.5 ± 0.8</td>
<td>0.9 – 1.7</td>
<td>40</td>
<td>41 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62 ± 2</td>
</tr>
<tr>
<td>Reungoat et al. (2011)(^2)</td>
<td>BAC</td>
<td>5</td>
<td>7.0 ± 0.1</td>
<td>n.m.</td>
<td>60</td>
<td>53 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81</td>
</tr>
</tbody>
</table>

Legend:
- n.m.: Not measured
- \(^1\) Author presented cumulative removals for ozone-biofiltration. Calculated biofilter removal from average influent concentrations reported.
- \(^2\) Obtained from experiment #4 only because DO was not a limiting factor.
- \(^3\) \(UV_{254}\) calculated from data reported.
4.9 Disinfection & Bacterial Re-growth

Table 4-5 represents the results from the three trials conducted on the effect of ozone and biofiltration on disinfection indicator microorganisms. MWWE total coliform counts were in the range of 80,000 to 400,000 MPN/100 mL. MWWE E. coli counts were in the range 20,000 to 105,000 MPN/100 mL. These counts appear to be much higher than those found from four samples taken in a previous study. Singh (2012) reported total coliform and E. coli counts of 10,000 – 100,000 and 2,500 – 21,000 MPN/100 mL respectively. However, two trials did have similar microbial counts to Singh (2012). Only the third trial had a much higher microbial count. Another sample not reported measured an E. coli count > 100,000 MPN/100 mL, which suggests that microbial counts can spike occasionally at LRPCP. The reason for this is unknown. All samples were collected within a one month period (mid-July to mid-August) and although it was hot the water temperature was comparable (22 – 24°C). In any case, during the warmer summer months it is more likely that E. coli counts could spike compared to the winter.

In terms of ozone’s disinfection ability, Table 4-5 indicates that total coliform and E. coli log-inactivation were in the range 2.0–2.9 and 2.3–3.2 respectively. Subsequent biofiltration, had < 1-log increase in re-growth. It is interesting that in trials 2 and 3 the UV$_{254}$ removal increased along with the log inactivation. A correlation between UV$_{254}$ reduction and E. coli log inactivation has been observed by other researchers (Singh, 2012). In general, Table 4-5 shows ozonation was able to reduce both total coliform and E. coli counts significantly, but subsequent re-growth did occur in biofiltration.
Table 4-5: Effect of ozone ozone and biofiltration on disinfection indicator microorganisms

<table>
<thead>
<tr>
<th>Experiment</th>
<th>TOD (mg/L)</th>
<th>Water Temp (°C)</th>
<th>DOC (mg/L)</th>
<th>UV&lt;sub&gt;254&lt;/sub&gt; (cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>UV&lt;sub&gt;254&lt;/sub&gt; removal %</th>
<th>Total Coliform MPN/100 mL (+) / (-)</th>
<th>Log</th>
<th>E. coli MPN/100 mL (+) / (-)</th>
<th>Log</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.5</td>
<td>22.6</td>
<td>n.m.</td>
<td>n.m.</td>
<td></td>
<td>79,253</td>
<td>21,953</td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>Post O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>747</td>
<td>- 2.0</td>
<td>117</td>
<td>- 2.3</td>
<td></td>
</tr>
<tr>
<td>BAC Sand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,520</td>
<td>+ 0.3</td>
<td>249</td>
<td>+ 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>721</td>
<td>- 0.0</td>
<td>161</td>
<td>+ 0.1</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.7</td>
<td>23.2</td>
<td>6.4</td>
<td>0.1139</td>
<td></td>
<td>105,150</td>
<td>31,367</td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>Post O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6.2</td>
<td>6.2</td>
<td>0.0835</td>
<td>27</td>
<td>899</td>
<td>- 2.1</td>
<td>87</td>
<td>- 2.6</td>
<td></td>
</tr>
<tr>
<td>BAC Sand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,253</td>
<td>+ 0.1</td>
<td>189</td>
<td>+ 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>745</td>
<td>- 0.1</td>
<td>134</td>
<td>+ 0.2</td>
</tr>
<tr>
<td><strong>Trial 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.5</td>
<td>23.4</td>
<td>5.8</td>
<td>0.1254</td>
<td></td>
<td>392,360</td>
<td>102,353</td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>Post O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5.7</td>
<td>5.7</td>
<td>0.0830</td>
<td>34</td>
<td>495</td>
<td>- 2.9</td>
<td>67</td>
<td>- 3.2</td>
<td></td>
</tr>
<tr>
<td>BAC Sand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,047</td>
<td>+ 0.3</td>
<td>329</td>
<td>+ 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>597</td>
<td>+ 0.1</td>
<td>141</td>
<td>+ 0.3</td>
</tr>
</tbody>
</table>

Legend:
(+): Increase (re-growth)
(-): Reduction (inactivation)
n.m.: Not measured
The effect of ozone and biofiltration on *E. coli* inactivation and re-growth is shown graphically in Figure 4-10. The results indicate that the disinfection limit of 200 MPN *E. coli*/100 mL was achieved in all three trials after ozonation. The disinfection limit was achieved previously for a similar TOD at LRPCP (Singh, 2012), which indicates that at a TOD ≈ 3.5 mg/L the Ontario MOE disinfection limit can be met.

However, there is concern with the final treatment train process unit being biofiltration that wastewater disinfection requirements may not be met. Biofiltration units provide a place for microorganisms to colonize on granular media and form a biofilm. Consequently, biofilters provide protection for bacteria and other pathogenic organisms to multiply. Figure 4-10 shows *E. coli* re-growth did occur in both biofilters following ozonation. Nevertheless, the sand biofilter actually met the LRPCP discharge limits (< 200 MPN *E. coli*/100 mL). In contrast, the BAC biofilter only met the disinfection limit once out of three trials. Overall, this demonstrates the BAC biofilter exhibited more re-growth than the sand biofilter.
No bacterial re-growth studies on sand biofilters could be found. However, Gerrity et al. (2011) studied the issue of bacterial re-growth for ozone-BAC in indirect potable water reuse (fecal coliform was measured instead of *E. coli*). The results are compiled in Table 4-6, along with the current study’s BAC biofilter. Gerrity et al. (2011) observed bacterial re-growth did occur following ozonation in the BAC biofilter as well, with re-growth as much as 1-log in fecal coliform. Despite this re-growth, all three sampling events conducted had post-BAC fecal coliform counts < 100 MPN fecal coliform/100 mL. However, Gerrity et al. (2011) used a higher TOD and also used ozone combined with hydrogen peroxide, which was able to reduce fecal coliform for the most part to < 10 MPN/100 mL following ozonation (Table 4-6); the *E. coli* count would be expected to be even lower. Ozonation in the current study was only able to
reduce *E. coli* for the most part to < 100 MPN/100 mL. Consequently, the disinfection limit of 200 MPN/100 mL was only met once after BAC biofiltration and was not surpassed enormously with the sand biofilter. The Ontario disinfection limit is based upon a monthly average, which can be exceeded in some samples as long as the monthly average is < 200 MPN *E. coli*/100 mL. Studies involving more samples would have to be conducted to see if the disinfection limit could be met over a monthly time period at LRPCP.

**Table 4-6: Ozone disinfection and potential bacterial re-growth following BAC biofiltration**

<table>
<thead>
<tr>
<th>Author</th>
<th>TOD (mg/L)</th>
<th>TOC/DOC Pre O₃ (mg/L)</th>
<th>Specific O₃ Dose</th>
<th><em>E. coli</em>/fecal coliform Post O₃ (MPN/100 mL)</th>
<th><em>E. coli</em>/fecal coliform Post BAC (MPN/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Study</td>
<td>3.5-3.7</td>
<td>5.8-6.4</td>
<td>0.55-0.60</td>
<td>&lt;100¹</td>
<td>189-329</td>
</tr>
<tr>
<td>Gerrity et al. (2011)</td>
<td>5 O₃: 3.5 H₂O₂</td>
<td>7.2-7.4</td>
<td>0.67-0.69</td>
<td>&lt;10²</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

Legend:

¹Two out of three trials < 100 MPN/100 mL
²Two out of three trials < 10 MPN/100 mL

If the *E. coli* monthly geometric mean exceeded the Ontario MOE disinfection limit, either a (1) higher ozone dose would have to be applied to achieve a higher level of disinfection or (2) the process flow would have to include a secondary disinfection unit. A higher ozone dose that reduces the *E. coli* closer to < 10 MPN *E. coli*/100 mL would allow fewer microorganisms to pass through the biofilters. Consequently, even if re-growth occurred in post-biofiltration, biofilter effluent still may be able to achieve the limit of 200 MPN/100 mL. If secondary disinfection were needed it would cause this treatment technology to have three unit processes: ozone → biofiltration → secondary disinfection. As a result, cost would rise because a third
treatment unit would be needed. Secondary disinfection could be achieved with common disinfection technologies, such as chlorine and UV radiation.

Comparing both biofilter’s *E. coli* re-growth, Figure 4-10 shows there was a difference in *E. coli* counts emitted from the two biofilters. The BAC biofilter had higher *E. coli* counts in all three trials as compared to the sand biofilter (Figure 4-10). Higher *E. coli* counts in the BAC effluent may indicate it supported more biomass than the sand biofilter. More biological activity in the BAC biofilter has already been suggested by DO observations. This will be discussed further in Section 4.10: ATP Activity Supported on Media.

In summary, bacterial re-growth did occur in all three trials with both biofilters. Thus, bacterial re-growth needs to be considered when operating ozone-biofiltration units. There is a need for further evaluation of the biofilter bacterial re-growth issue to examine the effect of varying seasonal conditions.

### 4.10 ATP Activity Supported on Media

Biological activity was quantified by measuring ATP on biofilter media during steady state. The goal was to determine whether GAC or sand media supported more biological activity. The results for each trial as well as the calibration curves are detailed in Appendix E. Figure 4-11 depicts graphically the biological activity supported on the sand and GAC media from the three trials, reported in ng ATP/g DW (dry weight of media). The mean biological activities are displayed in Table 4-7.
Figure 4.11: Effect of media on ability to support biological activity (ATP). Error bars represent ± standard deviation from the mean.

Table 4.7: Mean biological activity (ATP) supported on GAC and sand

<table>
<thead>
<tr>
<th></th>
<th>Trial 1 Oct. 30, 2012</th>
<th>Trial 2 Nov. 6, 2012</th>
<th>Trial 3 Nov. 13, 2012</th>
<th>Overall ATP/DW (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>862</td>
<td>804</td>
<td>803</td>
<td>823</td>
</tr>
<tr>
<td>SD</td>
<td>144</td>
<td>128</td>
<td>210</td>
<td>126</td>
</tr>
<tr>
<td>Sand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>609</td>
<td>464</td>
<td>416</td>
<td>496</td>
</tr>
<tr>
<td>SD</td>
<td>129</td>
<td>67</td>
<td>61</td>
<td>107</td>
</tr>
</tbody>
</table>

Legend:
ATP: adenosine triphosphate
DW: dry weight of media
SD: standard deviation
The data indicates GAC supported more biological activity than sand in all three trials. Overall, GAC supported 823 ng ATP/g GAC, whereas sand supported 496 ng ATP/g sand. This is analogous to two previous results in the current study (Figure 4-9, Figure 4-10). Higher DO reduction and *E. coli* counts in the BAC biofilter effluent also suggested the BAC biofilter exhibited more biological activity than the sand biofilter. When designing the biofilter pilot units, GAC and sand were chosen with the closest size specifications provided by a supplier. Their effective size (0.8-1.0 mm) indicates both media have very similar size specifications. Therefore, differences in attached biological activity can presumably be attributed to differences in media surface area or grain structure, not effective size.

Researchers have previously hypothesized that GAC may be able to accommodate a more dense or biologically active biofilm than non-adsorbing media (Lechevallier et al., 1992; Krasner et al., 1993). However, it has also been hypothesized that the area available for biomass attachment may be higher on sand compared to GAC because little bacteria (> 200 nm) could grow in GAC micropores (1-100 nm) (Urfer et al., 1997). These results suggest that GAC media can support more biological activity than sand media. Based on the data acquired the exact reason why GAC supported more biological activity could not be determined. However, this occurred for any number of reasons listed: (1) GAC’s higher surface area relative to sand means GAC could support more biomass, (2) the irregular grain structure of GAC provides better biomass attachment sites and protection from shear stress (Urfer et al., 1997), or (3) GAC’s ability to adsorb OM, providing food for biomass to metabolize at a later time encouraged a more active biofilm.

Table 4-8 compares the ATP bioactivity of the current study to previous reported values. No studies could be found reporting ATP bioactivity of a non-adsorbing media biofilter. The
data does indicate marginal variation in ATP bioactivity supported on GAC media. However, the BAC biofilter’s bioactivity in the current study is comparable to previous studies. The bioactivity observed (823 ± 126 ng ATP/g GAC) falls within the range of values previously reported. This indicates that despite the differences in water characteristics, bioactivity values are similar. This is most likely because only so much biomass can attach to a GAC granule, and bioactivity units are normalized per gram GAC media.

### Table 4-8: Comparison of biofilter biological activity (ATP) to previous studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Media</th>
<th>EBCT (min)</th>
<th>Hydraulic loading (m/ hr)</th>
<th>DOC influent (mg/L)</th>
<th>Scale</th>
<th>Bioactivity supported (ng ATP/g media)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>GAC</td>
<td>40</td>
<td>0.97</td>
<td>5.5 ± 0.8</td>
<td>Pilot</td>
<td>823 ± 126</td>
</tr>
<tr>
<td></td>
<td>GAC</td>
<td>12.5</td>
<td>6.5</td>
<td>0.96 ± 0.03</td>
<td>Full</td>
<td>609 ± 61</td>
</tr>
<tr>
<td>Velten et. al., 2007</td>
<td>GAC</td>
<td>16.5</td>
<td>8</td>
<td>0.96 ± 0.03</td>
<td>Pilot</td>
<td>1820 ± 147</td>
</tr>
<tr>
<td>Velten et. al., 2011</td>
<td>GAC</td>
<td>15.76</td>
<td>5.9</td>
<td>1.1 ± 0.04</td>
<td>Pilot</td>
<td>800 – 1830</td>
</tr>
<tr>
<td>Current study</td>
<td>Sand</td>
<td>40</td>
<td>0.97</td>
<td>5.2 ± 0.6</td>
<td>Pilot</td>
<td>496 ± 107</td>
</tr>
</tbody>
</table>

### 4.11 Genotoxicity & Toxic TPs

#### 4.11.1 Genotoxin Removal

Three trials were conducted to determine the effect ozone and both biofilters have on removing genotoxic compounds. The objective was to determine ozone’s effect on wastewater genotoxicity and to establish which biofilter can remove more genotoxic compounds. The UMU-Chromo test uses the induction ratio (IR) to quantify the amount of genotoxicity present within a sample. The UMU-Chromo test data are plotted as dose-response curves in Figure 4-12.
The dose is the array of concentration factors (CF_{overall}) processed from each sample’s solid phase extraction. It is important to emphasize two concepts about the graphs:

- As CF_{overall} ↑, IR ↑ (sample becomes more genotoxic)
- Samples are considered genotoxic when a given CF_{overall} induces an IR ≥ 1.5 (ISO, 2000)

Figure 4-12: UMU-Chromo test dose-response curves for three trials (a, b, c). Error bars represent the maximum and minimum values.
Figure 4-12 shows each dose-response curve is linear. The data indicates samples with less treatment (e.g., pre O₃ sample) have steeper slopes; these samples are more genotoxic. This means that samples with smaller slopes are less genotoxic and require a larger CF_{overall} to elicit an IR=1.5.

The linear dose response curves indicate which sample is more genotoxic relative to another. However, it is more desirable to have a single value to compare each pilot unit along the treatment train, rather than an array of points making a dose-response curve. To determine a single value to compare each pilot unit along the treatment train, the genotoxicity of each sample is displayed as the concentration factor required to elicit an IR=1.5 (CF_{IR=1.5}) (Macova et al., 2010). A higher CF_{IR=1.5} means the sample needed to be concentrated more to create a genotoxic effect. Therefore, a higher CF_{IR=1.5} indicates the sample is less genotoxic than a low CF_{IR=1.5}.

Results for each sample’s CF_{IR=1.5} are presented in Table 4-9. The data indicates that in all three trials, genotoxicity decreased in each stage of the treatment train. This can be seen by noticing the CF_{IR=1.5} became larger as the wastewater was treated further, indicating the sample needed to be concentrated more to elicit a genotoxic response. Table 4-9 shows the genotoxicity of the pre-ozone sample varied in all three trials. The pre-ozone sample needed to be concentrated between 0.3–13 times in order to elicit an IR=1.5, demonstrating MWWE variability. This was already observed through indicator microorganism counts.
Table 4-9: UMU-Chromo test CF required to elicit an IR = 1.5 (CF<sub>IR=1.5</sub>)

<table>
<thead>
<tr>
<th>extraction date</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>assay date</td>
<td>June 4, 5, 2012</td>
<td>June 19, 20, 2012</td>
<td>July 16, 17, 2012</td>
</tr>
<tr>
<td>CF&lt;sub&gt;IR=1.5&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre O&lt;sub&gt;3&lt;/sub&gt; (S1)</td>
<td>13.1</td>
<td>0.3</td>
<td>8.9</td>
</tr>
<tr>
<td>Post O&lt;sub&gt;3&lt;/sub&gt; (S2)</td>
<td>40.1</td>
<td>15.2</td>
<td>22.1</td>
</tr>
<tr>
<td>Sand (S4)</td>
<td>57.9</td>
<td>21.7</td>
<td>24.0</td>
</tr>
<tr>
<td>BAC (S3)</td>
<td>120.9</td>
<td>&gt;166.6</td>
<td>&gt;166.6</td>
</tr>
<tr>
<td>Blank (S5)</td>
<td>128.2</td>
<td>&gt;166.6</td>
<td>&gt;166.6</td>
</tr>
<tr>
<td>TOD (mg/L)</td>
<td>3.6</td>
<td>3.6</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Legend:
CF<sub>IR=1.5</sub>: concentration factor required to elicit an IR = 1.5
↑ CF<sub>IR=1.5</sub> α ↓ genotoxic effect

4.11.1.1 Effect of Ozone on Genotoxicity

For ozonation, a significant reduction in wastewater genotoxicity was observed for all three trials. Ozonated effluent had a CF<sub>IR=1.5</sub> between 15–40 (Table 4-9). For example, in trial 1, the post-ozone sample (S2) needed to be concentrated 40 times, whereas the pre-ozone sample (S1) only needed to be concentrated 13 times to elicit the same genotoxic response. The remaining genotoxicity after ozonation can be attributed to possible toxic TPs (organic or inorganic) or other genotoxic compounds that remained un-oxidized by ozone. However, the major concern with ozonation is whether unidentified TPs are produced that are more toxic than their parent compounds (Joss et al., 2008; Hollender et al., 2009). These results suggest that as a whole the TPs ozone produced were less genotoxic than their parent compounds. Similar results reporting less potent ozonated effluent was observed with the Microtox bioassay (Reungoat et
al., 2010; Reungoat et al., 2011; Reungoat et al., 2012), and the UMU bioassay (Macova et al., 2010). However, ozonated effluent is not always less potent depending on the toxic endpoint or test organism (Stalter et al., 2010). This study indicates that ozone was able to oxidize many genotoxic compounds into TPs that did not have the same bioactive genotoxic property of the parent compound.

4.11.1.2 Effect of Biofilters on Genotoxicity

Subsequent BAC and sand biofiltration further removed genotoxic compounds present in wastewater. Comparing both biofilters, the BAC outperformed the sand biofilter. This is demonstrated, for example, in trial 2. The BAC biofilter (S3) needed to be concentrated > 166.6 times, whereas the sand biofilter (S4) needed to be concentrated only 21.7 times to elicit the same genotoxic response. The sand biofilter had a CF$_{IR=1.5}$ between 22–58 (Table 4-9). In comparison, the BAC biofilter had a CF$_{IR=1.5}$ always > 121 and was less than the detection limit in trials 2 and 3. This is analogous to the better removal efficiencies of bulk organic parameters the BAC had over the sand biofilter. The BAC biofilter exhibited better performance most likely due to its additional ability to remove OM by bio-regeneration, and support more biological activity (indicated through ATP data).

A previous study indicated that toxicity removal might be comparable between a sand and BAC biofilter fed with ozonated wastewater as monitored using non-specific toxicity (Microtox) (Reungoat et al., 2011). One sampling campaign showed the sand biofilter removed 65%, while the BAC removed 60% of non-specific toxicity. However, in the second sampling event the sand biofilter removed 49 ± 3%, while the BAC removed 74 ± 10% non-specific toxicity. These results are somewhat conflicting. Both biofilters had similar non-specific
toxicity removals in the first sampling event, but the BAC biofilter removed more toxicity in the second sampling campaign. No reason for this was given (Reungoat et al., 2011). A more comparable removal of non-specific toxicity could have been observed by Reungoat et al. (2011) because of the difference in MWWE characteristics. Reungoat et al. (2011) studied MWWE treated with five additional process units before being ozonated for wastewater being used for non-potable reuse. It is expected that wastewater treated for reuse is of better quality than MWWE; hence reused water would contain less genotoxic compounds. Therefore, the BAC and sand biofilter may have exhibited more comparable performance by Reungoat et al. (2011) because there was not a large enough amount of genotoxic compounds in the feed to notice a significant difference in biofilter toxicity removals. However, it is difficult to compare the genotoxic content of the pre-ozonated wastewater between the two studies because two different bioassays with different test organisms and toxic endpoints were used. The current study indicates when specifically treating a lower water quality, such as MWWE, the BAC biofilter removed significantly more genotoxic compounds than the sand biofilter.

One note must be discussed about the results in Table 4-9. Genotoxicity in the BAC sample was detected in trial 1 most likely because extracts were not diluted enough. Although the concentration of DMSO was 10% as recommended by ISO (2000), matrix effects were observed because genotoxicity was detected in the blank sample (10% DMSO) at a $\text{CF}_{IR=1.5} = 128.2$. In trials 2 and 3, extracts were diluted more and the results show the blank did not exhibit any matrix effects. Regardless, Table 4-9 shows from all three trials that the BAC biofilter removed more genotoxic compounds than the sand biofilter.
4.11.2 Putting Genotoxicity Results into Perspective with Toxic TPs

Even though the current study shows as a whole the genotoxic potency reduced after ozonation, there is still the chance that a few toxic TPs may be produced. Currently, limited information is available on ozone TPs, whether they are more potent than their parent compounds and whether they are environmentally relevant at the concentration produced. However, studies indicate that bromate (von Gunten and Hoigne, 1994) and more recently NDMA (Schmidt and Brauch, 2008) TPs are a concern. These two TPs are now recognized as probable human carcinogens by either the CDPH, the USEPA or Health Canada. Accordingly, the USEPA has set a bromate maximum contaminant level (MCL) in drinking water of 10 µg/L annual average (USEPA, 2001a). Just like bromate, NDMA is most likely going to be regulated in the future in the USA according to CDPH (2006). In Canada, NDMA has already been given a maximum acceptable concentration (MAC) in drinking water of 40 ng/L (Health Canada, 2011). Although this study shows ozone does an effective job of oxidizing the majority of toxic OM into TPs that are less potent than their parent compounds, there are still a few TPs that actually become more potent. Standards in wastewater have not been made, but now that water standards are present there is a possibility wastewater may be regulated too.

The question then arises, if ozone reduces the genotoxicity of wastewater why would a biofilter need to follow ozonation to remove even more genotoxic compounds? Bromate and NDMA are the two most documented examples of toxic TPs produced by ozone. Hollender et al. (2009) reported that a sand biofilter biodegraded nitrosamines produced from ozonated wastewater. NDMA was formed during ozonation in concentrations up to 14 ng/L, but subsequent sand biofiltration was able to reduce NDMA by 50 ± 17% to < 10 ng/L, which is under the CDPH notification level (10 ng/L). In combination with the current study’s
observation that biofilters remove genotoxins still present after ozonation, Hollender et al. (2009) suggested that biofilters can also biodegrade NDMA. This is a significant finding of how useful biofilters can be, especially for future NDMA regulatory purposes. Studies conducted on whether bromate can be reduced by biofiltration are conflicting. Sundaram et al. (2009) suggests that bromate was removed by a BAC biofilter, however Gerrity et al. (2011) suggests that no bromate reduction was observed in the same BAC biofilter at a later date despite detecting anaerobic reducing microbes on the GAC media. Bromate may have been removed initially by adsorption and as the adsorption capacity reduced over time, bromate removal subsequently became insignificant. Regardless, peroxide addition has shown to be an effective barrier in bromate formation and was actually utilized by Sundaram et al. (2009) and Gerrity et al. (2011). The concept of NDMA produced during ozonation is still relatively new, but there is optimism that biofilters can biodegrade NDMA.

Overall, the findings of this study show genotoxic compounds were removed with each stage of the treatment train. Ozonation decreased genotoxicity, demonstrating that as a whole the TPs ozone created were less genotoxic than their parent compounds. Further biofiltration removed genotoxic OM present after ozonation, with the BAC biofilter removing more genotoxins than the sand biofilter. More studies are warranted to determine if adsorbing and non-adsorbing media biofilters can remove NDMA and bromate.

4.12 Use of Ozone-Biofiltration for Tertiary WWT in North America

As of now, few emerging contaminants have regulations in Canada and the United States (IJC, 2011). Currently, MWWTP regulations are based on solids, nutrients, dissolved biodegradable organic matter and pathogens. As a result, presently it is uncommon to use both
ozone and biofiltration for tertiary WWT in North America. However, there is an ever-increasing amount of evidence to suggest that at the very least EDCs may impact animals in surrounding ecosystems despite very low concentrations. Research still needs to be conducted to determine the toxicological relevance PPCPs have on humans and the surrounding wildlife. As for toxicity bioassays, there is uncertainty when extrapolating their results to more complex organisms. Until concrete toxicological data exist on the relevance of trace levels of PPCPs and EDCs, it will be difficult to establish regulations governing MWWE. In addition to determining the relevance of trace levels of CECs, there is a need to investigate the battery of available bioassays to determine their applicability to more complex organisms (e.g., humans). After this, regulators will be able to warrant whether additional regulations are necessary.

Currently, ozone-biofiltration units in North American MWWTPs are not commonly used because of their higher cost over common disinfection technologies, such as chlorine and UV radiation. Even though ozone-biofiltration has been implemented in wastewater treatment in Europe (Hollender et al., 2009; Stalter et al., 2011) unless trace levels of CECs are determined to be harmful in MWWE it is unlikely that ozone-biofiltration will be widely used in North American wastewater treatment. However, ozone-biofiltration is still useful presently in other applications. Currently ozone-biofiltration is most commonly utilized in water treatment to address disinfectant resistant pathogens, but it is also used in water re-use applications in regions with fresh water shortages (Gerrity et al., 2011; Lee et al., 2012; Reungoat et al., 2012). Regardless, if trace levels of CECs are determined to be harmful and MWWE is regulated further to protect human or animal health in the future, ozone-biofiltration is a feasible option in the group of technologies that are available to remove CECs and unidentified toxic compounds in wastewater.
CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the maturation phase of this study, it is concluded that:

- TSS, turbidity and head loss did not give any indication of biofilter operational problems. However, visual observations noticed a crust forming on the top couple centimetres of the bed that was not broken up during backwashing. The installed surface wash eliminated mud ball formation, while maintaining biofilter performance throughout the study.
- DO, DOC and UV$_{254}$ trends were observed. DO reduction gradually increased until steady state was reached in both biofilters. DOC and UV$_{254}$ removal declined in the adsorbing media biofilter, and rose in the non-adsorbing media biofilter until steady state was reached.
- DOC, UV$_{254}$ and DO percent removals took approximately 40 days to reach steady state values in both biofilters.

Based on the performance phase of this study, it is concluded that:

- The BAC biofilter outperformed the sand biofilter in terms of DOC, UV$_{254}$, and DO removal.
- UV$_{254}$ removal indicated the BAC removed more poorly biodegradable organic matter than the sand biofilter. This suggests that bio-regeneration may play an important role in removing OM in BAC biofilters.
- The sand biofilter was able to remove aromatic OM, indicating that not all UV$_{254}$ absorbing molecules are completely non-biodegradable.
- GAC supported more biological activity than sand media. This was reflected in higher BAC effluent E. coli counts, as well as larger DO and DOC removal.
- Bacterial re-growth occurred in both biofilters following ozonation. The sand biofilter achieved the disinfection limits established by the Ontario MOE < 200 MPN E. coli/100 mL. In contrast, the BAC biofilter was only under the
disinfection limit once out of three trials. Thus, bacterial re-growth needs to be considered when operating ozone-biofiltration units.

- Ozone reduced the genotoxicity of wastewater, suggesting that as a whole the TPs ozone produced were less genotoxic than their parent compounds. However, this does not mean that all TPs were less bioactive than their parent compounds. Caution should be observed because the TPs that exhibited less genotoxic properties on *Salmonella typhimurium*, may exhibit a different effect depending on the toxic endpoint or test organism (Stalter et al., 2010).
- Biofiltration further removed genotoxic compounds present after ozonation. The BAC biofilter removed significantly more wastewater genotoxins than the sand biofilter.

### 5.2 Recommendations

It is recommended that:

- Biofiltration studies be conducted with a higher hydraulic loading rate (between 6-14 m/hr). This is important to see if any operational problems occur from a more realistic solids loading rate. The peak solids loading in the current study was 2.7 mg/(s·m²). This is well below the recommended peak solids loading rate of 51 mg/(s·m²) (Ontario MOE, 2008b).
- Studies on biofilter bacterial re-growth be conducted to examine the degree of bacterial re-growth in varying seasonal conditions.
- Toxicity evaluations be conducted on CECs to gain a better understanding of the toxicological relevance of trace CEC levels on humans and the surrounding wildlife.
- A battery of toxicity tests be conducted on ozonated wastewater to gain a better understanding of the chemical properties of ozone TPs.
- Studies be conducted to determine how effective post-biofiltration can be in removing NDMA and bromate formed during ozonation.
- Biofilter investigations be carried out to fully understand the mechanisms used to remove OM, particularly the role of filter media on supporting biofilm activity and GAC’s adsorption capacity at steady state.
6.0 APPENDICES

6.1.1 Appendix A – Sampling Procedure

1. The head loss was recorded with a tape measure from both columns.
2. 2 Wheaton glass biochemical oxygen demand (BOD) bottles were filled with BAC effluent and sand biofilter effluent. Sampling commenced approximately 10 minutes later. The purpose was to acclimatize the BOD bottles to the water temperature. Without filling effluent in the BOD bottles and letting them sit for approximately 10 minutes, duplicate temperature readings were erratic. Following the above procedure, duplicate temperature readings were much more precise (+/- 0.1 °C).
3. 2 grab samples of BAC effluent were collected from the effluent sampling port, in Wheaton glass BOD bottles.
4. DO, pH and temperature were measured with Hach model HQ40d multimeter.
5. BAC effluent was then collected from the effluent sampling port in 1 L amber glass bottles capped with Teflon lined septa (to prevent contamination). The 1 L amber bottles were rinsed three times with sample before filling. The bottles were stored in a fridge until transported to the University of Windsor for analysis.
6. Steps 3 to 5 were repeated with sand biofilter effluent.
7. 2 Wheaton glass BOD bottles were filled with biofilter influent (ozonated effluent). Sampling commenced approximately 10 minutes later (for the reason described in Step 2).
8. 2 grab samples of biofilter influent were collected from the influent sampling port, in Wheaton glass BOD bottles.
9. DO, pH and temperature were measured with Hach model HQ40d multimeter.
10. Biofilter influent was then collected from the influent sampling port in 1 L amber glass bottles capped with Teflon lined septa (to prevent contamination) as described in Step 5.
11. Water flow rate was measured with a stopwatch, water collection container and a calibrated cylinder. The flow rate was then adjusted if need be to meet the desired flow rate.
6.1.2 Appendix B – Solid phase extraction SOP

Purpose

Aim to catch all compounds that pose a health hazard to humans and wildlife.

Scope

This method is to concentrate drinking water, surface water, and treated wastewater samples to ensure a better response for toxicity testing.

Sample Collection

1. Take 4 samples (inf O₃, eff O₃, eff BAC filter, eff Sand filter). Take 2L of inf O₃ sample and 3L of all other samples (11 full bottles). The amber bottles must be filled with no headspace to contain 1L.
2. Micropipette 490µL of HCl (36.5-38%) to all 1L sample bottles (for a final concentration of 5 mM for preservation). Percent recoveries were highest at pH 3 (Escher et al., 2005).
3. This should happen 11 times (11 samples bottles).
4. Store samples on ice <6°C in the dark (USEPA, 2007).
5. Bring all 11 sample bottles to the lab and store in fridge.
6. At U of W lab fill three 1L sample bottles with Milli-Q water.
7. Micropipette 490µL of HCl (36.5-38%) to all 1L blank bottles filled with Milli-Q water.
8. Store 3 blank bottles filled with Milli-Q water in fridge.
9. 14 1L bottles should now be in the fridge (11 filled with sample, 3 filled with Milli-Q water).
10. Begin SPE the following day.

Day 1

Filtration

1. Samples should be filtered in this order: blank, eff BAC filter, eff Sand filter, eff O₃, inf O₃.
2. Label 5 500mL vacuum flasks with the above labels. Label 1 vacuum flask with the label ‘scrap.’
3. Label 5 3L bottles with the above labels.
4. Attach tubing to 2L bottles aerated opening.
5. Connect tubing on 2L aerated bottles to silicone stopper (each silicone stopper has a piece of glass through its centre to connect the tubing.
6. Set up filter apparatus with scrap 500mL vacuum flask and new Whatman 934-AH 1.5 µm filter.
7. Rinse filter with 500mL of Milli-Q water to ensure any carbon is drained from the filter.
8. Rinse filter with ≈100mL of sample from ≈200mL sample bottle.
9. Remove and attach filter apparatus to clean vacuum flask.
10. Filter ≈400mL of sample into vacuum flask.
11. Pour ≈400mL of sample from the vacuum flask into a clean 3L bottle.
12. Repeat steps 8 & 9 until all 2L of sample is filtered.
13. Move on to next sample. Repeat steps 4-10 until all 5 samples are filtered.
14. 5 samples should be filtered: 4 samples + 1 blank.

SPE (t (min)= V (mL) / 10ml/min)

1. Set up 5 cartridges on SPE manifold. Cartridges are 1g Oasis HLB sorbent in 20 mL cartridges (Waters, Mississauga). Close valves.
2. Condition 10mL methanol through each cartridge. Flow rate is 1 drop/sec (1mL/min).
3. Condition 20mL of 5mM HCl through each cartridge. Flow rate is 1 drop/sec (1mL/min).
4. Disconnect top from SPE manifold (containing cartridges). Leave glass part of SPE manifold.
5. Place top of SPE manifold on 2 pieces of wood so it is elevated off the lab bench.
6. Connect pump tubing at outlet fitting and drain it into the sink.
7. Connect tubing on 2L aerated bottles to silicone stopper. Let silicone stopper rest without sealing it to the cartridge.
8. This part requires some skill. Gently lift each 2L bottle up individually to fill the connecting tube with water. Quickly jam silicone stopper into cartridge before water overfills cartridge. This forms an air tight seal. When the 2L bottle is placed back down on the counter there should be no backflow in water if the stopper is sealed to the cartridge.
9. Percolate all 5 samples under pump. Clamp suction side of tubing with metal clamps. Without metal clamps tubing will walk and pull over top of manifold. Q= 5–10 mL/min (USEPA, 2007). Q= 10 mL/min (Macova et al., 2010). Measure flow rate with a stop watch and graduated cylinder.
10. When water remaining in aerated bottles gets low, lean the bottle to ensure all the water is sucked out of the bottle.
11. If one sample finishes percolating before another, with ≈3-5cm of water head shut the pump off. Close the valve on this cartridge and disconnect the pump tubing from the pump. Then turn the pump back on to allow the rest of the samples to finish percolating.
12. Repeat above step for each sample as they finish percolating through their respective cartridges.
13. When all samples are finished, each cartridge should have ≈3-5cm of water head. At this point remove silicone stoppers from cartridges.
14. Connect top from SPE manifold (containing cartridges) to its glass bottom part of SPE manifold.
15. Dry cartridges for 5 min under vacuum (USEPA, 2007).
16. Remove the cartridges and, using Kimwipes, dry the inside of the SPE cartridges. Shake the cartridges firmly to remove water from the lower parts of the cartridges.
17. Place the cartridges back into the manifold and apply vacuum for ~2 min to remove any residual moisture.
18. Stop the vacuum after ~2 min.

Elution

19. Label 5 test tubes and 5 centrifuge tubes with labels given in Filtration Step 1.
20. Keep manifold connected, but close the vacuum.
21. Elute with 10 mL methanol, then 10 mL hexane:acetone (1:1) into 10 mL test tube. Initiate elution by vacuum if necessary and then continue by gravity. Elution can usually be carried out by just opening the valves and not using vacuum. Flow rate is 1 drop/2 sec (0.5 mL/min).
22. Pour all 5 test tubes into 5 labelled centrifuge tubes, when the test tubes get too full. Combine the methanol and hexane:acetone eluate.
23. When all eluate has dropped out by gravity, turn on vacuum and suck any remaining eluate out for ~2 min.
24. Rinse all 5 test tubes with 0.5 mL methanol and pour the 0.5 mL into the centrifuge tubes (to ensure all chemicals are transferred to centrifuge tubes).
25. There should be 5 centrifuge tubes (for 5 samples).
26. Store centrifuge tubes overnight in freezer. Cap with parafilm. Evaporate samples the following day.

Day 2

Evaporation

27. Take centrifuge tubes out of freezer and let them thaw.
28. Evaporate 20 mL eluate to dryness in gentle N₂ stream (Escher et al., 2005). Use Bill’s N₂ manifold under the fume hood. Connect blue tubing on luer fitting. Connect luer fitting to Bill’s manifold. \( P_{\text{at}} \approx 1 \text{ psi} \) (just enough pressure to hear the nitrogen flowing from the cylinder). Time required is 7-9 hr to evaporate to dryness. Need 3000 psi of N₂ (2 tanks).
29. White caking will occur around 10 mL mark. Leave white caking on the centrifuge tubes.
30. As eluate evaporates move the manifold down further towards the top of the eluate.
31. A colored cake should be present once sample has evaporated covering approximately the 1mL mark and down. This colored cake needs to be re-dissolved. The colored cake contains the toxic chemicals.

Reconstitute with 1mL of DMSO in all 5 centrifuge tubes (Day 2)

32. Samples should be reconstituted in this order: blank, eff BAC filter, eff Sand filter, eff O₃, inf O₃.
33. Add 0.5mL DMSO in centrifuge tube.
34. Swirl 0.5mL around the centrifuge tube to ensure all coloured chemicals are dissolved off the walls of the centrifuge tube. Leave the white cake at ~10mL mark.
35. Pasteur pipette 0.5 mL reconstitute from centrifuge tube to 1mL volumetric flask.
36. Add another 0.5mL DMSO in centrifuge tube.
37. Swirl 0.5mL around the centrifuge tube to ensure any remaining coloured chemicals are dissolved off the walls of the centrifuge tube. There should be no coloured chemicals caked on the centrifuge tube. All coloured chemicals should be dissolved. Use vortex gently if necessary. The vortex works really well to dissolve.
38. Pasteur pipette 0.5 mL reconstitute from centrifuge tube to same 1mL volumetric flask.
39. The volume should be 1mL or just under 1mL.
40. If the volume is under 1mL add drops of DMSO into centrifuge tube with Pasteur pipette. BE CAREFUL. Add only a small volume at a time to avoid exceeding 1mL.
41. Transfer reconstitute from centrifuge tube to 1mL volumetric flask with Pasteur pipette.
42. Repeat 36 and 37 until exactly 1mL is in the volumetric flask.
43. When exactly 1mL reconstitute exists, pasteur pipette this 1mL into a clean 1.5mL twist cap GC vial.
44. Rinse 1mL volumetric flask with Milli-Q water. Then rinse at least 5 times (5mL) with DMSO. Turn upside down and knock DMSO out on a Kimwipe. Use air valve in fume hood to spray air through 1mL volumetric flask to ensure it is dry.
45. Repeat for all samples.
46. Freeze extracts (<10°C) and analyze within 40 days (USEPA, 2007).
47. Overall, 4 samples + 1 blank each are concentrated 2000x-3000x.
6.1.3 Appendix C – UMU Chromo test SOP

Purpose

Measure the genotoxicity of wastewater samples with different levels of treatment.

Scope

This method is used to perform the UMU-Chromo test from Environmental Biodetection Products Inc. in drinking water, surface water, and treated wastewater.

Day 1

Bacteria

- Open bottles “A1” and “B”
- Add 200 µL of 1X glucose solution (C) to bottle “A1”
- Immediately transfer bottle “A1” to bottle “B” and mix
- Incubate at 37 °C overnight for 8 – 12 h. Time ____ :_____

Day 2

Bacteria

- Check bottle “B” at 600nm for UV absorbance (UVA) greater than 0.4-0.6. If the overnight growth is >0.4 proceed to next steps below. Blank with Milli-Q water. UVA is ______
- Add 200 µL of 1X glucose solution (C) to all 1X media (A2, A3, A4, A5)
- Re-inoculate using 5 mL from bottle “B” and 5 mL fresh TGA-medium (A2). Time ____ :_____
- Incubate at 37 °C for 1.5 h in CEI room 1211.
- Check UVA after 1.5h incubation at 600 nm. UVA at 1.5h should be > 80% of previous measure. UVA is ______

Preparation of Test Plate A

i. Take 4-NQO out of freezer and let defrost in a cupboard away from light
ii. Start 1h before re-inoculated bacteria finishes incubating
iii. Dilute sample extracts from SPE accordingly in sterile water
iv. Add 60 µL of 10X glucose solution (F) to 10X media (E)
v. Add 180 µL distilled water (K) to all well except A to F 1-3 and H 1-6
vi. Add 360 µL of first sample (diluted in sterile water) to A wells 1-3
vii. Repeat for remaining sample B to F
viii. Dilute samples A to F 1:2 ensuring proper mixing (eg. 8-channel pipette 60 µL first triplicate → column 1 to 4, then 2 to 5, then 3 to 6). Keep same pipette tips for the triplicate. Then change pipette tips for every dilution.
ix. Change pipette tips after this dilution is done
x. Repeat (vii) and (viii) for other 3 triplicates
xi. Discard 180 µL from A to F wells 10-12
xii. Add 153 µL distilled water (K) to control H wells 1-6
xiii. Add 27 µL 10% DMSO solvent to control H wells 4-6
xiv. Add 27 µL 4-NQO (D) to control H wells 1-3
xv. Add 20 µL of 10X TGA (E) to all wells A to H, 1-12.
xvi. Add 70 µL TGA culture medium (5 mL remaining from A2) to blank H wells 7-12 and mix
xvii. Once 1.5 hr bacteria incubation is done, add 70 µL of incubated 1.5 h bacteria to samples A to F wells 1-12
xviii. Mix from right to left (concentration dependent). Keep same pipette tips for the triplicate. Then change pipette tips for every dilution.
xix. Add 70 µL of incubated 1.5 h bacteria to control G wells 1-12 and H wells 1-6
xx. Mix well
xxi. Incubate at 37 °C for 2 h in CEI room 1211. Time ____:_____

Preparation of Test Plate B

i. Head to biology instrument room 1 hr before Plate A finishes incubating
ii. 30min before the Plate A finishes incubating, start Plate B
iii. Add 270 µL of TGA-culture medium (remaining A2, A3, A4, A5) to all wells, place lid
iv. Adjust Plate B temperature to 37 °C via incubator in biology building
v. Take Plate A out of the incubator when it finishes incubating and walk it to biology building.
vi. Take Plate B out of the incubator when Plate A arrives in the biology building.
vii. Add 30 µL from each well in Plate A to corresponding Plate B (4 [ ] to ↑ [ ]). Mix
viii. Measure initial Plate B at 600±20 nm using reader (reader says 590 nm)
ix. Incubate Plate B at 37 °C for 2 h. Time ____:_____
x. 30min before Plate B finishes incubating pour phosphate buffer (G) into amber vial containing ONPG powder (H). Shake 5 – 10 min and store in dark
xi. 30min before Plate B finishes incubating bring B-buffer (I) to room temperature (dissolve crystals formed) and add 35 µL of 2-mercaptoethanol (J)
xii. Add 120 µL B-buffer (I) to all wells of Plate C
xiii. Adjust Plate C temperature to 28±1 °C in incubator
xiv. Measure growth in Plate B at 600±20 nm using reader (reader says 590 nm)
Preparation of Test Plate C

i. Take Plate B and C out of the incubator when Plate B finishes incubating.

xv. Add 30 µL from each well in Plate B to corresponding Plate C (from ↓ [ ] to ↑ [ ]; meaning right to left)

xvi. Add 30 µL ONPG (H) to all wells of Plate C. Mix

xvii. Incubate for 30 min at 28±1 °C. Time ______:_______

ii. Take Plate C out of the incubator after it finishes incubating

iii. Add 120 µL stop solution (L) to all wells of Plate C.

iv. Measure absorption in Plate C at 420±20 nm using reader (reader says 405 nm)

v. Autoclave all plates for disposal.
6.1.4 Appendix D – Sand & GAC Specifications

6.1.4.1 Sand Supplier & Specifications

Carbon Enterprises, Inc. (CEI) Filtration

**Typical Lab Analysis**

Sand .80-1.20mm

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6.1.4.2 GAC Supplier & F-300 Specifications

Calgon Carbon Filtrasorb® 300

- Iodine Number, mg/g (Min.) 900
- Moisture, weight % (Max. %) 2
- Abrasion Number (Min.) 75
- Effective Size, mm 0.8-1.0
- Uniformity Coefficient (Max.) 2.1
- Sieve Size, U.S. Sieve Series, weight %
  
  | Larger than No. 8 (Max.) | 15 |
  | Smaller than No. 30 (Max.) | 4 |
  | Larger than No. 12 (Max.) | - |
  | Smaller than No. 40 (Max.) | - |
6.1.5 Appendix E – General Water Quality Parameters Monitoring

6.1.5.1 pH

Figure E-1: Secondary effluent pH entering the pilot plant throughout the study period

6.1.5.2 Temperature

Figure E-2: Secondary effluent temperature entering the pilot plant throughout the study period
6.1.5.3 Total Alkalinity

Figure E-3: Secondary effluent total alkalinity entering the pilot plant throughout the study period
6.1.6 Appendix F - BacTiter-Glo™ ATP Microbial Cell Viability Assay

6.1.6.1 ATP Calibration Curves

**Trial 1 - GAC**

\[ y = 4 \times 10^6 x - 3 \times 10^7 \]

\[ R^2 = 0.999 \]

**Trial 1 - Sand**

\[ y = 3 \times 10^6 x - 2 \times 10^7 \]

\[ R^2 = 0.9932 \]

**Trial 2 - Sand**

\[ y = 6 \times 10^6 x + 4 \times 10^7 \]

\[ R^2 = 0.9939 \]

**Trial 2 - GAC**

\[ y = 6 \times 10^6 x + 3 \times 10^7 \]

\[ R^2 = 0.9944 \]
Figure F-1: ATP calibration curves for each trial

**Trial 3 - Sand**

\[
y = 6 \times 10^6 x + 3 \times 10^7 \\
R^2 = 0.9948
\]

**Trial 3 - GAC**

\[
y = 5 \times 10^6 x + 5 \times 10^7 \\
R^2 = 0.9895
\]
### Detailed Results

#### Table F-1: Biological activity (ATP) supported on sand and GAC in each trial

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Legend:

ATP: adenosine triphosphate  
DW: dry weight of media
6.1.7 Appendix G – Full Scale & Pilot Plant Process Flow Diagram

Figure G-1: Full Scale & Pilot Plant Process Flow Diagram
7.0 REFERENCES


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