5-1-2017

Impacts of Ozone Dose and Empty Bed Contact Time on Bulk Organic Removal and Disinfection Byproduct Mitigation in Ozone-Biofiltration Systems

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IMPACTS OF OZONE DOSE AND EMPTY BED CONTACT TIME ON BULK ORGANIC REMOVAL AND DISINFECTION BYPRODUCT MITIGATION IN OZONE-BIOFILTRATION SYSTEMS

by

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Bachelor of Science in Environmental Engineering
Universidade Federal do Rio Grande do Norte
2015

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science in Engineering - Civil and Environmental Engineering

Department of Civil and Environmental Engineering and Construction
Howard R. Hughes College of Engineering
The Graduate College

University of Nevada, Las Vegas
May 2017

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

April 17, 2017

This thesis prepared by

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entitled

Removal and Disinfection Byproduct Mitigation in Ozone-Biofiltration Systems

is approved in partial fulfillment of the requirements for the degree of

Master of Science in Engineering – Civil and Environmental Engineering
Department of Civil and Environmental Engineering and Construction

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ABSTRACT

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In areas where water shortages have compromised water supplies, potable reuse is a promising solution. However, additional research is needed to identify and/or optimize cost-effective treatment technologies to demonstrate compliance with potable reuse regulations. Treatment trains employing reverse osmosis (RO) and advanced oxidation, a combination known as 'full advanced treatment' (FAT), are required by the California Division of Drinking Water (CDDW) for surface water augmentation and direct injection of recycled water into local aquifers. A maximum concentration of 0.5 mg/L of wastewater-derived total organic carbon (TOC) is also required by CDDW in all groundwater recharge applications. This appears to be very conservative when compared to typical TOC concentrations in conventional drinking waters. Although FAT can reliably achieve the TOC benchmark, the capital and operations and maintenance (O&M) costs may be unattractive and even prohibitive in some applications. Previous studies have shown that ozone-biofiltration systems are less costly and energy intensive but often achieve TOC removals of only 15-30%. This hinders compliance with the CDDW TOC requirement unless significant blending ratios are achieved. However, this issue may be overcome by optimizing operational conditions (e.g., ozone dose and empty bed contact time) or

by developing an alternative regulatory framework for bulk organic matter. As with conventional drinking water, the formation of disinfection byproducts (DBPs) is also a concern for potable reuse applications. When free chlorine is applied as a final disinfectant (e.g., in direct potable reuse applications), trihalomethanes (THMs) and haloacetic acids (HAAs), among other regulated and unregulated disinfection byproducts, are formed. The U.S. Environmental Protection Agency regulates four THMs (i.e., total THMs or TTHMs) and five haloacetic acids (i.e., the HAA5s) in drinking water at 80 and 60 μg/L, respectively.

The purpose of this research was to investigate the impacts of ozone dose and empty bed contact time (EBCT) on DBP formation in potable reuse applications, as well as to evaluate the possibility of using DBP formation potential as an alternative regulatory framework for TOC removal. A pilot-scale ozone-biofiltration system was operated with ozone/TOC ratios ranging from 0.1-2.5 and EBCTs ranging from 1-20 minutes. The biofiltration columns contained anthracite or biological activated carbon (BAC). Bench-scale chlorination was performed using the uniform formation conditions (UFC) approach, and quenched samples were analyzed for TTHMs and HAA5s. The data demonstrated that ozone-biofiltration achieved TOC removals ranging from ~15-30%, depending on operational conditions, but biofiltration without ozone consistently achieved <10% TOC removal. UFC testing demonstrated that ozone alone was efficient in transforming bulk organic matter and reducing DBP formation by ~10-30%. Ozone-biofiltration was able to reduce TTHM formation by ~20-35% and HAA5 formation by ~40-55%. Maximum TOC concentrations of 3.3 mg/L and 6.0 mg/L were identified as treatment targets for compliance with the U.S. EPA's TTHM and HAA5 regulations. Finally, microbial community characterization through sequencing of 16s rDNA indicated that *Bradyrhizobium* was the dominant genus in media samples collected from three biofilters. Minimal differences were

observed between columns containing BAC receiving non-ozonated vs. ozonated effluent, indicating that preozonation did not interfere on microbial community. According to PAC analysis, there was significant difference from anthracite and BAC samples, suggesting that origin of media used in this current study might have contributed to difference in microbial community.

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AOC - assimilable organic carbon

AOP - advanced oxidation process

ATP - adenosine triphosphate

AWPF - Advanced Water Purification Facility

BAC - biological activated carbon

BAP - biomass-associated products

BCAA - Bromochloroacetic acid

BDOC - biodegradable dissolved organic carbon

BOD - biochemical oxygen demand

CDDW - California Department of Drinking Water

CDPH - California Department of Public Heath

CLPP - community-level physiological profiles

COD - chemical oxygen demand

DBAA - dibromoacetic acid

DBP - disinfection byproduct

DCAA - dichloroacetic acid

DHAA - dihalogenated haloacetic acid

DNA - deoxyribonucleic acid

DOC - dissolved organic carbon

DON - dissolved organic nitrogen

DPR - direct potable reuse

EBCT - empty bed contact time

EDC - endocrine disrupter compounds

EEM - excitation emission matrix

EfOM - effluent organic matter

ESB - engineered storage buffer

FAT - full advanced treatment

FP - formation potential

GWRS - Groundwater Replenishment System

GAC - granular activated carbon

HAA - haloacetic acid

HAN - haloacetonitriles

HLR - hydraulic loading rate

HNM - halonitromethane

IOD - instantaneous ozone demand

IPR - indirect potable reuse

LRV - log removal value

MBAA - bromoacetic acid

MBR - membrane bioreactor

MCAA - chloroacetic acid

MCL - maximum contaminant level

MF - microfiltration

NOM - natural organic matter

NCBI - National Center of Biotechnology Information

NDMA - *N*-nitrosodimethylamine

NF - nanofiltration

NIWR - National Institute of Water Research

NPOC - non-purgeable organic carbon

NWRI - National Water Research Institute

OCSD - Orange County Sanitation District

OCWD - Orange County Water District

•OH - hydroxyl radicals

O&M - operations and maintenance

OTU - operational taxonomic unit

PAC - powdered activated carbon

PCA - principal component analysis

PCBs - polychlorinated byphenyls

PPCP - pharmaceutical and personal care products

QIIME - Quantitative Insights Into Microbial Ecology

RO - reverse osmosis

rRNA - ribossomal ribonucleic acid

SAT - soil aquifer treatment

SDS - simulated distribution system

SEC-OCD - size exclusion chromatography with online carbon detection

SMP - soluble microbial products

SNWA - Southern Nevada Water Authority

SUVA - specific ultraviolet light absorbance

TCAA - trichloroacetic acid

TDS - total dissolved solids

TOC - total organic carbon

TOrC - trace organic compound

TOX - total organic halide

TTHMs - total trihalomethanes

UAP - utilization-associated products

UF - ultrafiltration

UFC - uniform formation conditions

USEPA - United States Environmental Protection Agency

UV - ultraviolet light

Chapter 1 – Introduction

Climate change and population growth are major contributors to water shortages and compromised drinking water supplies, especially in semi-arid regions. According to the Southern Nevada Water Authority, the level of Lake Mead, the main source of drinking water for Southern Nevada, has decreased more than 130 feet since 2000 (SNWA, 2015). Under these types of conditions, the beneficial use of treated wastewater (i.e., water reuse) has become a critically important practice. In particular, projects incorporating surface water augmentation or groundwater replenishment with recycled water—commonly described as indirect potable reuse (IPR)—have overcome historical public perception issues and have been successfully implemented throughout the world (Gerrity et al., 2013) . However, research to improve the safety, reliability, and sustainability of advanced wastewater treatment technologies is still necessary for even wider adoption of potable reuse. This is particularly important for direct potable reuse (DPR) applications, which involve injecting advanced treated wastewater directly into drinking water distribution systems or blending either upstream or downstream of conventional drinking water treatment plants.

At this time, the U.S. Environmental Protection Agency (USEPA) has not yet established regulations specifically for potable reuse. As a result, the Clean Water Act, the Safe Drinking Water Act, and state-specific requirements (when applicable) have to be considered when designing potable reuse systems. Among existing state-level regulations (e.g., Florida, Nevada, Washington), the California Department of Drinking Water (CDDW) has established the most conservative requirements for groundwater recharge applications. One of the key requirements of these 'Title 22' regulations is a maximum of 0.5 mg/L of wastewater-derived total organic carbon (TOC) in water supplies impacted by recycled water. TOC is a parameter representative

of the bulk concentration of known and unknown organic chemicals in the water. This requirement appears to be very distinctive from typical TOC concentrations in surface water supplies, which is approximately 3 mg/L (Trussell et al., 2013).

Currently, reverse osmosis (RO) followed by advanced oxidation [e.g., ultraviolet light disinfection with peroxide (H_2O_2) addition], a treatment train recently defined as 'full advanced treatment' (FAT), is generally the only option capable of achieving TOC concentrations less than 0.5 mg/L in the effluent. This treatment train is required in potable reuse systems in California that employ surface water augmentation or direct injection of recycled water into local aquifers. For groundwater replenishment via spreading, agencies can use alternative treatment trains but must rely on high blending ratios to achieve the TOC benchmark. Although FAT is very efficient in reducing many contaminants, including TOC, the associated costs, elevated energy demand, and need for concentrate management may be prohibitive for many agencies (Tchobanoglous et al., 2015; Gerrity et al., 2014). On the other hand, Schimmoller et al. (2015) demonstrated that the 'triple-bottom-line' costs – which involve financial, social, and environmental elements – for alternative treatment trains employing ozone and biofiltration are significantly lower. Ozone-biofiltration have been employed in drinking water treatment due to its effect in transforming natural organic matter (NOM) into more bioamenable compounds, which enhances biodegradation in subsequent biofiltration process (Hozalski et al., 1999). Other studies have demonstrated that ozone-biofiltration is also very efficient in transforming and/or removing TOC and other trace organic compounds of interest from wastewater (TOrCs) (Selvy, 2015; Gerrity et al, 2014; Wert et al., 2009b; Gerrity et al, 2011).

Disinfection is also an important consideration when designing and implementing potable reuse systems. Though this process is essential to inactivate pathogens responsible for

waterborne diseases, the reactions between disinfectants (e.g., chlorine, chloramines, ozone, and chlorine dioxide) and the combination of organic (i.e., TOC) and inorganic (i.e., bromide, iodine) compounds present in treated wastewater are responsible for the formation of toxic disinfection byproducts (DBPs), such as trihalomethanes (THMs), haloacetic acids (HAAs), bromate, *N*-nitrosodimethylamine (NDMA), and other emerging DBPs (Richardson, 2003). After several toxicological studies conducted in laboratory animals demonstrated that DBP exposure causes damage to blood and kidneys (e.g., cancer), the USEPA regulated four THMs (chloroform, bromochloromethane, dibromochloromethane, and bromoform; collectively known as TTHMs) and five HAAs (monochloroacetic acid [MCAA], dichloroacetic acid [DCAA], trichloroacetic acid [TCAA], monobromoacetic acid [MBAA], and dibromoacetic acid [DBAA]; collectively known as HAA5s) at 80 µg/l and 60 µg/l, respectively. Bromate is also regulated in drinking water at 10 µg/l, but NDMA is only regulated at the state level (e.g., 10 ng/L in California), although it is listed on the USEPA's Contaminant Candidate List.

As previous studies have shown, ozone-biofiltration has the potential to transform and remove a significant portion of the bulk organic matter (i.e. TOC) (Hollender et al., 2009; Ratpukdi et al., 2010), one can hypothesize that this treatment combination will also reduce DBP formation upon final chlorination, as would be necessary in DPR applications similarly to disinfection of drinking water. Despite potential differences in origin of organic matter present in drinking water sources (i.e., originated from degradation and leaching of organic debris within the watershed), and in conventional treated wastewater (i.e., biorefractory natural organic matter in addition to autochthonous material originated from microbial activity during biological treatment (i.e., soluble microbial products)), ozonation has shown to be effective in mitigating DBP formation in both applications upon final disinfection (Xu et al., 2007; Farré et al., 2011;

Chu et al., 2012). However, further investigation is needed to determine appropriate operational parameters [e.g., ozone dose and empty bed contact time (EBCT)] for meeting regulatory guidelines and controlling DBP formation. As such, the primary objectives of this research are to (1) characterize the relationship between ozone dose, EBCT, TOC removal, and chlorinated DBP (i.e., THM and HAA) formation potential and (2) propose an alternative framework for TOC removal in potable reuse applications that is based on specific public health criteria. Ultimately, the required level of TOC reduction in potable reuse applications could be based on DBP compliance, which is consistent with the Safe Drinking Water Act, rather than more arbitrary TOC targets (i.e., 0.5 mg/L of wastewater-derived TOC). The USEPA's Stage 1 Disinfectant and Disinfection Byproducts Rule specifies required TOC reductions during drinking water treatment based on source water TOC and alkalinity. The hypothesis of the current research is that a similar framework could be applied to potable reuse systems, which would allow for broader acceptance and implementation of ozone-biofiltration systems.

This thesis includes a literature review followed by three independent chapters focused on (1) variables affecting TOC removal in ozone-biofiltration systems, (2) variables affecting DBP formation upon final chlorination in ozone-biofiltration systems, and (3) an assessment of microbial community structure in biofilter systems under different conditions. The specific research questions and hypotheses to be addressed in these chapters is as follows:

1. TOC removal in ozone-biofiltration systems:
    a. Research question: do higher ozone doses and higher EBCTs promote higher TOC removals in ozone-biofiltration systems?
    b. Hypotheses: as ozonation can lead to the transformation of bulk organics into more bioamenable hydrophilic compounds (Hollender et al., 2009;

Snyder et al., 2014), higher ozone doses would increase the concentration of biodegradable organic carbon, which can be completely degraded in subsequent biofiltration process. Longer EBCTs allow more time for water to be in contact with the microbial community on the biofilter, which could possibly enhance TOC reduction. Thus, the hypotheses are that higher ozone doses and higher EBCTs would lead to higher reduction of TOC concentration.

2. DBP formation in ozone biofiltration systems

    a. Research question 1: do different parameters (i.e., ozone doses and EBCTs) influence DBP mitigation/formation (i.e., TTHMs and HAA5s) upon final free-chlorine disinfection?

    Hypothesis 1: as studies have revealed that main precursors of TTHMs and HAA5s are related high molecular weight and hydrophobic moieties (Xu et al., 2011; Snyder et al., 2014), ozonation followed by biofiltration would be effective in mitigation of aforementioned regulated DBPs, as ozonation is very effective in transforming hydrophobic compounds into more hydrophilic moieties with low molecular weight.

    b. Research question 2: based on DBP formation, what TOC removal is necessary to achieve compliance with U.S. EPA maximum contaminant levels in drinking water for TTHMs (80μg/L) and HAA5s (60μg/L)? Is the 0.5 mg/L TOC-benchmark established by CDDW the maximum TOC concentration necessary achieve DBP compliance with regulated DBPs? Comparing final DBP formation results in advanced treated wastewater

employing ozone-biofiltration with DBP formation in drinking water presented in the literature, is it possible to adopt an alternative framework for TOC removal in direct potable reuse applications similar to what is delimited in drinking water applications (i.e., USEPA's Stage 1 Disinfectant and Disinfection Byproducts Rule)?

Hypothesis 2: as typical TOC concentrations in most U.S. drinking waters are approximately 3 mg/L, TOC concentrations as low as 0.5 mg/L established by CDDW are not crucial to achieve compliance with DBP MCLs.

3. Microbial community structure in ozone biofiltration systems
    a. Research question: what are the microbial community characteristics in biofiltration during advanced treatment with pre-ozonation? does microbial community change in biofilters in terms of media type, pre-oxidation process, as well as depth of biofilters?

    Hypothesis: As pilot-scale non-ozonated biofilter was acclimated and fed with membrane bioreactor effluent from wastewater treatment, it is expected a similar microbial community to that present in wastewater biological treatment. As ozone is a powerful disinfectant, it would contribute to selection of specific microbes in ozonated biofilters.

Chapter 2 - Background and Literature Review

2.1 – Potable Reuse

Potable reuse consists of using treated wastewater as a direct drinking water supply (i.e., direct potable reuse or DPR) or for augmentation of source water supplies (i.e., indirect potable reuse or IPR). Unplanned IPR, also referred to as *de facto* reuse, has been unintentionally implemented for decades. It consists of the discharge of conventionally treated wastewater by community 'A', which is located upstream of the drinking water supply of community 'B' (Figure 1**)**. The Mississippi River is an example of unplanned IPR, with more than 10 states simultaneously discharging wastewater and withdrawing raw water for treatment (Gerrity et al., 2013). Planned potable reuse, including both IPR and DPR, has become more common in recent years and involves the beneficial use of treated wastewater as a reliable drinking water supply within the same community (Rodriguez et al., 2009). The Montebello Forebay groundwater replenishment project located in Los Angeles County, California, has been in operation for over 50 years and is one of the pioneers in adopting planned potable reuse (Khan, 2015). In fact, Los Angeles County was one of the first communities to have its major water supply deliberately replenished by municipal wastewater (Khan, 2015). Other planned potable reuse projects are also active in Namibia, Australia, and many other parts of the U.S., such as California, Texas, Nevada, Virginia, Georgia, and Florida (van Leeuwen et al., 2003; Rodriguez et al., 2009; Gerrity et al., 2013; Khan, 2015).

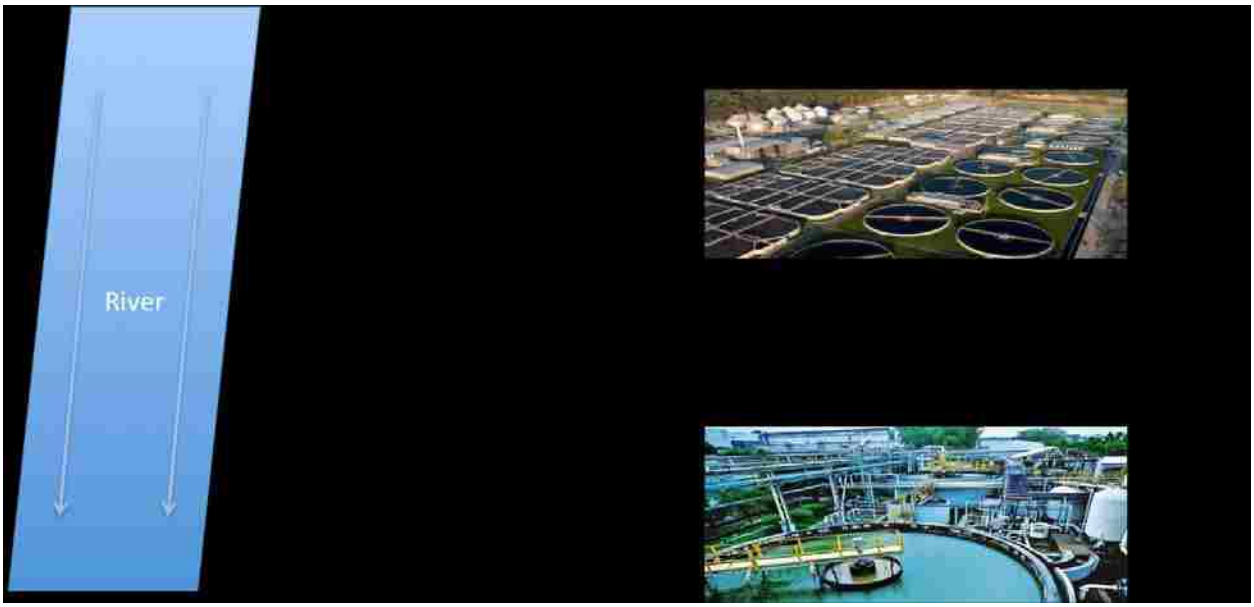

Figure 1- Unplanned/De facto reuse schematic

As mentioned above, planned potable reuse can happen in two ways: direct and indirect potable reuse. IPR involves the deliberate augmentation of a community's water supply with treated wastewater, in which the receiving surface water or aquifer is utilized as an environmental buffer. After conventional wastewater treatment (e.g., primary sedimentation, biological treatment, secondary sedimentation, filtration, disinfection), the effluent can be further purified with innovative and/or advanced treatment processes such as membrane filtration, ozonation, and biofiltration before it is discharged to the receiving water body. The aforementioned Montebello Forebay project is an example of an IPR project, where conventionally treated wastewater is used for groundwater replenishment as well as a seawater intrusion barrier. Another example is the Groundwater Replenishment System (GWRS) in Orange County, California, where conventionally treated wastewater from the Orange County Sanitation District (OCSD) is further treated at the Orange County Water District's (OCWD)

Advanced Water Purification Facility (AWPF) with microfiltration (MF), reverse osmosis (RO), and an advanced oxidation process (AOP; UV/H_2O_2). In the GWRS, the advanced treated wastewater is either sent to spreading grounds or directly injected into the local aquifer for groundwater replenishment and for the formation of a seawater intrusion barrier. Particularly in cases like the GWRS in which the wastewater is purified to extremely high standards, discharge to an environmental buffer may actually decrease the quality of the water due to exposure to natural or anthropogenic contaminants in the environment (Leverenz et al., 2011).

DPR consists of introducing advanced treated wastewater directly into a drinking water distribution system, or blending it either upstream or downstream of a conventional drinking water treatment plant. In DPR applications, it is recommended that the final product water be retained in an engineered storage buffer (ESB) to allow sufficient time to guarantee water quality compliance before directing it for public consumption. Compared with some IPR systems, DPR may reduce the logistical complexity associated with recovering the treated water for drinking water purposes. In other words, IPR systems may accrue significant capital and operational costs associated with pumping the water to and from the environmental buffer. DPR systems have the potential to eliminate the environmental buffer and possibly reduce overall costs, or allow for redirecting budgets toward treatment upgrades. DPR is historically uncommon, but the city of Windhoek, which is the capital of Namibia, has been one of the pioneers of this practice (Haarhoff and Van der Merwe, 1996; du Pisani, 2006). Prior to the implementation of DPR in Windhoek in 1968, a four-year study was performed by the National Institute of Water Research (NIWR) in South Africa to ensure that the city would not suffer health impacts from using treated wastewater as a drinking water supply (Haarhoff and Van der Merwe, 1996). Considering the success of this system (Haarhoff and Van der Merwe, 1996; du Pisani, 2006), Windhoek

serves as an example that DPR can be a sustainable and reliable alternative to conventional drinking water supplies.

Despite the success of numerous benchmark systems, there are still certain factors, such as public acceptance, financial constraints, and restrictive or nonexistent regulatory frameworks, that can hinder implementation of potable reuse. However, with respect to public health, there is no evidence to suggest that planned IPR or DPR cause appreciable increases in public health risks compared to conventional drinking water systems (Sloss et al., 1996; Rodriguez et al., 2009). In fact, some studies indicate that planned IPR or DPR may actually result in decreased public health risks due to the advanced treatment and expanded water quality monitoring efforts typically employed in these systems (National Research Council, 2012). Table 1 summarizes the diverse potable reuse projects currently in operation throughout the world.

Table 1 - Potable Reuse Projects

Project	Location	Potable Reuse Application	Treatment Train	Reference
Montebello Forebay Groundwater Project	Los Angeles, CA	IPR – Groundwater Recharge	Secondary treatment, chloramination, and spreading (i.e., soil aquifer treatment)	Sloss et al, 1996
Groundwater Replenishment System	Orange County, CA	IPR – Groundwater Recharge	Secondary treatment, microfiltration, reverse osmosis, UV/H_2O_2, and spreading or direct injection	GWRS
Gwinnett County Department of Public Utilities	Lawrenceville, GA	IPR – Surface Water Augmentation	Secondary treatment, pre-ozone, biological activated carbon, post-ozone	Gerrity et al., 2013
Upper Occoquan Service Authority (UOSA)	Fairfax County, VA	IPR – Surface Water Augmentation	Secondary treatment, lime clarification, two-stage recarbonation, sand filtration, granular activated carbon, ion exchange, post carbon filtration, chlorination, dechlorination	Rodriguez et al., 2009
Hueco Bolson Recharge Project –Fred Harvey Water Reclamation Plant	El Paso, TX	IPR – Surface Water Augmentation	Secondary treatment, ozonation, granular activated carbon, chlorination, storage	Gerrity et al., 2013
Village of Cloudcroft Advanced Treatment	Cloudcroft, NM	DPR – Drinking Water Distribution System	Secondary treatment, RO/ UV-peroxide, blending with raw water, storage, ultrafiltration, UV disinfection, granular activated carbon, Disinfection	Tchobanoglous et al., 2011
Big Spring Water Reclamation Plant	Big Spring, TX	DPR – Drinking Water Distribution System	MF,RO, UV-peroxide, blended with raw water upstream drinking water treatment plant	Tchobanoglous et al., 2011
Water Reclamation Plant at South Caboolture	Queensland, Australia	IPR – Surface Water Augmentation	Biological denitrification/ preozonation/ coagulation/flocculation/ dissolved air-flotation/sand filtration, ozone/BAC	Van Leeuwen et al., 2003
Goreangab Reclamation Plant	Windhoek, Namibia	DPR – Drinking Water Distribution System	Pre-ozonation/ Dissolved air Flotation/ Sand filtration/ Ozonation Granular activated Carbon/ Ultrafiltration/ Chlorination	Haarhoff and Van der Merwe, 1996; du Pisani, 2006

Potable reuse systems normally incorporate multi-barrier advanced treatment in order to guarantee reliability (i.e., ability to provide water that always meets or exceeds the public health

protection) with redundancy (i.e., the use of measures outside minimum requirements to ensure that treatment objectives are met), robustness (i.e., the ability to address an extensive variety of contaminants and to resist failures), and resiliency (i.e., ability to treatment train effectively adapt to failure) (Pecson et al., 2015). Microbial inactivation plays a role in selecting the treatment process and in designing a reliable multi-barrier advanced treatment train. The following section will focus on inactivation of critical pathogens in potable reuse applications.

2.1.1 - Microbial Inactivation in Potable Reuse

Inactivation of microbial pathogens is essential prior to distributing recycled water in potable reuse applications. The primary target pathogens are typically *Cryptosporidium*, *Giardia*, and enteric viruses. *Cryptosporidium* and *Giardia* are small protozoan parasites that are hard to remove from water due to their small size and resistance to disinfection when present in (oo)cyst form. They are easily removed by exclusion filtration (e.g., microfiltration, ultrafiltration, nanofiltration and RO). Because these aforementioned protozoa are most resistant to different types of disinfection, their inactivation may guarantee inactivation of the bacteria. Viruses are microscopic parasites smaller than bacteria and they lack the capacity to reproduce outside of a host (e.g., bacteria). They can be inactivated by primary disinfectants such as free chlorine, UV radiation and ozone. Some pathogenic viruses can be found in water supplies (e.g., adenovirus) and if ingested, it can cause gastroenteritis, conjunctivitis, and respiratory diseases (Kuo et al., 2010).

Microbial inactivation is typically described in terms of a log removal value (LRV), which is calculated by taking the log of the ratio of influent and effluent pathogen concentrations. The California “Title 22” regulations for water reuse mandates LRVs of 12-10-10 for viruses, C*ryptosporidium*, and *Giardia*, respectively, as well as a target total coliform

concentration of less than 2.2 most probable number (MPN) per 100 mL (i.e., method based in serial dilution tests and useful for estimating low concentrations of organisms). Australia has regulated the same three pathogens with required LRVs 9.5-8-8, respectively, (EPHC, 2008). A panel of public health experts organized by the National Water Research Institute (NWRI) in the U.S. indicated that an additional 9-log inactivation of total coliform bacteria is warranted to ensure adequate bacteriological water quality (Crook et al., 2013). These LRVs can be demonstrated with engineered treatment processes, such as ozonation, chlorination, UV disinfection, and/or membrane filtration, or in the environmental buffer in IPR applications. For example, a 1-log virus inactivation credit is awarded by California per month of aquifer storage time, and a 10-log inactivation credit is awarded for *Cryptosporidium* and *Giardia* assuming the agency provides adequate disinfection to achieve 5-log viral inactivation (e.g., a chlorine CT values of at least 450 mg-min/L), <2.2 MPN/100 mL of total coliform bacteria, and at least 6 months of aquifer storage time (CDPH, 2014). The aforementioned NWRI panel also indicated that demonstrating compliance with the *Cryptosporidium* requirement would presumably satisfy the *Giardia* requirement as well, considering *Cryptosporidium* is more difficult to treat due to its smaller size and greater resistance to disinfection (Crook et al., 2013).

In order to provide proper treatment and inactivation of microorganisms, a multi-barrier treatment train is essential. Table 2 provides a summary of estimated LRVs for treatment processes typically incorporated into potable reuse systems (Trussell et al., 2016).

Table 2 - Expected LRV for different microbial pathogens in potable reuse treatment trains

Unit Process	Expected Log Removal Value		
	Enteric viruses	***Cryptosporidium***	**Total coliform bacteria**
Conventional Activated Sludge (CAS)	1	0	2
Microfiltration (MF)	0	4	4
Ultrafiltration (UF)	1	4	4
Reverse Osmosis (RO)	2	2	2
Biological Activated Carbon Filtration (BAC)	0	0	0
Ozone	6	1	4
UV	6	6	6
UV/H_2O_2	6	6	6
Free Chlorine	6	0	4

Table adapted from Trussell et al., 2016

2.2 – Emerging Treatment Processes for Potable reuse

A common advanced treatment train that often meets the aforementioned standards reliability involves membrane filtration (MF), reverse osmosis (RO), and advanced oxidation (UV-H_2O_2). However, due to the high costs associated with these technologies (e.g., high energy demand, brine disposal management), more sustainable alternative advanced treatment technologies are desired. Ozonation followed by biofiltration has shown to be a potential emerging alternative treatment process in potable reuse applications and it will be discussed in the following section. As disinfection is a critical process in inactivating waterborne diseases in drinking water treatment, it also poses a very important step in potable reuse applications (i.e,., specially DPR). Disinfection processes (i.e., chlorine, chloramine, ozonation) and its implications (e.g., formation of disinfection by-products) will also be discussed in further sections.

2.2.1 - Ozonation

Ozone is an unstable gas formed by three oxygen molecules and acts as powerful oxidant and disinfectant for the inactivation of viruses, bacteria, and protozoa (Rakness et al., 1993; Gerrity and Snyder, 2011). Its oxidative capability is efficient for transforming larger biorefractory organic matter (e.g., phenols, anilines, alkoxy- and alkylbenzenes, olefins, and deprotonated amines) into smaller oxygen-rich compounds (Linlin et al., 2011; Farré et al., 2011; Li et al., 2017). Ozone is capable of breaking carbon-carbon chains and converting recalcitrant organic matter into biodegradable dissolved organic carbon (BDOC) or assimilable organic carbon (AOC). Where AOC represents the more readily biodegradable fraction of TOC and BDOC represents both mineralized and assimilable organic carbon within TOC (Escobar and Randall, 2001). UV absorbance and specific UV absorbance (SUVA), particularly at a wavelength of 254 nm, are parameters known to indicate aromatic carbon content (Weishaar et al., 2003). Wert et al. (2009a) observed that as ozone dose increased, both UV absorbance and SUVA decreased, demonstrating that ozonation is capable of transforming aromatic carbon content into simpler moieties (i.e., carboxylic acids, aldehydes, and ketones). Other studies expanded on this concept and demonstrated the utility of using changes in surrogate parameters like UV_{254} absorbance to predict or verify the performance of ozone systems (Wert et al., 2009a). In disinfection, ozone is capable of damaging bacterial cells and viral capsid sites, thus releasing genetic material (i.e., RNA, DNA) (Rakness et al., 1993). Ozone has been shown to be a stronger disinfectant able to inactivate viruses and organisms resistant to other disinfectants (i.e., chlorine, chloramine), such as *Cryptosporidium* oocysts and *Giardia* cysts. Although ozone is a powerful disinfectant, it does not provide a sufficiently stable residual to prevent microbial regrowth in

distribution systems. In fact, the generation of BDOC and AOC during ozonation can actually promote regrowth in distribution systems (Van der Kooij et al., 1989; Hammes et al., 2007).

Ozonation of wastewater has been identified as a second order biphasic process, in which the initial reactions in the first 30 seconds can be described as the instantaneous ozone demand (IOD) phase and the subsequent reactions can be described as the decay phase (Wert et al., 2009b). During the first phase, ozone is rapidly consumed by reactions with bulk organic matter and nitrite. Nitrite reacts rapidly with ozone according to a mass ratio of 1.1 mg O_3/mg NO_2 (Wert et al., 2009b). Although it is a second order process, the IOD can either be described as a pseudo first order process due to the relatively constant concentration of reactive bulk organic matter or even an immediate reduction in dissolved ozone residual, hence the 'instantaneous' designation. Although the subsequent phase is second order as well, the decay is also often described as a pseudo first order reaction.

During the demand and decay phases, ozone decomposes into hydroxyl radicals (•OH), O_2, and OH^-. The formation of •OH has been attributed to decomposition of ozone during reactions with specific organic moeities, including amines, phenols, and alkoxylated aromatics (Nöthe et al., 2009). The combination of •OH and molecular ozone is particularly effective for the oxidation of a wide range of TOrCs, including endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs). The efficiency of ozone for TOrC oxidation is directly related to the applied ozone dose, the ozone and •OH scavengers present in the water matrix, and the second order rate constants describing the reactions between ozone or •OH and the target compounds (Nöthe et al., 2009). Although some compounds react slowly with ozone ($k_{O3} < 10\ M^{-1}s^{-1}$), oxidation may still be favorable through •OH pathways because •OH is less selective and reacts rapidly with many organic and inorganic compounds. Because of

the complexity of the various reactions involved during ozone oxidation and the variability between water matrices, particularly in wastewater applications, the O_3/TOC ratio, which standardizes the ozone dose to the bulk organic matter content of the water matrix, is often used to predict the performance of the treatment process (Lee et al., 2013). In other words, the same O_3/TOC ratio will achieve similar bulk organic matter transformation and TOrC attenuation in diverse wastewater qualities (Lee et al., 2013).

Wert et al. (2009b) studied the oxidation of 31 TOrCs during ozonation of three different tertiary wastewater effluents. Results demonstrated 20-90% attenuation of ozone-susceptible compounds ($k_{O3} > 10^5\ M^{-1}s^{-1}$), such as carbamazepine, diclofenac, naproxen, sulfamethoxazole, and triclosan, with O_3 doses as low as ~2 mg/L (O_3/TOC = 0.2). The same was observed by Hollender et al. (2009) with O_3/TOC of 0.36. Ozone-resistant compounds ($k_{O3} < 10\ M^{-1}s^{-1}$) that are susceptible to radical oxidation ($k_{\bullet OH} > 10^9\ M^{-1}s^{-1}$), such as diazepam, atrazine, and ibuprofen, were oxidized only at higher ozone doses (~6 mg/L; O_3/TOC = 1). Similar results were also observed by Gerrity et al. (2014) with an O_3/TOC of 1.5 This demonstrates that ozone can be effective for some treatment goals even at lower doses.

Beyond disinfection and TOrC oxidation, ozone is also gaining popularity for emerging applications [e.g., reduction of organic fouling on microfiltration membranes (Stanford et al., 2011)], particularly because it is an efficient and cost-effective treatment option (Tchobanoglous et al., 2015; Schimmoller et al., 2015). However, ozone implementation is also hindered by a number of issues. Notably, the use of ozone in some water matrices may lead to the formation of carcinogenic DBPs, such as *N*-nitrosodimethylamine (NDMA) (Hollender et al., 2009; Marti et al., 2015) and bromate (Hollender et al., 2009; Li et al., 2017; Gerrity et al., 2011b) (both further discussed on section 2.4.1). Also, because ozone decomposes quickly, it cannot provide a

persistent disinfectant residual in distribution systems. Thus, other types of disinfectants (e.g., chlorine and chloramines) may also have to be used when the final product water is intended for potable uses. Finally, ozone-based treatment trains are unable to reduce concentrations of total dissolved solids (TDS), which is one of the reasons RO is popular in potable reuse applications.

2.2.2 - Biofiltration

Biofiltration consists of the use of media providing surface area for biological attachment and growth (i.e., a biofilm). The media acts as a filter to remove particulates and suspended solids, whereas the attached biofilm consumes biodegradable organic matter [i.e., biodegradable dissolved organic carbon (BDOC)] by facilitating oxidation-reduction reactions. In the U.S., granular media filtration started as early as 1872, with the primary objective being the removal of particulates from drinking water. Historically, granular media filters in conventional drinking water applications have been dosed with residual disinfectant to hinder biological growth. However, due to the presence of assimilable organic carbon (AOC) in finished drinking water, bacterial regrowth in distribution systems is sometimes a significant problem, particularly in systems employing ozonation (Van der Kooij et al., 1989). As a result, some drinking water systems have started to adopt biofiltration (Schneider and LeChevallier, 2017) to remove organics that might result in the formation of disinfection byproducts upon final disinfection (Chu et al., 2012; Basu et al., 2015) or promote bacterial regrowth in distribution systems (Page et al., 2006).

Oxidation, particularly via ozonation, has been shown to enhance biofiltration treatment efficacy (discussed further in section 2.3). This is because ozonation is effective in converting recalcitrant organic matter into biodegradable dissolved organic carbon (BDOC) or assimilable organic carbon (AOC) (Hollender et al., 2009). In particular, biofilters can attenuate potentially

toxic transformation products formed after ozonation (Hollander et al., 2009; Stalter et al., 2010) and can also achieve significant removal of biodegradable trace organic compounds (TOrCs), disinfection byproducts (DBPs), and DBP precursors (Reungoat et al. 2011; Farré et al. 2011; Reaume et al., 2015). The removal of bulk and trace organics during biofiltration is dependent on a variety of factors related to biofilm growth and activity, including nutrient loading, dissolved oxygen, and pH levels (Lazarova and Manen, 1995; Wang et al., 2008). Other design parameters governing efficacy of the system include the type of granular media, empty bed contact time (EBCT), hydraulic loading rate (HLR), and backwashing conditions.

Biofilters are often single or dual-media. The most common biofiltration applications include biological activated carbon (BAC), anthracite and/or sand filtration, riverbank filtration, and soil aquifer treatment (SAT) (Reungoat et al., 2011; Mckie et al., 2011). In these biofilters, there is often a layer of media near the surface where treatment/contaminant removal is most efficient. This rapidly forming layer is known as the *schmutzdecke* (Page et al., 2006). Studies of different media [e.g., anthracite, granular activated carbon (GAC)] have demonstrated high levels of microbial activity in this upper zone of the biofilter (Gibert et al., 2013; Selvy, 2015). Performance in the deeper layers the biofilter is dependent on residual organic concentrations and oxic conditions, which are partially controlled by EBCT. The EBCT is an estimate of the time in which the wastewater is in contact with the biofilm attached to the media. It essentially represents the theoretical hydraulic retention time of an empty system with the same dimensions as the biofilter and is calculated by dividing the total volume of the filter bed by the flow rate. In other words, the EBCT neglects the effects of the media on flow paths and residence time. In water treatment studies involving biofiltration, EBCT is a useful design parameter and is frequently used for comparing biofilter performance (Reaume et al. 2015; Basu et al. 2015).

Studies have observed an increase in organic matter removal as EBCT in the biofilter increased (Lechevallier, 1992; Reungoat et al., 2011; Trussell et al., 2016). Typical values for EBCT in biofiltration systems range from 9 to 45 minutes (Haarhoff and Van der Merwe., 1996; Reungoat et al., 2012; Gerrity et a., 2013). The HLR, also known as superficial velocity through the filter (units of m^3/m^2-h or m/h), is calculated by dividing the flow rate by the surface area of the top of the filter (Crittenden et al. 2005). Typical values for HLR range from 0.5 to 8 m/h (Lautenschlager et al., 2014; Zhang et al., 2010; Knopp et al., 2016).

Because filtered solids and biofilm growth accumulate over time, biofilters can only be operated for a certain period of time before maintenance is required (i.e., the filter run time). The accumulation leads to increased head loss in the biofilter, thereby hindering water flow or requiring greater pumping rates. Therefore, backwash cycles are necessary to restore the filter to its original condition at the beginning of the filter run. For the backwash procedure, clean water (and/or air) flushes back through the filter at a high rate to remove attached solids. The backwash flow rate must be great enough to push excessive solids out from the filter, but not so great that media is lifted out of the filter column or excessive biomass is detached from the media (in a biofiltration system) (Crittenden et al., 2005; Basu et al., 2015). In most water and wastewater treatment plants, backwashes occur multiple times per week. Backwash frequency can be determined based on time to breakthrough and/or accumulation of excessive head loss (Simpson, 2008). A target level of biological growth can be maintained by varying nutrient loading, dissolved oxygen concentrations in the influent, pH levels, and backwash frequency. Hydraulic bumps are also very effective in biofilters in order to remove gas binding accumulation. It consists of backwashes of short duration that are sufficient to remove air accumulation in the biofilters. This process is essential when adopting pre-ozonation process. As the final product after ozonation is oxygen, gas binding

accumulation in the filter media may hinder water flow through the filter and consequently decrease the filtration efficiency. Trussell et al. (2016) found that performing a hydraulic bump every 4 hours was sufficient to avoid gas binding in the biofilters following the ozonation process. When excessive microbial growth is not desired in the biofilters, longer backwash cycles with a higher flow rate maybe used to remove biomass attached to filter media.

2.2.2.1 – Anthracite

Anthracite is described as "hard coal" because it has a high carbon content and the least amount of volatile matter compared to other types of coal (Crittenden et al., 2005). Anthracite is often used as a medium in biofiltration due to its large effective size (i.e., 0.8-2.0 mm), which serves as an effective surface for biological community attachment and growth, and its relatively low uniformity coefficient (i.e., 1.3-1.7), which minimizes stratification following backwashes. Yang et al. (2011) determined the specific surface area of anthracite to be approximately 250 m^2/g. Anthracite does not offer significant adsorption capacity, but it is effective for the removal of fine suspended solids and offers a surface for biofilm development to promote biodegradation. It is often used in dual-media filter applications in combination with sand.

2.2.2.2 – Activated Carbon

Activated carbon is a common adsorbent and it is manufactured from natural carbonaceous material, such as coal, peat, and coconuts by several processes such as high temperatures (i.e., 800°C) and steam. Activated carbon is manufactured with lower particle sizes (i.e., 20-50μm), referred to as powdered activated carbon (PAC) and higher particle sizes, referred as granular activated carbon (GAC). Virgin GAC is known for its complex pore structure, which is generally effective for the adsorption of organic and even some inorganic contaminants. GAC has an effective size ranging from 0.55-0.75 mm, a density of 450 g/L, and a

uniformity coefficient of <1.9 (Xu et al., 2007; Chu et al., 2012). Most importantly, one gram of GAC is able to provide a surface area of about 600 -1200 m^2 (Crittenden et al., 2005; Simpson 2008; Gilbert et al., 2013; Knopp et al., 2016), thereby offering an abundance of adsorption sites. GAC is often packed in a bed column where water flows through, whereas PAC can be directly applied to the water and it is usually removed by sedimentation or filtration. Both forms of activated carbon can be used for the removal of taste and odor as well as toxic organic compounds (Crittenden et al., 2005). The primary disadvantage of activated carbon is that its adsorption capacity is quickly exhausted in wastewater applications, thereby requiring frequent replacement or regeneration (Schneider and LeChevallier, 2017).

The high surface area of activated carbon is also conducive to biofilm development, which is obviously advantageous for biofiltration. In fact, studies have demonstrated that GAC supports more dense microbial communities than other types of media (Basu et al., 2015). In systems that do not require adsorption and instead can rely on biodegradation alone, there may not be a need to replace or regenerate the carbon—even for decades in some applications (Gerrity et al., 2013). In these applications, the media is referred to as biological activated carbon (BAC) to reflect the importance of biodegradation over adsorption. Biofiltration with activated carbon has been shown to be efficient for removing bulk organics (i.e., TOC) (Reungoat et et. 2012; Gerrity et al. 2014), pharmaceuticals (Farré et al, 2011; Reungoat et al. 2011), and other TOrCs (Gerrity et al. 2011).

Simpson (2008) demonstrated that the lifecycle of activated carbon can be summarized in three stages. The first stage is characterized by high removal of organic matter through adsorption. In the second stage, the adsorption capacity starts to diminish due to saturation with organic matter, and removal starts to decrease (i.e., onset of contaminant breakthrough

conditions). Simultaneously during stages one and two, bacteria attach to the media and start to develop a biofilm. The third stage is characterized by steady biomass presence and complete exhaustion of the activated carbon (i.e., minimal to no adsorption capacity), after which the removal of organic matter is almost exclusively due to biodegradation and is often much lower than earlier stages. Despite its diminished removal capacity, exhausted GAC—now considered BAC—can operate for decades without replacement (Gerrity et al., 2013).

2.2.3 Combined Ozonation and Biofiltration Systems

Combining ozone and biofiltration provides an opportunity to leverage the synergistic benefits of preoxidation and biodegradation. In potable reuse applications, this combination is the most common alternative to RO-based treatment trains (e.g., FAT) due to its lower costs (Gerrity et al., 2014), reduced energy demand (Schimmoller et al., 2015), and potential for significant reductions in bulk organic matter (e.g., TOC) and TOrCs (Gerrity et al., 2014). As described in previous sections, ozonation has the potential to transform non-biodegradable organic matter into BDOC, which can be removed in the downstream biofiltration process (Xu et al., 2007; Linlin et al., 2011). Hollender et al. (2009) observed an increase of 100-500 µg/L of AOC after ozonation and then demonstrated its removal by the subsequent biofiltration process. When preceded by ozone, biofilters can achieve TOC reduction of up to 60% (Rachwal, 1988; Xu et al., 2007; Reungoat et al., 2012; Selvy, 2015;). Table 3 summarizes TOC reductions reported in several studies. Observing the data presented in Table 3, there is not yet an established relationship between ozone/TOC and EBCT that enhances TOC removal. However, the level of treatment attained by this combination seems to depend on combinations of influent water quality, ozone dose (Van der Kooij et al., 1989; Hammes et al., 2007), and EBCT (Selvy, 2015).

Table 3 - Typical TOC removal percentage in different studies

Reference	Influent DOC (mg/L)	Effluent DOC (mg/L)	TOC/DOC Removal (%)	O_3 Dose (mg/L)	O_3/TOC Ratio	EBCT (min)
Xu et al., 2007[c]	3.86	2.65	30	2-2.5	0.52 – 0.65	30
Gerrity et al., 2011	7.3	4.9	33	5[a]	0.8	30
Reungoat et al., 2012	4.2- 5.8	2.2 – 3.0	48	5	0.4-0.5	45
Linlin et al., 2011	6.4	2.6	60	6	0.6-1.0	N/A[b]
Chu et al., 2012[c]	3.1	2	35	2	0.6	15
Knopp et al., 2016	10.7	7.3	32	10	0.87	28
Trussell et al., 2016	6	3.9	38	5	0.94	18

a. In this study there was additional of 3 mg/L of H_2O_2
b. Slow sand filtration (SSF) with velocity of 0.12 m/h
c. Study with surface water/drinking water

There are several benchmark facilities, including the Fred Hervey Water Reclamation Plant in El Paso, Texas, and the F. Wayne Hill Water Resources Center in Gwinnett County, Georgia, that have demonstrated the historical success of ozone-biofiltration (Gerrity et al., 2013). These examples are highlighted in Figure 2, which describes several possible treatment train configurations for potable reuse (van Leeuwen et al. 2003; Gerrity et al, 2013). With the recent growth in the potable reuse industry expected to continue into the future, there may be more widespread implementation of ozone-biofiltration because of its sustainability benefits. For example, Gerrity et al. (2014) concluded that adopting ozone-BAC in a full-scale application treating 10 million gallons per day could lead to capital and O&M cost savings of up to $51 million and $4 million per year, respectively.

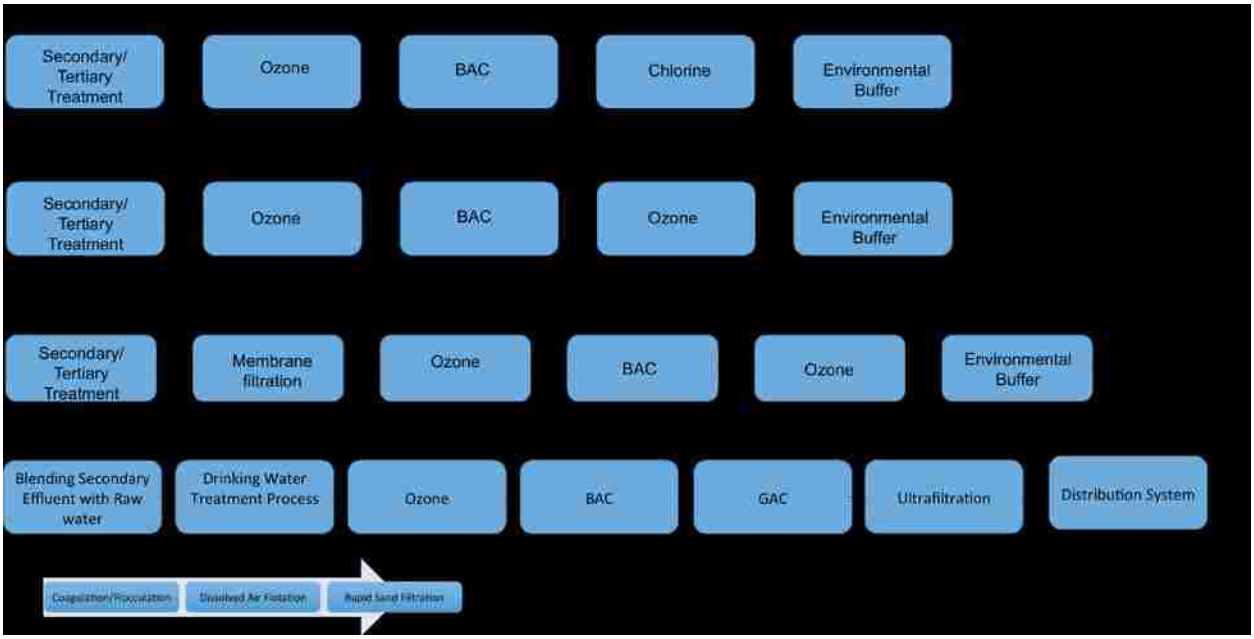

Figure 2 – Examples of potable reuse treatment trains throughout the world

(a) EI Paso, Texas USA; (b) Gwinnett County, Georgia, USA; Caboolture, Queensland, Australia (c) Gwinnett County, Georgia, USA; pilot scale treatment in Reno, Nevada, USA (excludes final ozone step); Las Vegas, Nevada, USA (excludes BAC and final ozonation) (d) Goreangab, Namibia (DPR application)(Adapted from Gerrity et al., 2013)

TOC concentrations in secondary effluent typically ranging from 5-9 mg/L, but after full-scale ozone-BAC, effluent TOC concentrations may range from 1.5-3 mg/L, depending on influent water quality and operational conditions, and possibly as low as 0.5 mg/L with SAT (Gerrity et al., 2013). The Fred Hervey Water Reclamation Plant in El Paso achieved average TOC concentrations averaging 3.2 mg/L with an ozone dose of ~5mg/L and a 16-min EBCT (Gerrity et al, 2013). In Australia, full-scale ozone-BAC achieved final TOC concentration ranging from 2- 4 mg/L with O3/TOC ranging from 0.2-0.8 and EBCTs ranging from 9-45 min (Reungoat et al, 2012). Finally, the F. Wayne Hill Water Resources Center in Georgia typically achieves TOC concentrations of 4 mg/L with an ozone dose of ~3 mg/L (for pre-ozonation) and

an EBCT of 15 minutes. Currently, it is not entirely clear how these systems can be better engineered to control effluent TOC in these systems. Thus, additional studies involving controlled variation of ozone dose and EBCT are needed to characterize their relative impacts and potentially optimize ozone-BAC systems to maximize TOC removal.

2.3 – Chlorination and Chloramination Disinfection

Microbial pathogens causing gastrointestinal illness, particularly *Giardia*, *Cryptosporidium*, and enteric viruses, are often found in raw surface waters and wastewater. They can cause a range of adverse, health effects such as diarrhea, vomiting, cramps, and even chronic conditions or sequelae (USEPA, 2015). Therefore, disinfection is essential to achieve sufficient levels of inactivation of these microbial pathogens in drinking water applications. Disinfection for water treatment occurs in two stages: (1) primary disinfection to achieve public health targets and regulatory requirements and (2) secondary disinfection to achieve a disinfectant residual in the distribution system (Crittenden, 2012). The residual concentration is essential to minimize bacterial regrowth in distribution systems.

The first continuous use of chlorination for disinfection occurred in Middelkerke, Belgium, in 1902, and disinfection was eventually adopted in the United States in 1908 (Crittenden, 2012). Chlorine disinfection was first accomplished with solid calcium hypochlorite but then the availability of chlorine gas allowed for large-scale disinfection applications (Crittenden, 2012). By 1941, chlorination was used in 85% of the drinking water systems in the U.S. (Crittenden, 2012). Full-scale drinking water treatment plants in North America typically target chlorine residuals ranging from 0.7-4 mg/L (LeChevallier et al., 1996). In the U.S., the Stage 1 Disinfectant and Disinfection Byproducts Rule established a maximum chlorine residual

of 4 mg/L as Cl_2 (EPA, 2015), but 1 mg/L has been identified as a useful target for secondary disinfection (Metz et al., 2011).

Chloramination is a disinfection application that combines chlorine and ammonia to generate chloramines. Chloramines are generally less effective for primary disinfectant than free chlorine (Crittenden et al., 2005), though its use has a lower potential for THM and HAA formation (Hua and Reckhow, 2007). Chloramines are also known to achieve better penetration of biofilms in distribution systems (Norton and LeChevallier, 1997). Many utilities in the U.S. have actually transitioned from chlorine to chloramine to enhance overall water safety while facilitating compliance with drinking water standards (USEPA, 2015).

2.4 – Disinfection Byproducts

In the 1970s, it was discovered that the oxidation of organics and inorganics present in source waters often result in the formation of toxic disinfection byproducts. Depending on the oxidant used in water treatment (e.g., ozone, chlorine, chloramine), unique byproducts can be formed, such as NDMA during ozonation and chloramination, bromate during ozonation, and THMs and HAAs during chlorination. Formation and speciation of DBPs are dependent on the source water conditions, such as the pH, ammonia, applied dose of the disinfectant, and the concentrations of bromide, iodide, and bulk organic matter (Richardson, 2003).

2.4.1 – Ozone Disinfection Byproducts

NDMA is a nitrosamine that sometimes forms during ozonation. Many studies have linked the formation of NDMA to the oxidation of specific precursor compounds, such as dimethylamine and dimethylsulfamine (Marti et al., 2015). The USEPA classified NDMA as a probable human carcinogen in 1987, and a lifetime risk of 10^{-6} has been linked to an NDMA concentration of 0.7 ng/L in drinking water supplies. To balance the risks of developing cancer

with other practical concerns (e.g., analytical detection limits and a lack of cost effective treatment options), the California Department of Public Health established a notification level of 10 ng/L (CDPH, 2013).

NDMA precursors have not been completely characterized, which makes it difficult to accurately predict its formation in complex wastewater matrices in potable reuse applications. Formation in some systems may be at the low ng/L level (Gerrity et al., 2015), but some studies have reported NDMA formation of more than 100 ng/L after ozonation (Sgroi et al., 2015; Farré et al., 2011; Trussell et al., 2016). Gerrity et al. (2014) observed peak NDMA concentrations ranging from 95-125 ng/L for O_3/TOC varying from 0.25 to 1.0. Despite the potential for direct NDMA formation during ozonation, other studies have shown that chloramine-induced NDMA formation can be reduced by pre-ozonation (Hua and Reckhow, 2007). In other words, unique precursors appear to be responsible for NDMA formation with different disinfectants. Although photolysis (i.e., ultraviolet irradiation) is the most common treatment option for NDMA mitigation (Sgroi et al., 2015), studies have shown that biological treatment can be effective in reducing NDMA concentrations to regulatory or public health targets (Hollender et al., 2009; Webster et al, 2013; Gerrity et al., 2014). Trussell et al. (2016) observed an increase in NDMA removal in biofilters with increased EBCT: 70% and 90% removal for EBCTs of ~10 and ~20 minutes, respectively. However, additional studies are needed to identify optimal conditions for NDMA removal with biofiltration to reliably achieve compliance with relevant guidelines and regulations (Gerrity et al, 2014).

Bromate is another toxic DBP formed from reactions of bromide with ozone (and hydroxyl radicals) (von Gunten, 2003). Currently, the USEPA and the California Division of Drinking Water regulate this contaminant as 10 µg/L. Previous studies demonstrated no

significant removal of bromate after biofiltration (Trussell et al., 2016), which is a concern for ozone-BAC systems, but it may be possible to modify oxic conditions to promote bromate reduction to bromide. However, ozonated waters are often supersaturated (e.g., dissolved oxygen >20 mg/L) so it may not be practical to rely on biofiltration as a barrier to bromate exposure. Instead, modifications to the ozone process are typically the most effective option for controlling bromate formation. Some studies found that O_3/TOC ratios less than 0.8-0.9 might ensure that the bromate concentration remains below the USEPA MCL of 10 µg/L (Li et al., 2017; Trussell et al., 2016; Snyder et al., 2014). Supplementing the ozone process with H_2O_2 has also been shown to control bromate formation (Gerrity et al., 2011). Li et al. (2017) even developed an empirical equation correlating bromate formation and bulk organic matter transformation (via changes in fluorescence) to help utilities monitor bromate formation.

2.4.2 – Chlorinated Disinfection Byproducts

Reaction between organic or inorganic compounds and free chlorine cause the formation of chlorinated, brominated, and iodinated DBPs (Krasner, 2009). The most common DPBs formed during chlorination are THMs and HAAs, but other emerging and currently unregulated DBPs are also gaining increasing attention, such as halonitromethanes (HNMs), haloacetonitriles (HANs), and haloacetaldehydes (Krasner, 2009). Chloramination has been viewed as an alternative to chlorination because of the potential for lower THM and HAA formation. Hong et al. (2013) observed that chloramination suppresses THM formation and reduces HAA formation. However, other emerging DBPs can be formed during chloramination, such as dihalogenated HAAs (DHAAs), iodinated DBPs, and NDMA. Toxicological studies revealed that iodinated DBPs, which are typically present at lower concentrations, may be more toxic than their chlorinated counterparts (Plewa et al., 2004).

Because of the high level of toxicity associated with halogenated DBPs, the USEPA regulated four species of THMs [chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$)] and five HAAs [chloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetic acid (MBAA), dibromoacetic acid (DBAA)]. Bromochloroacetic acid (BCAA), among others, is also formed during chlorination but is not yet regulated in drinking water. The chemical structures of these compounds are shown in Figure 3 and Figure 4. Research has demonstrated a relationship between elevated concentrations of halogenated DBPs and adverse impacts on pregnant women in addition to higher rates of bladder, colon, and rectal cancers (Krasner, 2009). In the Stage 1 Disinfectant and Disinfection Byproducts Rule (D/DBPR), the USEPA established a maximum contaminant level (MCL) of 80 µg/L for the TTHMs and 60 µg/L for the HAA5s in drinking water. The Stage 2 D/DBPR strengthened the regulation to enforce site-specific, rather than system-wide, running annual averages for the TTHMs and HAA5s.

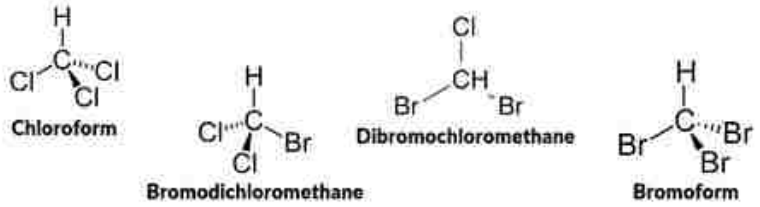

Figure 3 - Chemical structures of the four regulated trihalomethanes

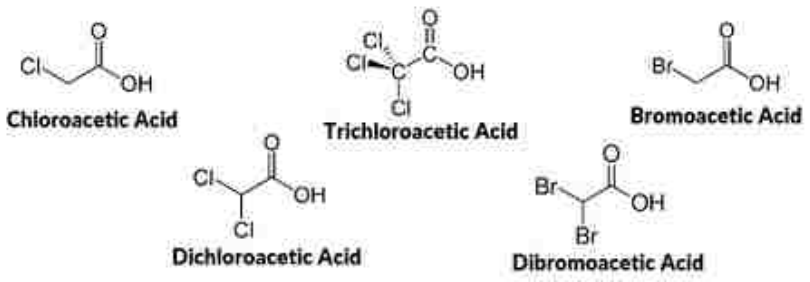

Figure 4 - Chemical structures of the five regulated haloacetic acids

Typical concentrations of TTHMs and HAA6s (i.e. including BCAA) range from 20 to 200 µg/L in natural source waters, depending on TOC concentrations, as shown in Table 4 (Summers et al., 1996). Lower TOC concentrations generally yield less formation of DBPs upon chlorination, thereby implying a possible correlation between TOC and DBP formation. Studies have also demonstrated that higher temperatures are likely to increase DBP formation, and changes in pH affect the distribution of species (Summers et al., 1996). Increase in trihalomethanes formation was observed with increase in pH due to hydrolysis of chlorinated intermediates (Summers et al., 1996). In the other hand, formation of haloacetic acids decreased with increase in pH.

Table 4 - Typical Chlorinated DBP concentration after UFC DBP formation assessment

Source Water	TOC (mg/L)	TTHM (µg/L)	HAA6 [a] (µg/L)
Ohio River (Ohio)	1.3	58	27
Salt River project (Arizona)	2.2	68	37
Manatee Lake (Florida)	4.1	151	82
Passaic River (New Jersey)	3.2	73	70
Lake Gaillard (Connecticut)	1.5	31	29
Florida groundwater	10	238	142
Harsha Lake (Ohio)	3.6	95	78
Miami Whitewater Lake (Ohio)	4	104	62
Great Miami River (Ohio)	3.2	97	55

Table adapted from Summers et al., 1996. The uniform formation conditions (UFC) approach was used to assess DBP formation in different surface waters.
a. HAAs analysis included aforementioned HAAs species and bromochoroacetic acid (BCAA)

Because of the potential carcinogenicity of DBPs, it is important to study technologies capable of reducing their concentrations or formation potential. In addition to conventional drinking water applications, this is particularly important for DPR, in which the final product water is intended for human consumption. Waters with high humic acid or aromatic content and more abundant compounds with higher molecular weights generally result in higher concentrations of THMs and HAAs upon final chlorination. In particular, higher molecular weight aromatics are more reactive with chlorine and more likely to form halogenated DBPs (Xu et al., 2007). This has significant implications for wastewater effluents used in potable reuse applications. However, research has shown that pre-ozonation can reduce total organic halide (TOX) formation, including THMs and HAAs, by up to 70% upon final chlorination and chloramination (Xu et al., 2007; Hua and Reckhow, 2007). This provides further justification for incorporating ozone into potable reuse treatment trains.

2.5 - DBP Formation Assessment

Historically, the USEPA has used simulated distribution system (SDS) testing to evaluate DBP formation potential in drinking water applications. SDS testing consists of reproducing site-specific distribution system conditions, such as temperature, water age (i.e., incubation time), pH, and chlorine dose/residual, in a bench-scale setup (Summers et al., 1996). Although SDS testing is a very efficient approach for assessing site-specific DBP formation, the varying test parameters make it difficult to compare different systems. Thus, two new approaches are becoming increasingly common for assessing and comparing DBP formation: the formation potential (FP) approach and the uniform formation conditions (UFC) approach.

The FP approach consists of dosing the target disinfectant (e.g., free chlorine) at high concentrations and for long incubation times to determine the 'maximum' expected DBP formation and to account for all potential precursors in the water. The FP test conditions typically include 7 days of incubation at 25°C, a pH of 7, and a target chlorine residual of 3-5 mg/L at the end of the incubation period (Summers et al, 1996). Although this approach is useful for maximizing DBP formation, the results are not necessarily consistent with what would actually be expected under normal conditions in a drinking water distribution system.

Instead, the UFC approach can be used to provide a more accurate estimate of DBP formation under normal conditions in the drinking water distribution systems. For the UFC approach, the water is incubated in a dark environment for 24 hours at 20°C, the water is buffered at pH 8. A target concentration of 1 mg/L of free chlorine residual must be obtained at the end of the incubation period.

2.6 – Conclusions

Several successful case studies supports potable reuse applications as a sustainable and reliable alternative to conventional drinking water supplies. However, in order to guarantee reliability, potable reuse must be adopted upon a multi-barrier advanced treatment train. Ozone-biofiltration technology has demonstrated to be a promising technology in advanced treatment because it is cost-effective when compared to common adopted advanced treatment with membrane filtration (i.e., FAT). Furthermore, it brings $2-$4 millions in O&M savings per year. Ozone-biofiltration is also effective in decreasing bulk organics (i.e., TOC) concentrations in water, main precursors of disinfection by-products upon final disinfection. Therefore, ozone-biofiltration preceding final disinfection with free chlorine can be promising in mitigating regulated disinfection by-products such as THMs and HAAs. However, further study is essential to determine optimum operational parameters, as well as determine TOC-benchmark necessary to accomplish compliance with DBP regulations for drinking water.

Chapter 3 - Impacts of Ozone dose and Empty Bed Contact Time on Bulk Organics removal

3.1 - Introduction

Water shortages due to climate change and population growth are compromising drinking water supplies in many parts of the world, but potable reuse may be a viable alternative for drinking water supply augmentation or even replacement. Potable reuse involves conventional or advanced treatment of municipal wastewater prior to indirect (i.e., IPR) or direct (i.e., DPR) reuse. In the IPR scenario, the treated water goes into an environmental buffer (i.e. rivers, lakes, dams, groundwater aquifer), whereas in the DPR scenario, the product water goes directly into the drinking water distribution system or is blended either upstream or downstream of a drinking water treatment plant. Operating since 2008, the Groundwater Replenishment System (GWRS) in Orange County, CA, is an example of an IPR application. The GWRS receives conventionally treated wastewater from the Orange County Sanitation District (OCSD); further purifies the water with 'full advanced treatment' consisting of microfiltration (MF), reverse osmosis (RO), and an advanced oxidation process (AOP); and then returns the water to the aquifer via spreading basins or direct injection. The project was originally intended as a seawater intrusion barrier but is now a critical drinking water source for the local community. On the other hand, the Goreangab Water Reclamation Plant, in operation since 1969 in the City of Windohoek, Namibia, is one of the pioneers in DPR (du Pisani, 2006). The advanced treated wastewater bypasses the environment and serves as the primary source of drinking water for the community.

One of the critical treatment targets in potable reuse is bulk organic matter, specifically effluent organic matter (EfOM). EfOM consists of an assortment of both particulate and dissolved recalcitrant organic compounds that persist through conventional wastewater

treatment. Biologically recalcitrant compounds generally consist of amines, phenols and alkoxylated aromatics [e.g., polychlorinated byphenyls (PCBs), toluene, benzene, and atrazine) (Simpson, 2008). Bacteria are generally unable to absorb these high molecular weight compounds, thereby hindering biodegradation. EfOM includes natural organic matter from the local water supply and a suite of wastewater-derived organics, including soluble microbial products (SMPs), trace organic compounds (TOrCs) resistant to biodegradation (e.g., carbamazepine, primidone, and sucralose) (Gerrity et al., 2014; Michael-Kordatou et al., 2015), and their transformation products (Michael-Kordatou et al., 2015).

A major challenge for the potable reuse industry is characterizing this EfOM and determining its public health relevance, with the ultimate goal of developing regulatory guidelines for bulk and trace organics. Surrogate analyses such as total organic carbon (TOC), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) can be used to quantify and characterize the EfOM (Michael-Kordatou et al., 2015). Even simpler spectroscopic surrogates, such as UV_{254} absorbance (Wert, Rosario-Ortiz and Synder, 2009), specific UV absorbance (SUVA) (Weishaar et al., 2003; Hua and Reckhow, 2007), fluorescence (Wert et al., 2009; Gerrity et al., 2012; Hao et al., 2012; Li et al., 2016; Li et al., 2017); and size exclusion chromatography with online carbon detection (SEC-OCD) have shown promise. These surrogates have been correlated with molecular weight, aromaticity, and organic composition (e.g., humic-like, fulvic-like, protein-like) and applied as online water quality monitoring tools in potable reuse applications (Weishaar et al., 2003; Gerrity et al., 2012).

In addition to EfOM transformation and/or removal, considerable levels of microbial inactivation must be achieved to adequately protect public health in potable reuse applications. Although disinfection is essential, reactions between EfOM and various disinfectants (e.g. ozone,

chlorine, and chloramine) are responsible for the formation of toxic disinfection byproducts (DBPs), such as bromate, trihalomethanes (THMs), and haloacetic acids (HAAs) (Farré et al., 2011; Liu at al., 2010; Chu et al., 2012). Studies have shown that exposure to DBPs in drinking water can lead to bladder and colorectal cancer (Villanueva et al., 2004; Krasner, 2009; Kogevinas et al., 2011). Even though regulated DBPs are the focus of drinking water treatment, emerging disinfection byproducts, such as brominated and iodinated compounds (e.g., bromonitromethanes, iodo-trihalomethanes, iodo-acids) as well as *N*-nitrosodimethylamine (NDMA), are also of toxicological concern. Therefore, as with conventional drinking water treatment systems, it is also important to study the formation/mitigation of these DBPs in potable reuse systems. In fact, it may be more critical for potable reuse because of the complex composition of EfOM in treated wastewater.

Thus far, the United States Environmental Protection Agency (USEPA) has not yet established a set of regulations for potable reuse. Instead, potable reuse regulations have been primarily developed at the state level. For example, the California Division of Drinking Water (CDDW) determined that reverse osmosis (RO) followed by advanced oxidation, a treatment train now identified as “full advanced treatment” (FAT), is to be employed in potable reuse systems that directly inject recycled water into local aquifers or drinking water reservoirs. This treatment train is effective in achieving California’s regulatory benchmark of 0.5 mg/L of wastewater-derived TOC without additional blending of water (CDPH, 2014). In fact, research has shown that RO is able to achieve an average TOC removal of 90% (Kim et al., 2002; Drewes et al., 2003). Systems not using FAT are unable to augment drinking water reservoirs but may replenish groundwater supplies via spreading basins—but not direct injection. In these spreading applications, the agency must often employ significant blending ratios or demonstrate that

natural percolation, a process known as soil aquifer treatment (SAT), has adequately reduced the TOC concentration to achieve the regulatory benchmark. This regulation seems very conservative considering the median TOC concentration for U.S. drinking water is approximately 3.2 mg/L (Trussell et al., 2013; Snyder et al., 2012). In comparison, the water reuse regulations in Florida specify a maximum TOC concentration of 3 mg/L, and water reuse guidelines published by the USEPA recommend a maximum TOC concentrations of 2 mg/L (Schimmoller et al., 2015), although the USEPA value is not enforceable. Because of potential human and aquatic health implications associated with TOrCs, certain indicator compounds are also regulated in potable reuse applications. However, few TOrCs are regulated at the federal level in the U.S. Exceptions include atrazine, for example, which is a commonly used herbicide in agricultural applications that is regulated at 3 µg/L.

Although FAT is highly effective in reducing TOC concentrations, among other contaminants, the costs associated with RO are often cost-prohibitive. In fact, the addition of RO to an advanced treatment train represents an incremental cost of $\$2.99/10^3$ gallons (Tchobanoglous et al., 2015), whereas conventional drinking water treatment can be estimated at approximately $\$1.50\text{-}\$2.00/10^3$ gallons (Tchobanoglous et al., 2015). This is due to the high capital costs for the membrane system coupled with high energy consumption during operation and the need for concentrate management (Gerrity et al, 2013). As a result, more sustainable potable reuse treatment trains need to be investigated.

Studies have demonstrated that ozone followed by biofiltration [e.g., mono- or multimedia filtration, biological activated carbon (BAC), and soil aquifer treatment (SAT)] has the potential to transform and remove a significant portion of the EfOM in potable reuse applications. Studies of size exclusion chromatography with organic carbon detection (SEC-

OCD) have demonstrated that ozonation is able to transform particulate, hydrophobic, and microbial-derived organic matter (>20kD) into lower molecular weight humics (~1kD), acids, (>350D), and building blocks (0.300-0.500kD) that can subsequently be removed by biodegradation (Snyder et al., 2014). For example, Gerrity et al. (2012) observed TOC reductions of up to 33% after ozone-BAC, while Reungoat et al. (2012) observed reductions of up to 50% for dissolved organic carbon (DOC) and 90% for some TOrCs.

The differences in bulk organic reduction observed in various studies can presumably be linked to the composition of the water matrix, the applied ozone dose, and the EBCT in the biofiltration system (Chu et al., 2012; Gerrity et al., 2011; Gerrity et al., 2014; Kim et al., 1997; Linlin et al., 2011; Reaume et al., 2015). Li et al. (2017) found that increases in ozone exposure (i.e., O_3/TOC ratios > 0.4) can lead to extensive breakdown of aliphatic and aromatic structures and other electron-rich targets into lower molecular weight compounds, which increases the amount of biodegradable dissolved organic carbon (BDOC) (Xu et al., 2009). Similarly, Hollender et al. (2009) observed an increase in assimilable organic carbon (AOC) of 100-500 µg/L after the ozonation process , but the AOC was completely removed by the subsequent biofiltration process.

As previous DBP studies have shown, THM and HAA formation is correlated with the presence of aromatic moieties (Weishaar et al., 2003; Krasner, 2009). Therefore, because ozone targets these electron-rich moieties and converts them to more bioavailable fractions that can be removed with biofiltration (Selvy, 2015; Reungoat et al., 2012; Linlin et al., 2011; Santos et al., 2013), ozone-BAC is a promising combination for DBP control in systems employing chlorination as a primary and/or secondary disinfectant. In fact, studies have demonstrated DBP precursor reductions of 50-70% after ozone-BAC (Xu et al., 2009; Linlin et al., 2011). Despite

the synergism of ozone-BAC and the potential for significant transformation and removal of bulk organic matter, final effluent TOC concentrations are generally much greater than 0.5 mg/L—the TOC benchmark established by the CDDW. Therefore, additional optimization studies are needed to develop strategies to maximize TOC removal for potable reuse applications.

Although RO-based treatment trains generally achieve superior water quality, many agencies are considering ozone-BAC because of its lower capital and operations and maintenance (O&M) costs. Gerrity et al. (2014) estimated capital and O&M savings of up to $51 and $4 million, respectively, when ozone-BAC is adopted instead of RO. Agencies are more likely to implement ozone-BAC and exploit these cost benefits if the ozone-BAC system can be engineered to achieve water qualities that are more consistent with RO-based alternatives (e.g., FAT). There are certainly limitations to the comparison (e.g., no reduction in total dissolved solids with ozone-BAC), but it may be possible to achieve comparable levels of bulk and trace organics by optimizing the operational conditions. The hypothesis of the current research is that increasing the ozone dose will generate more BDOC, and increasing the EBCT will allow for greater TOC removal. Ultimately, this should reduce the formation of DBPs upon final disinfection. This chapter specifically discusses the relationship between ozone dose, EBCT, and TOC removal, and the next chapter will address the resulting impacts on DBP formation.

3.2 - Materials and Methods

3.2.1 - Pilot-Scale Reactor

A 1-liter-per-minute pilot-scale ozone-biofiltration system was constructed and operated at a full-scale water reclamation facility in the Las Vegas area. The reactor was fed with full-scale membrane bioreactor (MBR) filtrate. The full-scale treatment process consisted of coarse bar screens, grit removal, fine screens (2 mm), and a membrane bioreactor (MBR) with

biological nutrient removal, a solids retention time (SRT) of 8-10 days, and a nominal pore size of 0.04 μm.

To generate ozone, gas from an oxygen concentrator (AirSep, Denver, CO) was passed through a Magnum-600 air dryer (Ozone Solutions Inc., Hull, IA) and then to a Nano dielectric ozone generator (Absolute Ozone, Edmonton, AB, Canada). Ozone gas transfer was accomplished with a Venturi injector (Mazzei, Bakersfield, CA). The ozonated water then traveled through a series of 4-ft long contactors, of which four were 1 inch in diameter and eight were 2 inches in diameter, to allow for complete ozone decay. Teflon tubing was installed at the top of each ozone contactor for ozone off gassing, and the off gas was directed to a catalytic ozone destruct unit (Ozone Solutions Inc., Hull, IA).

Following the serpentine contactors, the ozonated water was collected in a storage tank and then pumped into biofiltration columns using a MasterFlex peristaltic pump (Cole Palmer, Vernon Hills, IL). The storage tank allowed for partial degassing of the water, which reduced air trap in the biofilters. The parallel biofilters consisted of 1-inch columns containing either 0.95-mm-diameter exhausted granular activated carbon (GAC) (Norit 820, Cabot Corporation, Alpharetta, GA) or 1.2-mm-diameter anthracite media. Additionally, an exhausted GAC column was fed non-ozonated MBR filtrate to evaluate bulk organic removal in the absence of pre-ozonation. The F. Wayne Hill Water Resources Center in Gwinnett County, GA, provided the exhausted GAC, which had been used in full-scale wastewater treatment for over 10 years. The San Jose Creek Water Reclamation Plant in Los Angeles, CA, provided the anthracite media. The media height in the biofilters was approximately 30 inches, and the flow rate was controlled by needle valves located at the bottom of each column and by the peristaltic feed pumps. The

EBCT was determined by dividing the media bed volume by the water flow rate. The layout of the pilot-scale reactor is illustrated in Figure 5.

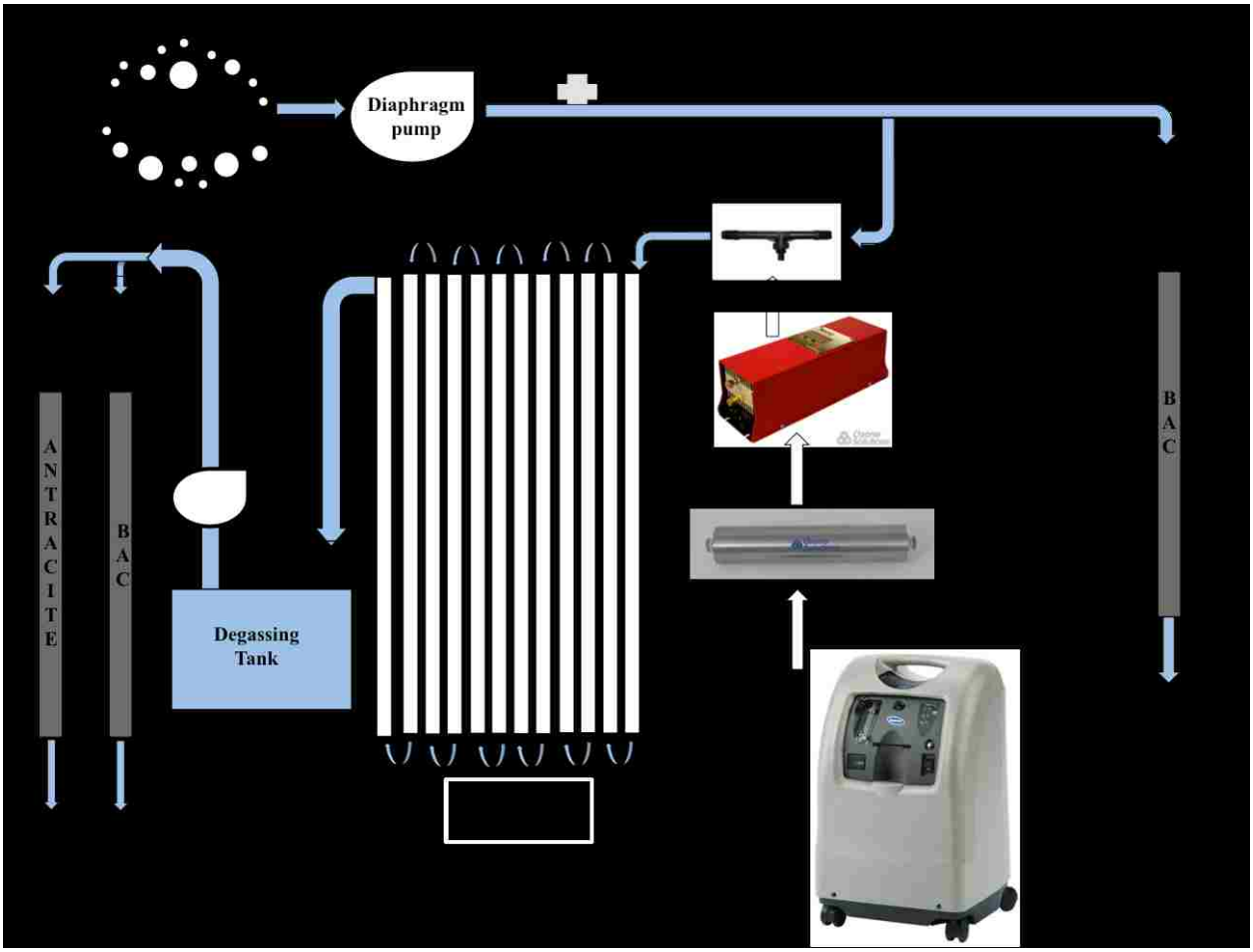

Figure 5 - Schematic of pilot-scale reactor

Multiple studies in the literature have reported empirical relationships between applied ozone dose, specifically the O_3/TOC ratio, and changes in UV_{254} absorbance in wastewater applications (Buffle et al., 2006; Wert et al., 2009b; Gerrity et al., 2012; Selvy, 2015). The correlations developed by Gerrity et al. (2012) and Selvy (2015) are shown in Figure 6, along with the corresponding logarithmic regression models (**Equation 1**). The regression model

presented in Selvy (2015) was developed using the same MBR filtrate as the current study. Therefore, this model used to determine the O_3/TOC ratios applied in the current study.

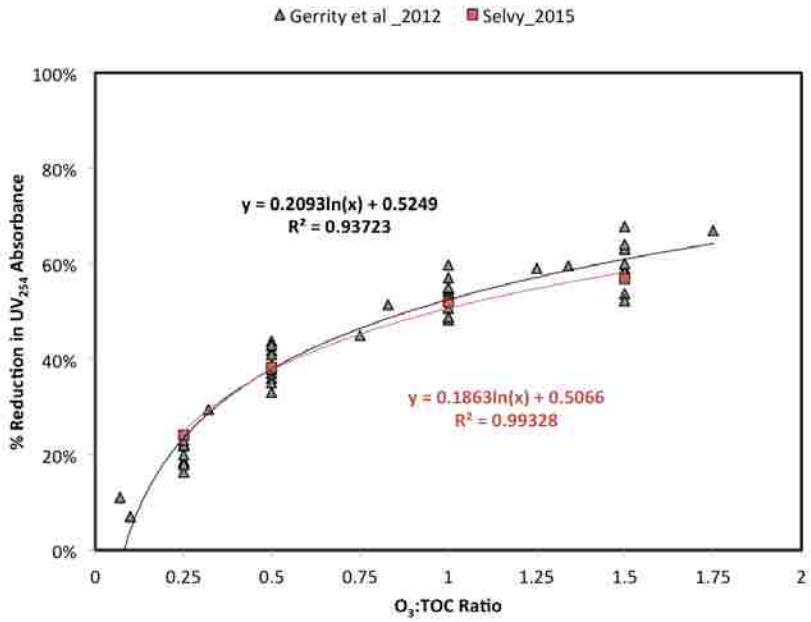

Figure 6 - Relationships between UV_{254} absorbance and O_3/TOC ratio

Developed from 10 different secondary wastewater effluents (Gerrity et al., 2012) and the MBR filtrate used in the current study (Selvy, 2015).

$$UV_{254}\ Absorbance\ Reduction\ (\%) = 0.1863 \ln\left(\frac{Applied\ O_3\ dose}{TOC}\right) + 0.5066 \qquad \textbf{Equation 1}$$

$$R^2 = 0.99$$

Backwash frequency in the biofilters was based on observations of process performance and was controlled by head loss accumulation, presumably due to biofilm accumulation. MBR

filtrate was used as the backwash water and was fed for a duration of 10 minutes using a peristaltic pump.

3.2.2 - Start-up

Two biofilters (anthracite and BAC) were fed ozonated MBR effluent over a period of 27 days. The reactor was operated with an average O_3/TOC ratio of 0.2 and an EBCT of 2.5-5.0 minutes to promote microbial growth. TOC removal was selected as a surrogate for microbial growth and activity in the biofilters. Since the media was in use during a previous project (Selvy, 2015), an acclimation period of three weeks was sufficient to achieve steady state conditions, as indicated by stabilization of TOC removal. Once stead state conditions were achieved, an initial kinetics test was performed, as indicated in Table 5. Following the first kinetics test, the system was shutdown for approximately 1 month prior to another 2-week startup period, during which the system was operated with an O_3/TOC ratio of 0.9 and an EBCT of 5 minutes. A second kinetics test was then performed, as indicated in Table 5.

Table 5 - Testing and kinetics test events

Period	Events	Dates
Phase 1 (SP1)	Start-up and acclimation period	3/22/2016 – 4/18/2016
	Kinetics test 1	4/18/2016
S	Shutdown	4/30/2016 – 6/3/2016
Phase 2 (SP2)	Start-up and acclimation period	6/3/2016 – 7/18/2016
	Kinetics test 2	7/18/2016

3.2.3 - Kinetics Tests

Two kinetics tests were performed during the study. For each test, the EBCT was increased stepwise while the O_3/TOC ratio was held constant (O_3/TOC = 2.25 for test 1 and O_3/TOC = 0.74 for test 2). After each operational adjustment, samples were collected after the

experimental EBCT had elapsed three times to allow adequate time to achieve steady state conditions. For each test, 3-4 sampling events were performed at EBCTs ranging from 2-20 minutes. Table 6 summarizes the operational targets for each kinetics test.

Table 6 - Operational conditions for each kinetic test and order of performance

Test 1	O_3/TOC	EBCT (min)			
1	2.25	2	10	15	-
2	0.74	2	5	10	20

3.2.4 - Analytical Methods for Quantification and Characterization of EfOM

For this study, organic matter characterization was accomplished with UV and fluorescence spectroscopy. According to Crittenden et al. (2005), the amount of light absorbed by the components in a solution at a specified wavelength is the measure of absorbance. Absorbance of water is usually measured at a wavelength of 254 nm because this particular wavelength is indicative of the structural characteristics of the bulk organic matter, particularly bonding arrangements in the molecule (i.e., aromaticity) (Weishaar et al., 2003; Wert et al., 2009b). Higher UV absorbance can be attributed to double bounds and recalcitrant components (Xu et al., 2009). While absorbance relates to the energy absorbed by the constituents in a water matrix, fluorescence relates to the energy released in form of light by the constituents in a water matrix (Li et al., 2016). Fluorescence spectroscopy has been used to characterize the origin of bulk organic matter present in the water (i.e., autochthonous (microbial) vs. allochthonous (terrestrial) origin or humic-like vs. fulvic-like vs. protein-like) (Chu et al., 2012; Li et al., 2016; Li et al., 2017). These measurements have been shown to be useful tools for monitoring bulk organic matter transformation (Wert et al., 2009b; Gerrity et al., 2012), TOrC oxidation (Gerrity et al., 2012), and DBP formation (Weishaar et al., 2003; Li et al., 2016).

During the study, absorbance and fluorescence spectra were developed using an Aqualog spectrofluorometer (Horiba, Edison, NJ). The excitation-emission matrices (EEMs) were created for each sample by scanning over an excitation range between 240 nm and 470 nm with an emission wavelength increment of 0.82 nm. Data processing included corrections for the inner filter effect and Rayleigh masking and development of the EEMs in Matlab (MathWorks, Natick, MA). The fluorescence data were standardized to the Raman peak area, which was based on excitation wavelength at 350 nm and emission range from 212 nm to 620 nm in deionized water. Raman correction standardizes the fluorescence intensities of experimental samples. This method allows for direct comparisons between different samples analyzed in different laboratories.

The EEMs were divided into three regions to further characterize the organic matter. Fluorescence in region I is often associated with soluble microbial products (SMPs), region II is associated with fulvic-acid-like compounds, and region III represents humic-like constituents, as shown in Figure 7 (Gerrity et al., 2011).

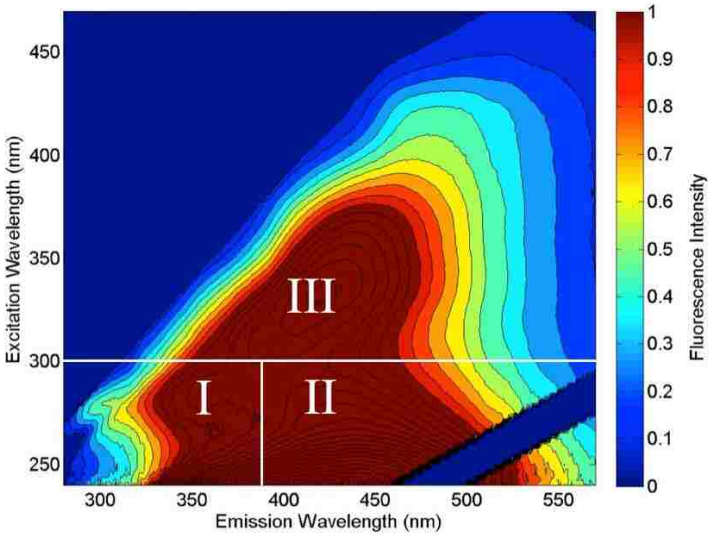

Figure 7 - Characterization of EfOM based on fluorescence.

TOC concentration was used to quantify bulk organic matter present in the samples. TOC was measured as non-purgeable organic carbon (NPOC) using a Shimadzu TOC V-csn (Kyoto, Japan). TOC samples were collected in 40 mL amber vials with Teflon-lined lids and analyzed in duplicate or triplicate (less than 5% relative standard deviation). All samples were acidified with 2 N hydrochloric acid (HCl) to reduce the pH to less than 2. This step ensures inorganic carbon (i.e., carbonates are unstable at pH < 2) is transformed into carbon dioxide that can be sparged by carrier gas inside the analyzer. The remaining carbon is then combusted in the presence of a platinum catalyst in the furnace of the TOC analyzer, and the resulting CO_2 is measured by a nondispersive infrared detector and reported as the TOC.

3.2.5 - Nutrient Quantification

Samples for ammonia analysis were collected once a week throughout the entire study and analyzed with Hach Method 10023 (salicylate method), which allows for low-range

quantification (0.02-2.5 mg/L NH_3-N). Samples were also collected for nitrite, nitrate, and phosphate once a week during the two start-up periods to evaluate nutrient cycling. Hach Method 8039 (cadmium reduction method) was used for high-range nitrate detection (0.3-30 mg/L NO_3-N), Hach Method 8507 (diazotization method) was used for low-range nitrite detection (0.002-0.3 mg/L NO_2-N), and Hach Method 8048 (ascorbic acid) was used for phosphate detection (0.02-2.5 mg/L – PO_2^{-3}). Ammonia and nitrite was measured using a DR900 multiparameter handheld colorimeter (Hach, Loveland, CO), whereas nitrate was measured using a DR5000 spectrophotometer (Hach, Loveland, CO). Phosphorus measurement was also done using a DR900 multiparameter handheld colorimeter (Hach, Loveland, CO)

3.2.6 - Ozone Residual

Hach Method 8311 (indigo method) was used to measure high-range (0.01 to 1.50 mg/L) and low-range (0.01 to 0.25 mg/L) dissolved ozone concentrations. The analysis was performed with a DR900 multiparameter handheld colorimeter (Hach, Loveland, CO).

3.3 - Results and discussion

3.3.1 - MBR Filtrate Water Quality

For the MBR filtrate, the average temperature was 27°C, the dissolved oxygen was 2.8±0.5 mg/L, the pH was 6.9±0.3, and the TOC concentration was 7.9±0.4 mg/L. Ammonia concentrations in the influent varied from 0.03 to 4 mg-N/L. High ammonia concentrations resulted from a failure of a dissolved oxygen sensor in the full-scale treatment plant, which compromised oxygen delivery and nitrification in the activated sludge process. This failure occurred during the initial startup period (Table 5), during which ammonia concentrations ranged from 0.7 to 4 mg-N/L. During the second startup phase, ammonia concentrations were more representative of a properly operating MBR system, with an average concentration of 0.05 mg-

N/L and one sample at an elevated concentration of 0.7 mg-N/L. The NO_3-N concentration in the MBR filtrate (i.e., pilot influent) varied from 5 to 9 mg-N/L, and the concentration of nitrite varied from 0.01 to 1.2 mg-N/L during the study. The average phosphate concentration was 8.7±2.2 mg/L. Detailed influent water quality during start-up phase 1 and 2 is summarized in Table 7.

Table 7 – General water quality Summary for the MBR Filtrate (i.e., Pilot Influent)

	Influent SP1	Influent SP2
Temperature	27°C	
DO (mg/L)	2.8 ± 0.5	
pH	6.9	
TOC (mg/L)	7.9 ± 0.4	
PO_4^{3-} (mg/L)	8.7 ± 2.2	
NH_4^+/NH_3 (mg-N/L)	1.6 ± 1.3	0.05 ± 0.04
NO_2^- (mg-N/L)	0.8 ± 0.4	0.2 ± 0.1
NO_3^- (mg-N/L)	6.1 ± 0.9	6.4 ± 1.5

3.3.2 - Nutrient Monitoring

Ammonia concentration in all columns increased after biofiltration, indicating that ammonification was occurring inside the biofilters. This result contradicts other research indicating ozone-BAC is effective for enhanced ammonia removal (Chu et al., 2012). The increase in ammonia appeared to be related to operational time prior to backwashing. After backwashing, the production of ammonia in the biofilters decreased significantly in all columns, thereby indicating possible detachment of the bacteria responsible for ammonification (discussed later in chapter 5).

During the first startup phase, the nitrate concentration increased after ozone and then decreased after biofiltration, as shown in Table 8 and Figure 8. The increase in nitrate after

ozonation indicates possible oxidation of ammonia in the MBR filtrate to nitrite-nitrate, as demonstrated by Rahmadi and Kim (2013). The decrease in nitrate after biofiltration is not typical of aerated biofilters and requires more investigation. Denitrifying filters are commonly used in drinking water and sensitive wastewater applications, but this process requires minimal dissolved oxygen to achieve appropriate redox conditions. Ozonation results in supersaturation of the water with dissolved oxygen, which would not typically be conducive to denitrification. After backwashing was performed prior to the second startup period, increases in nitrate were sometimes observed across the biofilter columns (Figure 9), suggesting nitrification of ammonia and/or nitrite by the microbial community. Phosphate concentrations did not change significantly during ozone-biofiltration.

Table 8 - Changes in nitrogen speciation after ozonation in different phases

	Average Concentrations			
	Influent SP1	**Ozonated Effluent SP1**	**Influent SP2**	**Ozonated Effluent SP2**
Ozone/TOC		0.2		0.9
NH3-N (mg/L)	1.6 ± 1.3	1.0 ± 0.8	0.05 ± 0.04	0.23 ± 0.1
NO2-N (mg/L)	0.8 ± 0.4	1.21 ± 0.9	0.2 ± 0.1	0.02 ± 0.03
NO3-N (mg/L)	6.1 ± 0.9	8.6 ± 2.2	6.4 ± 1.5	6.5 ± 1.34

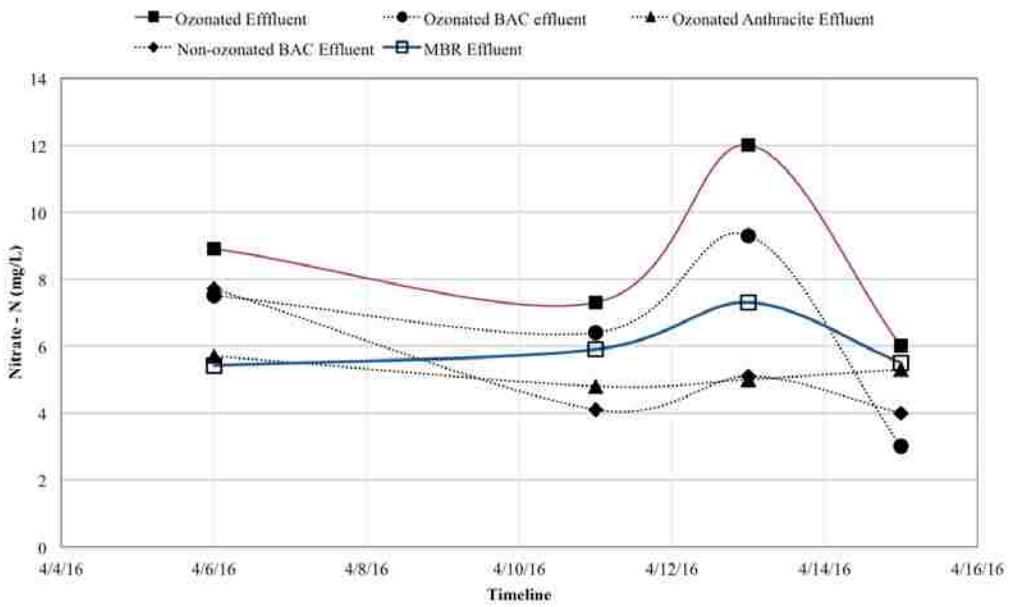

Figure 8 - Nitrate concentration during first start-up phase

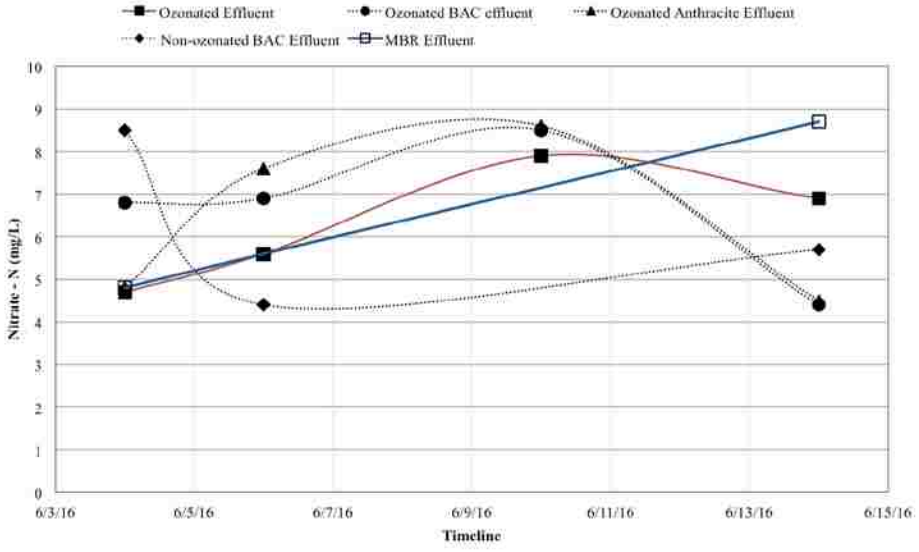

Figure 9 - Nitrate concentration during second startup phase

3.3.3 - Bulk Organic Transformation and Removal

3.3.3.1 - Ozonation and Bulk Organic Transformation

During the study, the O_3/TOC ratio ranged from 0.13 to 2.25. The ozonated effluent had an average TOC concentration of 7.8±0.3 mg/L, which was comparable to the concentration in the influent (7.9±0.4 mg/L). Other studies have concurred that typical ozone doses are insufficient to achieve any significant level of organic mineralization. Therefore, reductions in TOC from ozone alone are not anticipated, but ozone is expected to transform the bulk organics with larger molecular weights into simpler, more bioavailable molecules (Linlin et al., 2011). This step is essential for enhancing biodegradation (i.e., TOC removal) in the biofilter (Reungoat et al., 2012; Santos et al., 2013; Stalter et al., 2010). Surrogates such as UV absorbance and fluorescence can be used to demonstrate the reduction in aromaticity and bulk organic matter transformation, as shown in Figure 10 and Table 9. Ozone doses were able to decrease UV_{254} absorbance by 15% in lower ozone doses (O_3/TOC = 0.13) and 62% in higher ozone doses (O_3/TOC = 2.25).

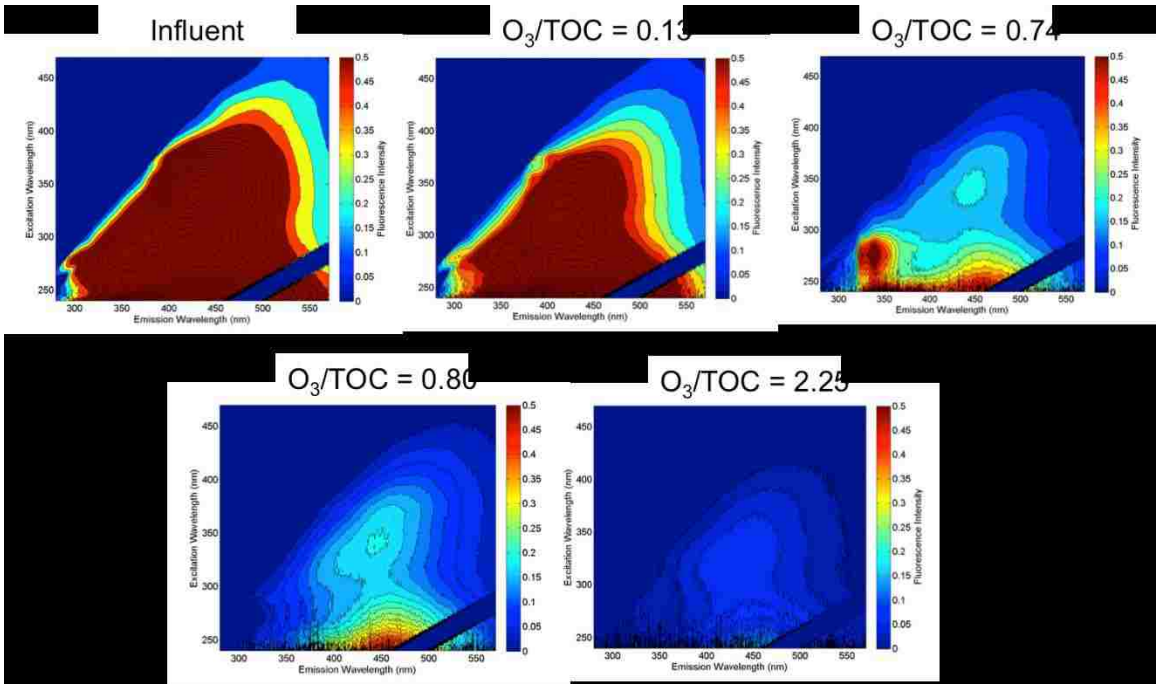

Figure 10 - Comparison of EEMs of water with increasing ozone dose

Table 9 – Total and regional fluorescence comparison for different O_3/TOC ratios

	Fluorescence (AFU)				
Ozone/TOC	**Influent**	**0.13**	**0.74**	**0.8**	**2.25**
Total	53,831	32,513	10,966	6,616	4,824
Region I - SMP	18,328	11,095	4,950	1,302	1,161
Region II - Fulvic-like	25,106	15,309	4,356	3,795	2,638
Region III- Humic-like	10,397	6,109	1,659	1,518	1,026

3.3.3.2 - Biofiltration and Baseline Bulk Organic Removal

Without preozonation, the BAC control achieved limited TOC removal (average of 7-9%). Longer EBCTs also had minimal impact on TOC removal, as shown in Table 10. Selvy (2015) performed kinetic tests across a larger range of EBCTs and showed that TOC removal plateaus when an optimum EBCT is achieved, as shown in Figure 11. However, the plateau is

more apparent when the feed water is ozonated prior to biofiltration. Without pre-ozonation, the limited quantity of bioavailable organics is consumed rapidly by the microbial community near the top of the biofilter, which essentially represents short EBCTs. Ozonation process is able to increase the biodegradable dissolved organic carbon, which enhances the organics removal in biofiltration process.

Table 10 - Average TOC removal in the absence of pre-ozonation as a function of EBCT.

EBCT (min)	Average TOC Removal (%)
2	7 ± 2
5	7 ± 2
10	8 ± 5
20	9 ± 5

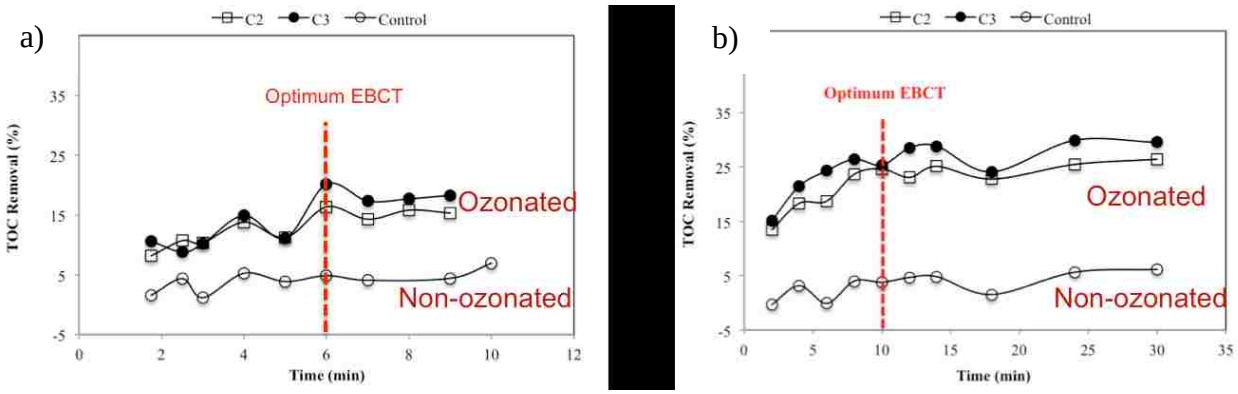

Figure 11 - TOC removal during kinetics tests across a range of EBCTs

a) O_3/TOC ratio = 0.35; b) O_3/TOC ratio = 1.12. C2 = ozone+anthracite, C3 = ozone+BAC, Control = BAC without ozone (Selvy, 2015)

In the absence of ozone, BAC alone achieves small decreases in total and regional fluorescence as well as UV_{254} absorbance compared to ozonated samples (shown later). A decrease of only 10% in UV_{254} absorbance is observable. An EEM comparison between MBR filtrate (i.e., pilot influent) and BAC without pre-ozonation (EBCT = 20 minutes) is shown in Figure 12. The corresponding reductions in fluorescence are summarized in Table 11.

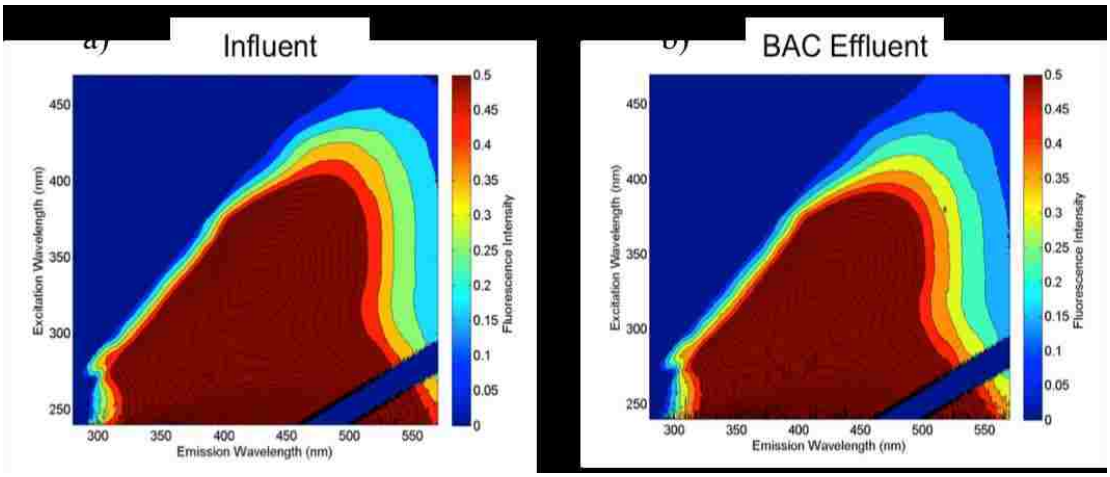

Figure 12 - Comparison of EEMs of MBR filtrate with and without biofiltration

a) EEM related to MBR effluent. b) EEM related to biofiltration with 20 minutes EBCT.

Table 11 - Fluorescence after biofiltration without pre-ozonation

	Fluorescence (AFU)		Reduction
	Influent	BAC (20 min)	%
Total	46,132	38,334	17%
Region I - SMP	14,937	13,073	12%
Region II - Fulvic-like	21,454	17,370	19%
Region III- Humic-like	9,741	7,892	19%

AFU = arbitrary fluorescence units

3.3.3.3 - Combined Ozonation and Biofiltration

3.3.3.3.1 - Start-up

During the initial start-up phase (O_3/TOC = 0.2 and EBCT = 5 min), TOC removal decreased from the ozonated BAC column (maximum TOC removal) to the ozonated anthracite column (median TOC removal) to the non-ozonated BAC column (minimum TOC removal). TOC removals of 10% and 5% were achieved in the ozonated BAC and ozonated anthracite, respectively (Table 12). The reactors were then shut down for a short period of time before a second start-up phase was initiated. For the second start-up phase (O_3/TOC = 0.9 and EBCT = 5 min), the average TOC removals in the ozonated BAC and ozonated anthracite were 15% and 6%, respectively (Figure 13). Collectively, these data confirm that (1) acclimation occurs rapidly in biofiltration systems, (2) pre-ozonation improves TOC removal in downstream biofiltration processes, and (3) BAC achieves better TOC removal than anthracite.

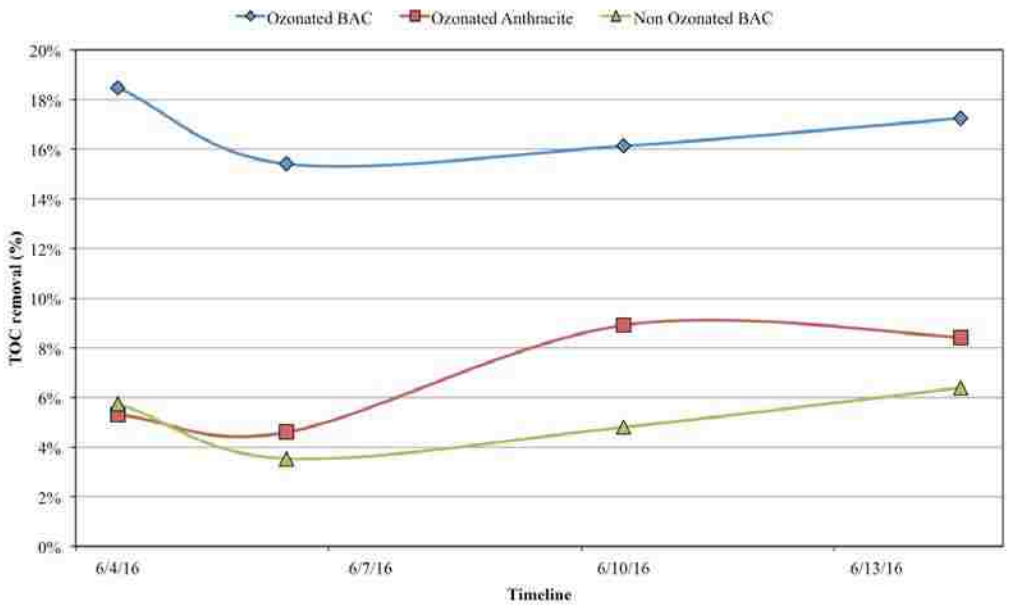

Figure 13 –TOC removal during acclimation period

Second start-up with ozone/TOC = 0.9 and EBCT = 5 minutes

Table 12 - Average TOC removal during start-up phases with an EBCT of 5 minutes

Ozone/TOC	0.2	0.9
Ozonated BAC	10±3%	15±3%
Ozonated Anthracite	5±3%	6±2%

3.3.3.3.2 - Kinetics Tests

Two kinetics tests were performed during the study. The first kinetics test was performed with an O_3/TOC ratio of 2.25. From a practical perspective, this is likely an upper limit for ozone dosing in full-scale wastewater applications, so this would theoretically represent a maximum level of biodegradable organic carbon being fed into the biofiltration columns. The results of each kinetics test can be seen in Figure 14. As demonstrated during the start-up phases, higher O_3/TOC ratios allowed for greater TOC removal during biofiltration, and BAC was superior to

anthracite. The TOC removal percentages and final TOC effluent concentrations are summarized in Table 13. Minimum TOC concentration achieved in this system was 5.4 mg/L in the current. Thus, in waters with high bulk organics concentrations, a polishing treatment after ozone-biofiltration process would be necessary to considerably decrease the TOC concentration. If system was to be employed in California, a high blending ratio would be necessary to achieve 0.5 mg/L TOC-benchmark.

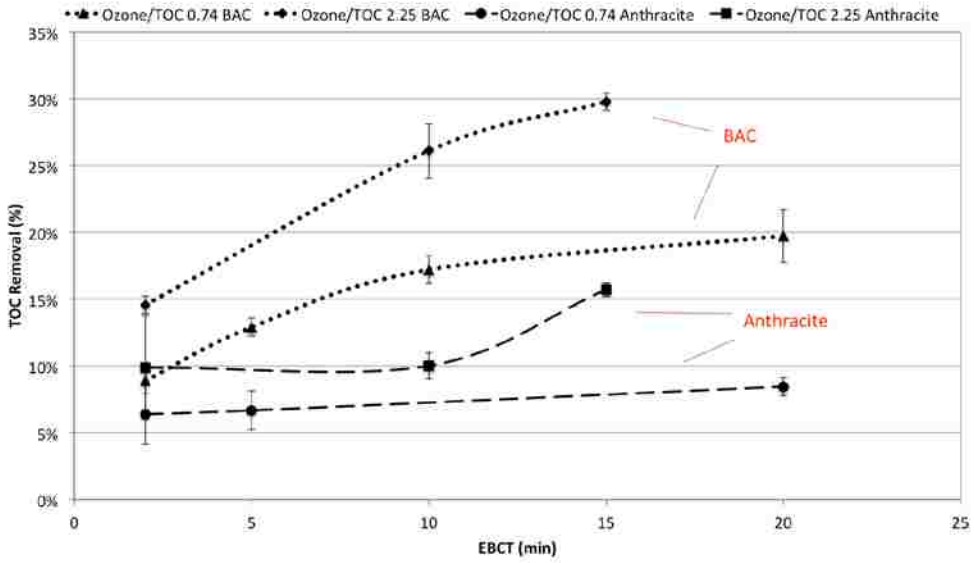

Figure 14 - TOC removal results from kinetics test

Table 13 – Conditions for maximum TOC removal and final minimum TOC effluent

		1.2-mm Anthracite		0.95-mm BAC	
O_3/TOC	**EBCT (min)**	**%**	**TOC Effluent Concentration (mg/L)**	**%**	**TOC Effluent Concentration (mg/L)**
0.74	20	10	6.1[a]	20	5.4
2.25	15	16	6.7[b]	30	5.5

a. TOC influent concentration was 7.4 mg/L
b. TOC influent concentration was 8.5 mg/L

Therefore, as previously shown by Selvy (2015), TOC removal appears to plateau at longer EBCTs (Table 14). However, the 'optimum' EBCT also appears to be positively correlated with O_3/TOC ratio. Similar to the start-up phases, BAC was superior to anthracite in terms of TOC removal, and this has also been observed in other research (Chien et al., 2008). In fact, the BAC achieved double the TOC removal in the current study, although the differences were smaller in Selvy (2015). Because GAC used in the biofilters were collected from a full-scale wastewater treatment plant that was use for over 10 years, we assumed that adsorption capacity was exhausted. However, due to outstanding performance of biofilter containing activated carbon over biofilter containing anthracite, it is possible that adsorption was still available in the activated carbon. Differences in available surface area may also explain the superior performance of the activated carbon. Previous studies estimated the surface area of a similar type of bituminous coal-based activated carbon to be 1000 m^2/g (Gibert et al., 2013; Yang et al., 2011), while anthracite was considerably lower at 250 m^2/g (Yang et al., 2011). Therefore, the activated carbon and its porous structure may support more dense microbial populations than sand or anthracite (Basu et al., 2015), thereby increasing biodegradation rates. However, in order to quantify microbial activity in both biofilters, ATP analysis was performed and results are shown in Table 15. There was not significant difference in microbial activity quantification between BAC and anthracite filters that would support that the outstanding performance of BAC filter was due to higher microbial density. Thus, further characterization would still be needed in order to evaluate difference in biofilters performance, such as adsorption capacity test.

Table 14 - Comparison of optimum conditions and treatment efficacy

O_3/TOC	Optimum EBCT	TOC removal with anthracite	TOC removal with BAC	Minimum TOC Achieved
0.35	6 min	16%	20%	6.4 mg/L
0.62	9 min	19%	22%	5.7 mg/L
1.12	10-12 min	25%	25%	5.0 mg/L

Adapted from (Selvy, 2015)

Table 15 - ATP analysis in biofilters during the study.

Date	ATP (pg/g) Top (5 inches from surface)			ATP (pg/g) Bottom (19 inches from surface)		
	Ozonated BAC Effluent	Ozonated Anthracite Effluent	Non-Ozonated BAC Effluent	Ozonated BAC Effluent	Ozonated Anthracite Effluent	Non-Ozonated BAC Effluent
2/25/16	-	-		1.88E+03	2.54E+04	1.72E+04
3/22/16	-	-	-	1.81E+04	1.61E+05	4.83E+04
4/15/16	1.26E+05	1.15E+04	5.93E+04	1.62E+05	4.13E+04	1.06E+04
4/30/16	Shutdown					
6/3/16	Restart-up					
6/18/16	-	-	-	7.56E+04	1.55E+05	2.04E+05
7/17/16	1.51E+05	3.41E+05	4.75E+05	7.54E+04	2.26E+05	9.80E+04

Despite significant reductions in absorbance and fluorescence due to pre-ozonation, increases in UV absorbance and fluorescence were observed after biofiltration. As summarized in Table 16, region I fluorescence, which is often linked to soluble microbial products, increased by 81% after biofiltration with an EBCT of 20 minutes (Figure 15**a**) and by 63% after biofiltration with an EBCT of 10 minutes (Figure 15**b**). This is consistent with a previous study (Snyder et al., 2014), as soluble microbial products result from substrate metabolism during biomass growth [i.e., utilization-associated products (UAPs)] and from cell lysis during biomass

decay [i.e., biomass-associated products (BAPs)] (Michael-Kordatou et al., 2015). Chu et al. (2012) also observed an increase in fluorescence after BAC due to a shift from allochthonous organics to authochthonous organics due to microbial activity.

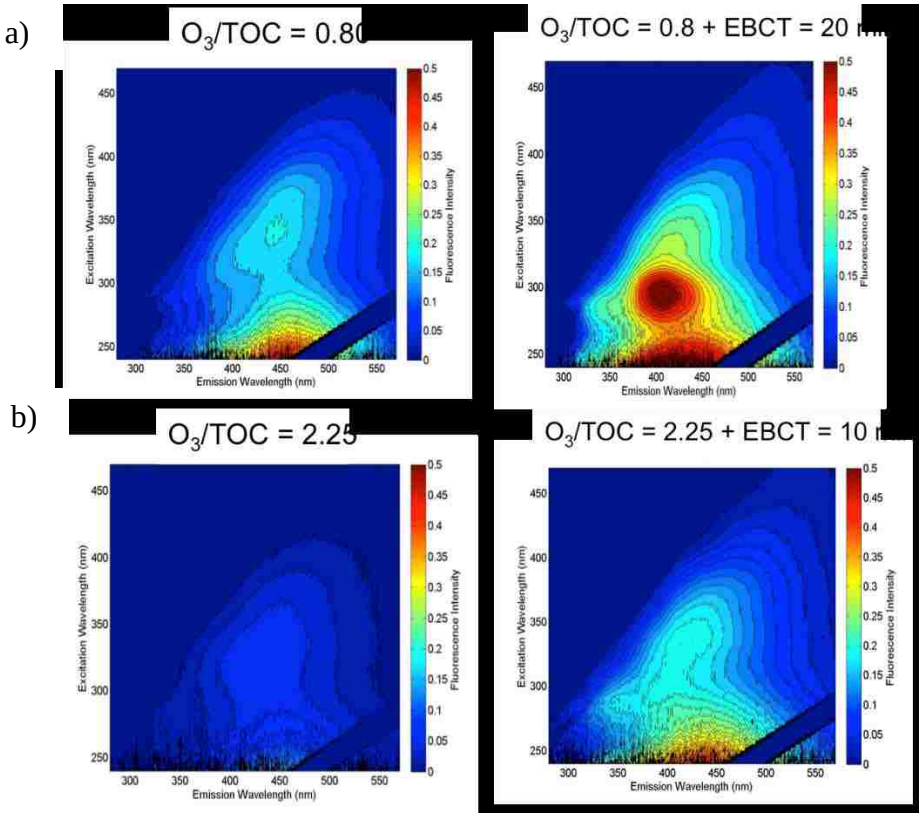

Figure 15 - Comparison of EEMs after ozonation+biofiltration

Table 16 – Total and regional fluorescence (AFU) after ozonation and biofiltration

	O_3/TOC = 0.8	EBCT = 20 min	O_3/TOC = 2.25	EBCT = 10 min
Total Fluorescence	6,617	11,510	2,046	7,897
Region I - SMP	1,303	3,561	473	2,506
Region II - Fulvic-like	3,795	5,959	1,130	3,883
Region III- Humic-like	1,519	1,990	443	1,509

AFU = arbitrary fluorescence units

3.4 – Conclusions

Even though TOC removal concurred with previous studies (Gerrity et al., 2011; Selvy, 2015; Knopp et al., 2016), the ozone-BAC process was not sufficient to achieve wastewater-derived TOC concentrations approaching 0.5 mg/L, as required by CDDW for potable reuse applications with no blending. As mentioned earlier, this 0.5-mg/L requirement may be overly conservative considering that typical TOC concentrations in drinking water are around 3 mg/L (Trussell et al., 2003; Snyder et al., 2012). Even so, a polishing process (e.g., adsorption with GAC, ion exchange) might still be required to achieve TOC concentrations in ozone-BAC effluents that are consistent with typical drinking waters. However, some studies have shown that the remaining organic matter after biological treatment may be composed of dissolved organic nitrogen (DON), which may be difficult to remove (Farré et al., 2011). Currently, it is not clear whether this residual organic matter poses significant concerns for public health. An analysis of disinfection byproduct formation potential would be useful for further characterizing the risks associated with this effluent TOC and is presented in the following chapter. This chapter, which focused on operational conditions in ozone-BAC systems and their impacts on TOC removal, resulted in the following conclusions:

- Ozone alone achieves significant TOC transformation, as determined by reductions in absorbance and fluorescence, but it does not reduce the TOC concentration in the treated effluent (i.e., no mineralization of bulk organics).
- TOC removal rapidly (EBCT < 10 minutes) plateaus at less than 10% for biofiltration without pre-ozonation. The minimum effluent TOC concentration achieved with biofiltration alone was 7.1 mg/L.

- As the pre-ozone dose increased, TOC removal also increased but then appeared to plateau at longer EBCTs, which is consistent with previous research (Selvy, 2015). TOC removal with ozone-biofiltration was up to 20% greater than with biofiltration alone.
- BAC was superior to anthracite with respect to TOC removal, presumably adsorption capacity was not completely exhausted in activated carbon, leading to greater removal of organics. Further analysis (quantification of adsorption capacity) is needed in order to evaluate this hypothesis.
- An O_3/TOC of 2.25 and an EBCT of 15 minutes achieved 30% TOC removal and a minimum effluent TOC concentration of 5.5 mg/L. This concentration is still considerably higher than the 0.5 mg/L TOC-benchmark in California, but is close to the median TOC concentration of 3 mg/L for drinking waters in the U.S. Therefore, additional polishing of ozone-BAC effluents may still be necessary unless an alternative TOC removal framework is developed for potable reuse applications.

Chapter 4 - Impacts of Ozone Dose and Empty Bed Contact Time on Disinfection Byproduct Mitigation

4.1 - Introduction

The disinfection process in water treatment is essential for the inactivation of pathogenic microorganisms responsible for waterborne diseases, such as cholera, typhoid, and dysentery. Disinfectants operate by oxidizing critical components of target microorganisms, including cell walls/membranes and genetic material, thereby hindering or preventing their ability to infect and colonize a human host. Despite their efficacy in reducing the risk of waterborne disease, disinfectants also react with a wide range of inorganic (e.g., bromide) and organic [e.g., natural organic matter (NOM)] constituents commonly found in water, which ultimately leads to the formation of potentially toxic disinfection byproducts (DBPs). Many DBPs pose risks to public health due to their potential carcinogenicity (Richardson, 2003), as research has shown that exposure to some DBPs can lead to bladder and colorectal cancer (Villanueva et al., 2004; Krasner, 2009; Kogevinas et al., 2011).

There are several commonly used disinfection processes in water treatment, such as chlorination, chloramination, and ozonation, and each process is responsible for the formation of a relatively unique class of DBPs. For example, chlorine disinfection typically results in the formation of regulated trihalomethanes (THMs) and haloacetic acids (HAAs) at the μg/L level, but a large percentage (~50%) of the total organic halides (TOX) formed during chlorination have not yet been identified (Richardson, 2003). Chloramination is often used to avoid or minimize the formation of THMs and HAAs but can lead to the formation of *N*-nitrosodimethylamine (NDMA) at the ng/L level. NDMA concentrations in drinking water distribution systems have been shown to be as low as 16 ng/L and as high as 630 ng/L after

chloramination (Krasner et al., 2013). Ozone is one of the most powerful oxidants in drinking water treatment and is particularly effective against disinfectant-resistant pathogens, such as *Cryptosporidium* and *Giardia*, but ozonation of bromide-containing waters results in the formation of bromate at the μg/L level. A survey of different drinking water treatment plants in Switzerland found that bromate levels above 10 μg/L (i.e., the USEPA maximum contaminant level) generally only occur with typical ozone dosing conditions when bromide levels in the source water are >50 μg/L (von Gunten and Salhi, 2003). The average bromide concentration in Switzerland was found to be 25 μg/L (von Gunten and Salhi, 2003), thereby suggesting minimal risk of excessive exposure to bromate. However, ozonation in bromide-containing wastewaters might pose a challenge for implementation of ozone-biofiltration systems in potable reuse applications.

Free chlorine is the most common disinfectant used in water treatment because of its broad efficacy as a primary disinfectant and ability to maintain a relatively stable residual for secondary disinfection in the distribution system. However, its reaction with organic carbon, particularly higher molecular weight humic compounds, present in the water leads to the formation of regulated and unregulated DBPs. In general, THMs and HAAs are the two major classes of halogenated DBPs that form during chlorination (Krasner et al., 2006). In the U.S., the four regulated THMs, specifically chloroform, bromoform, bromochloromethane, and dibromochloromethane, comprise the total trihalomethanes (TTHMs), which are regulated collectively at 80 μg/L. Reactions between organic matter and chlorine also result in the formation of haloacetic acids (HAAs). In the U.S., the five regulated HAAs, specifically monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA),

monobromoacetic acid (MBAA), and dibromoacetic acid (DBAA), comprise the HAA5s, which are regulated collectively at 60 µg/L.

One study of drinking water sources with TOC concentrations ranging from 1-4 mg/L observed average TTHM concentrations of 85 ± 34 µg/L and HAA6 concentrations of 55 ± 20 µg/L after chlorination (Summers et al., 1996). In another study, Krasner et al. (2006) conducted a DBP survey of 12 drinking water treatment plants and found that in raw waters with a median TOC concentration of 5.8 mg/L, the maximum TTHM concentration was 164 µg/L. On the other hand, DBP formation potential testing of secondary wastewater effluent resulted in a TTHM yields of 23 µg/mg-DOC and an HAA yield of 21 µg/mg-DOC (Sirivedhin and Gray, 2005; Liu et al., 2010). For low DOC concentrations consistent with the aforementioned drinking water studies, expected TTHM concentrations would be 69 µg/L for a DOC of 3 mg/L and 138 µg/L for a DOC of 6 mg/L. The corresponding HAA5 concentrations would be 63 µg/L and 126 µg/L, respectively. Therefore, DBP formation in wastewater matrices appears to be higher, which might be expected because of the complexity of the effluent organic matter (EfOM), although it can be challenging to directly compare studies that use different approaches to assess DBP formation potential.

The regulated THMs and HAAs represent only a small fraction of the TOX found in water after chlorination (TTHMs = 20% and HAA5s = 10%) (Richardson, 2003). The remaining TOX is currently unregulated (e.g., bromochloroacetic acid, chloral hydrate, halonitromethanes, haloacetonitriles, cyanogen chloride, and haloacetadehydes) or currently unknown. (Richardson, 2003; Krasner 2009). Relative toxicity is also an important consideration when assessing the significance of DBP formation. Genotoxicity and cytotoxicity studies have found that brominated compounds are generally more cytotoxic (i.e., leading to cell death) and genotoxic

(i.e., causing genetic mutations) than their chlorinated analogs (Plewa et al., 2002). Moreover, unregulated iodinated THMs (e.g., $CHCl_2I$, CHBrClI, $CHBr_2I$, $CHClI_2$, $CHBrI_2$, and CHI_3) can be more toxic than their brominated and chlorinated counterparts (Richardson, 2003; Krasner, 2009). Recent studies have also indicated that other emerging DBPs, such as halonitromethanes (HNMs), haloacetonitriles (HANs), and haloacetadehydes, are more toxic than currently regulated DBPs based on *in vitro* mammalian cell assays (Muellner et al., 2007).

Considering that disinfection is an essential process in drinking water treatment, DBP formation poses a significant concern for agencies considering adoption of potable reuse. Potable reuse is a promising option for water supply augmentation in places currently facing drought conditions. Potable reuse has been studied extensively and even implemented in several states across the U.S., including Arizona, California, Colorado, Florida, Nevada, Texas, and Virginia, and also across the globe, including Namibia, Australia, and Singapore (Gerrity et al., 2013). However, potable reuse can pose unique or magnified challenges depending on the level of treatment provided prior to reuse. Although treatment trains with ozone-biofiltration or reverse osmosis have been deemed ‘equivalent’ on the basis of public health criteria (Trussell et al., 2016), the final product water in each system will likely be very different, particularly with respect to organic content. Final disinfection with chlorination, for example, will likely lead to different DBP profiles.

The implications of the finished water quality will also differ in indirect potable reuse (IPR) versus direct potable reuse (DPR) systems. IPR is characterized by replenishing surface or groundwater with treated wastewater to augment the water source of a community. On the other hand, DPR involves directly introducing advanced treated water into drinking water distribution systems, or blending it either upstream or downstream of drinking water treatment plants. When

considering DPR applications, DBP mitigation poses even greater concerns because of the lack of an environmental buffer. Thus, all public health criteria and drinking water regulations must be satisfied by the advanced treatment processes.

Even though the USEPA has not yet established a set of regulations for potable reuse at the federal level, regulations have been defined at the state level in some places. The California Division of Drinking Water set a maximum of 0.5 mg/L of wastewater-derived TOC and mandated the use of reverse osmosis (RO) followed by advanced oxidation—a treatment train known as "full advanced treatment" (FAT)—for direct injection into local aquifers or for surface water augmentation (CDPH, 2014). Justification for these stringent requirements includes ensuring compliance with DBP regulations and also addressing unregulated and even unknown contaminants that might be unique to wastewater matrices. The Groundwater Replenishment System (GWRS) in Orange County, California, is an example of an FAT system composed of microfiltration (MF), RO, and advanced oxidation with UV/H_2O_2. This treatment train essentially guarantees compliance with the 0.5-mg/L TOC benchmark and minimal DBP formation, with the exception of NDMA.

However, other places use different approaches to regulate bulk organic matter in potable reuse applications. For examples, Florida has a TOC limit of 3 mg/L (Schimmoller et al., 2015), which might allow for alternative treatment trains, assuming other MCLs could still be met (e.g., TTHM and HAA5 MCLs). Alternatives to FAT are particularly appealing because of the high costs, energy consumption, and brine disposal requirements associated with RO-based treatment trains. In fact, adoption of ozone-biofiltration instead of FAT could allow for capital and annual O&M savings of $25-$51 million and $2-$4 million, respectively, for a 10 million-gallon-per-day potable reuse facility (Gerrity et al., 2014). As demonstrated in the previous chapter, ozone

is capable of transforming organic matter into simpler, smaller, and more bioavailable molecules that could subsequently be removed in a downstream biofiltration system. In fact, published studies have demonstrated that ozone-biofiltration can achieve up to 50% bulk organic removal (Gerrity et al, 2011; Pisarenko et al, 2012; Gerrity et al, 2014). This would presumably lead to reduced DBP formation upon final chlorination, although this has not yet been studied in sufficient detail.

As such, this study investigates the impacts of operational parameters in ozone-biofiltration systems, specifically ozone dose and empty bed contact time (EBCT), on the formation and mitigation of DBPs upon final disinfection with free chlorine. Experimental results from the previous chapter revealed that higher ozone doses coupled with longer EBCTs enhanced TOC removal (maximum of 30%), but the impact on DBP formation was not evaluated during those experiments. This phase of the research couples the evaluation of ozone dose and EBCT with both TOC removal and DBP formation potential. Another aspect of this phase of the research was the potential development of an alternative framework for TOC removal in potable reuse applications. In conventional drinking water applications, the USEPA's Stage 1 Disinfectant and Disinfection Byproducts Rule (D/DBPR) mandates certain levels of TOC removal based on source water TOC and alkalinity (Table 17), with the ultimate goal of controlling DBP formation to ensure MCL compliance. Accordingly, a similar approach could be proposed for potable reuse applications.

Table 17 - U.S. EPA Stage 1 D/DBPR for TOC removal

Source Water TOC (mg/L)	Source Water Alkalinity (mg/L as $CaCO_3$)		
	0 - 60	**> 60 to 120**	**> 120**
> 2.0 to 4.0	35.0%	25.0%	15.0%
> 4.0 to 8.0	45.0%	35.0%	25.0%
>8.0	50.0%	40.0%	30.0%

4.2 - Materials and Methods

4.2.1 – Pilot Unit

The configuration of the pilot-scale ozone-biofiltration system (referred to as PR1 in this phase of the research) was identical to the one described in the previous chapter. However, the various feed and effluent waters were also chlorinated in this phase of the research to evaluate THM and HAA formation (chlorination protocol described later). Additional samples were collected from a similar 7.6-liter-per-minute pilot-scale ozone-biofiltration system (PR2) that was located at a different water reclamation facility in the Las Vegas valley. PR2 received tertiary effluent (anthracite biofilters) from the full-scale treatment plant, ozonated the water at a dose of ~3.2 mg/L (O_3/TOC = 0.7), and then fed the ozonated effluent into three parallel biofiltration columns (BAC with EBCT = 10 min, BAC with EBCT = 20 minutes, and anthracite with EBCT = 10 minutes).

4.2.2 – Sampling Events

Sampling in PR1 occurred after an acclimation period of three weeks to allow for stabilization of the microbial community in the biofilters. TOC removal was used as a surrogate to characterize microbial growth and activity during the acclimation period. For each sampling

event, the ozone dose was adjusted by changing the flow rate of the oxygen concentrator. This altered the efficiency of the ozone generator, which ultimately changed the ozone feed gas concentration and the applied ozone dose. The applied ozone dose was estimated as an O_3/TOC ratio using correlations with changes in UV_{254} absorbance previously developed by Selvy (2015) for the same wastewater matrix. The relationship is shown in **Equation 1**. After any operational adjustments, sufficient time was allowed for the ozonated water to travel through the contactors and then the biofiltration columns and achieve new steady state conditions (i.e., 3 times the theoretical hydraulic retention time). Samples were collected for O_3/TOC ratios ranging from 0 to 2.25 and EBCTs from 2 to 20 minutes (n = 78). Table 18 describes each sampling condition and the corresponding number of samples.

Table 18 - Sampling points and corresponding number of samples

Sample Point	Ozone/TOC	EBCT (min)	Number of samples (n)
MBR Filtrate	-	-	7
Non-Ozonated BAC	-	2 - 20	25
Ozonated Effluent	0.1 – 2.25	-	7
Ozonated BAC	0.1 – 2.25	2 - 20	25
Ozonated Anthracite	0.1 – 2.25	2 - 20	25

4.2.3 – Quantification and Characterization of Nutrients and Bulk organics

Similar to previous chapter, organic matter characterization was accomplished with UV and fluorescence spectroscopy. Absorbance and fluorescence spectra were developed using an Aqualog spectrofluorometer (Horiba, Edison, NJ). The excitation-emission matrices (EEMs) were created for each sample by scanning over an excitation range between 240 nm and 470 nm

with an emission wavelength increment of 0.82 nm. Data processing included corrections for the inner filter effect and Rayleigh masking and development of the EEMs in Matlab (MathWorks, Natick, MA). Raman correction was also performed in order to allow for direct comparisons between different samples analyzed in different laboratories. The EEMs were divided into three regions (previously shown in Figure 7) to further characterize the organic matter. TOC concentration was used to quantify bulk organic matter present in the samples. TOC was measured as non-purgeable organic carbon (NPOC) using a Shimadzu TOC V-csn (Kyoto, Japan). TOC samples were collected in 40 mL amber vials with Teflon-lined lids and analyzed in duplicate or triplicate (less than 5% relative standard deviation). All samples were acidified with 2 N hydrochloric acid (HCl) to reduce the pH to less than 2. Low range ammonia analysis was done for each sample using Hach Method 10023 (salicylate method; 0.02-2.5 mg-N/L) and a DR 900 multiparameter handheld colorimeter (Hach, Loveland, CO).

4.2.4 – Chlorination and Uniform Formation Conditions Approach (UFC)

In order to estimate DBP formation, typical distribution system conditions such as pH, temperature, time, and disinfectant residual must be simulated in bench-scale tests. These bench-scale experiments can employ different approaches, such as the formation potential (FP) test or the uniform formation conditions (UFC) approach (Summers et al., 1996). The FP approach targets maximum DBP formation by employing higher chlorine doses than might typically be used in a full-scale application (e.g., chlorine residuals of 3-5 mg/L) and longer incubation time (e.g., 2-7 days). This approach has been shown to be less characteristic of real distribution system conditions (Summers et al., 1996). On the other hand, the UFC approach adopts conditions that are more characteristic of real systems, and the conditions are consistent across different studies, thereby enabling more direct comparisons of DBP formation between different

water sources. In the UFC approach, the water sample is adjusted to pH 8 using borate buffer, chlorinated, and incubated in the dark at 20°C for 24 hours. The UFC approach targets 1 mg/L of free chlorine residual at the end of the incubation period. The UFC approach was selected for this study to achieve DBP formation results that would be consistent with an actual drinking water application.

Because some samples had higher concentrations of ammonia either due to operational upsets at full-scale or apparent formation during biofiltration, an assessment of breakpoint chlorination requirements was performed to estimate chlorine doses necessary to achieve the target free chlorine residual (1-mg/L after 24 hours). Similar to the aforementioned O_3/TOC dosing framework, chlorine dosing was evaluated in the context of chlorine/ammonia ratios (i.e., to address demands due to reactions with ammonia) and chlorine/TOC ratios (i.e., to address demands due to reactions with bulk organic matter). The relationships developed during this preliminary chlorine demand testing were used during the subsequent DBP formation assessments.

As mentioned earlier, DBP assessment was performed with the UFC approach (Summers et al., 1996). Chlorine-demand-free 250-mL amber bottles were used. Before chlorination, the water samples were buffered to pH 8.0 with 2 mL/L of pH 8.0 borate buffer. A 1000-mg/L (as Cl_2) free chlorine (HOCl) stock solution was prepared with 7.5% available free chlorine sodium hypochlorite solution and stored at room temperature. The stock solution was also buffered with the borate solution to achieve a hypochlorite solution at pH 8. Decay of the free chlorine stock solution was monitored to ensure proper dosing conditions. After chlorination, the water samples were incubated in the dark at ~20°C for 24 hours. After incubation, the free chlorine residual was measured using Hach Method 8021 with a DR900 multiparameter handheld colorimeter (Hach,

Loveland, CO). Samples were transferred to 40 mL amber vials and quenched with 65 mg of ammonium chloride for subsequent THM analysis or 0.25 ml of sodium thiosulfate (8%) for subsequent HAA analysis.

4.2.5 – THM and HAA Quantification

DBP samples were sent to a third-party laboratory (Eurofins Eaton Analytical, Monrovia, CA) for analysis. Concentrations of HAAs were determined using gas chromatography with electron capture detection (Standard Method 6251B), and THM concentrations were measured using capillary column gas chromatography mass spectrometry (USEPA Method 524.2).

4.3 Results and Discussion

4.3.1 – Influent Water Quality

Influent samples for PR1 were characterized by an average temperature of 27°C, a dissolved oxygen concentration of 2.8±0.5 mg/L, and a pH of 6.9±0.3. The average TOC concentration and UV_{254} absorbance were 7.9±0.4 mg/L and 0.162 ± 0.011 cm^{-1}, respectively. The ammonia concentration in the influent varied from 0.03 to 4 mg/L during the experimental period due to operational upsets in the nitrification process in the full-scale MBR feeding the pilot. Table 7 summarizes the influent water quality for PR1 during the study. Influent samples for PR2 (i.e., tertiary wastewater effluent from a separate full-scale facility) were characterized by a DOC concentration 4.5 mg/L with negligible ammonia and nitrite.

4.3.2 – Development of a Chlorine Dosing Framework

Because significant ammonia concentrations were detected in some samples, breakpoint chlorination and chlorine demand tests were performed to develop a chlorine dosing framework for this application. Specifically, a multivariate linear regression was developed to estimate required chlorine doses when targeting a free chlorine residual of 1±0.4 mg/L after 24 hours in

the presence of bulk organic matter (i.e., TOC) and ammonia. **Equation 2** represents the relationship between TOC concentration, ammonia concentration, and target chlorine dose for the UFC testing approach. Figure 16 demonstrates the similarity between the required chlorine dose determined during experimentation and the predicted chlorine dose using the multivariate regression equation. Typical breakpoint conditions require chlorine/ammonia (i.e., mg Cl_2/mg N) of 7-8:1 or greater (McDonald, 2003; Metcalf and Eddy, 2003), which is consistent with the mass ratio observed in this study (>12:1) due to the high chlorine demand by the ammonia concentration present in some samples. However, the UFC approach requires consideration of demand due to other constituents, with bulk organic matter being the most significant constituent in wastewater applications. The regression model indicates an approximate 1:1 demand caused by the bulk organic matter. By accounting for both of these critical water quality parameters, it is possible to estimate the chlorine dose yielding a 1-mg/L free chlorine residual after 24 hours of incubation. This reduces the number of required bottles/samples compared to a purely trial-and-error approach.

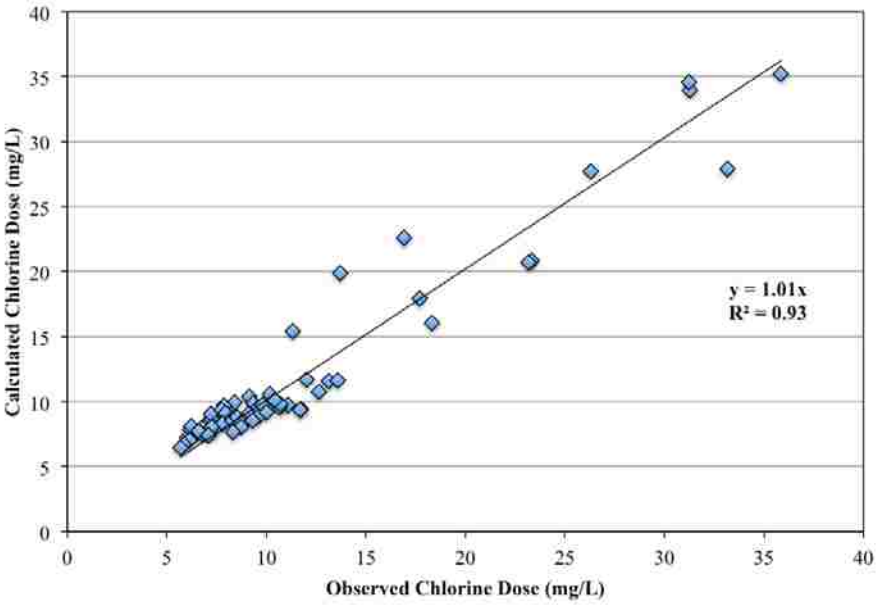

Figure 16 - Multivariate chlorine dose correlation

$$\text{Chlorine dose (mg/L as } Cl_2) = 8.2 \times NH_3 \text{ (mg-N/L)} + 1.2 \times \text{TOC (mg/L)} \quad \textbf{Equation 2}$$

4.3.3 – Bulk Organic Transformation and DBP Formation

Prior to evaluating DBP formation potential in the pilot-scale effluents, ambient DBP levels were quantified in the MBR filtrate before and after chlorination. Ambient concentrations of both TTHMs and HAA5s were below 2 µg/L. After chlorination with the UFC approach, the MBR filtrate generated in average 226±23 µg/L of TTHMs and 139±28 µg/L of HAA5s. The next phase of testing involved evaluations of DBP mitigation with ozone, biofiltration, and ozone-biofiltration.

4.3.3.1 – Ozonation Only

There was no significant removal of TOC after ozone alone (approximately 2% on average) in both PR1 and PR2. However, the reduction in UV_{254} absorbance and fluorescence indicated considerable transformation of organic matter after ozonation (Table 19 and Figure 17). Previous research has shown that typical ozone dosing conditions are generally ineffective for mineralizing bulk organics but is able to transform recalcitrant organics into simpler, more bioavailable, oxygen-rich moieties (e.g., carboxylic acids, aldehydes, and ketones), thereby enhancing biodegradation in downstream biological processes (Linlin et al., 2011; Reungoat et al., 2012; Santos et al., 2013; Stalter et al., 2010).

On average, ozone alone was capable of reducing TTHM formation by 13% and HAA5 formation by 31% upon chlorination. Similar research with natural water and secondary effluent observed reduction in TTHMs of 17-48% (Hua and Reckhow, 2007) and 12-18% (Linlin et al., 2011) after pre-ozonation, respectively. Previous studies have also shown that UV absorbance and fluorescence can be used as surrogates to estimate DBP precursor abundance and subsequent formation (Chen and Westerhoff, 2010). Effluent organic matter (EfOM) contains significant quantities of degradation products and soluble microbial products (SMPs), and it has been categorized as a significant source of precursors for chlorine DBPs (Krasner, 2009). Specific ultraviolet absorbance (SUVA), which is UV absorbance standardized to the total or dissolved organic carbon concentration, is also used for organic characterization efforts. Higher SUVA values are characteristic of higher aromatic content (e.g., humic substances), which has been shown to be a principal predictor of chlorinated DBP formation (Weishaar et al., 2003). In the current study, higher ozone doses led to reductions in UV_{254} absorbance, SUVA, and

fluorescence, thereby indicating a decrease in aromatic content of the wastewater (Table 19). These changes were also correlated with reductions in DBPs, as shown in Figure 18.

Table 19 – Changes in bulk organic surrogate parameters during ozonation

O_3/TOC[a] ratio	% reduction in UV_{254} Absorbance	SUVA (L/mg-m)	Total Fluorescence reduction (%)
Influent	-	2.04 ± 0.07	-
0.07	2%	2.10	6%
0.13	15%	1.73	42%
0.74	44%	1.12	80%
0.80	46%	1.02	86%
0.94	51%	1.02	86%
1.50	55%	0.95	91%
2.25	62%	0.80	96%

a. Ozone/TOC ratios were estimated based on correlation with UV_{254} absorbance according to **Equation 1**

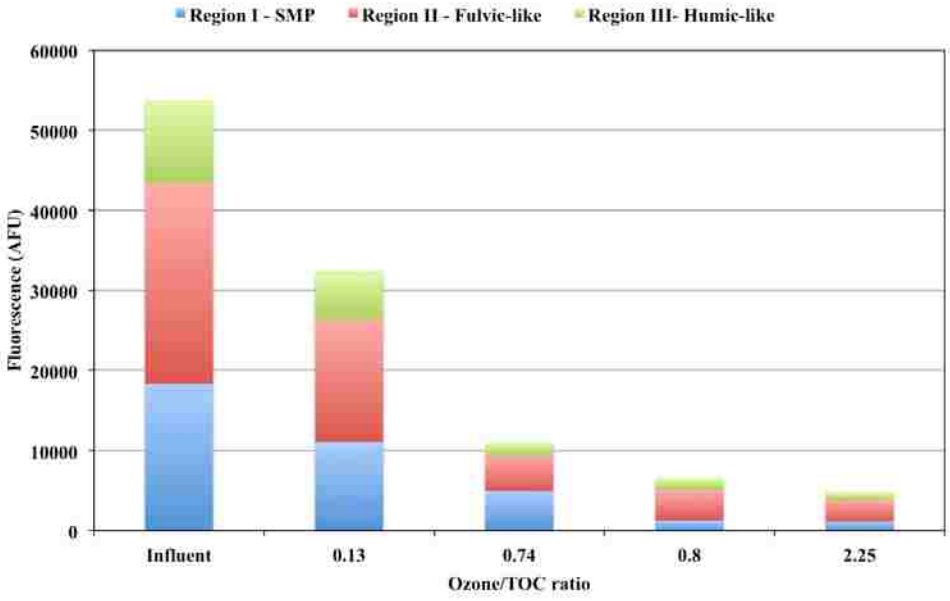

Figure 17 - Fluorescence according to each region

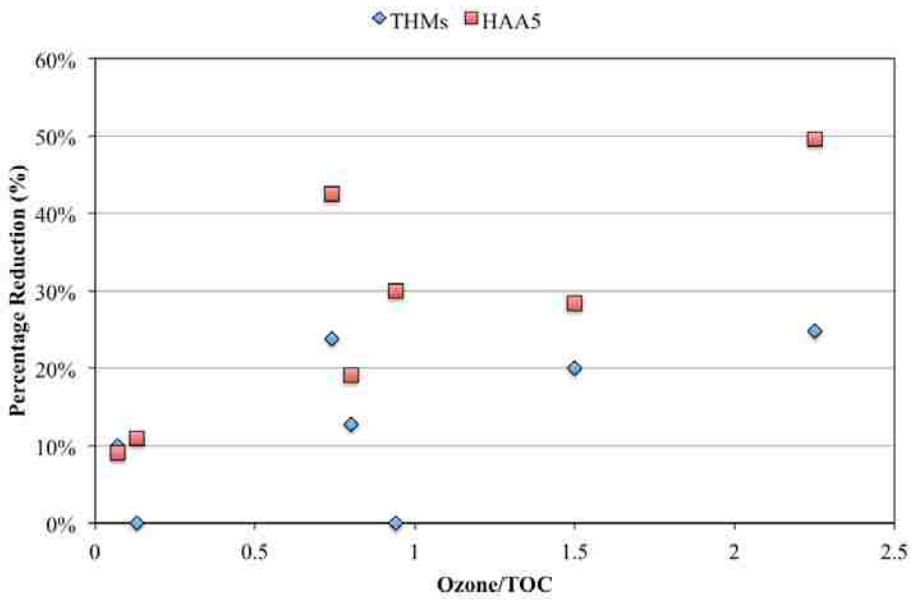

Figure 18 - Percentage removal attributable due to ozonation only

4.3.3.2 – Biofiltration and Ozone-Biofiltration

No direct correlation was found between longer EBCTs with biofiltration alone and DBP mitigation, as shown in Figure 19 (TTHMs) and Figure 20 (HAA5s). However, preozonation enhanced DBP mitigation during biofiltration. The reduction in DBP formation plateaued at relatively short EBCTs, which was consistent with the relationship between EBCT and TOC removal. On average, the combination of ozone and biofiltration achieved an 18% reduction in TTHMs and a 34% reduction in HAAs , whereas biofiltration alone was able to achieve a 9% reduction in TTHMs and a 15% reduction in HAAs. As mentioned earlier, pre-ozonation transforms particulate, hydrophobic, and microbially-derived organic matter into non-humic, lower molecular weight, and more biodegradable compounds (Reaume et al., 2015; Hollender et al., 2009), thereby achieving initial reductions in DBP formation potential, and then the subsequent biofiltration process actually removes the organic precursors from the water, thereby

achieving even greater reductions in DBP formation potential. Biofiltration alone is able to remove some precursors, but without the pre-ozonation step, many of the biologically recalcitrant compounds may still react with free chlorine to form DBPs.

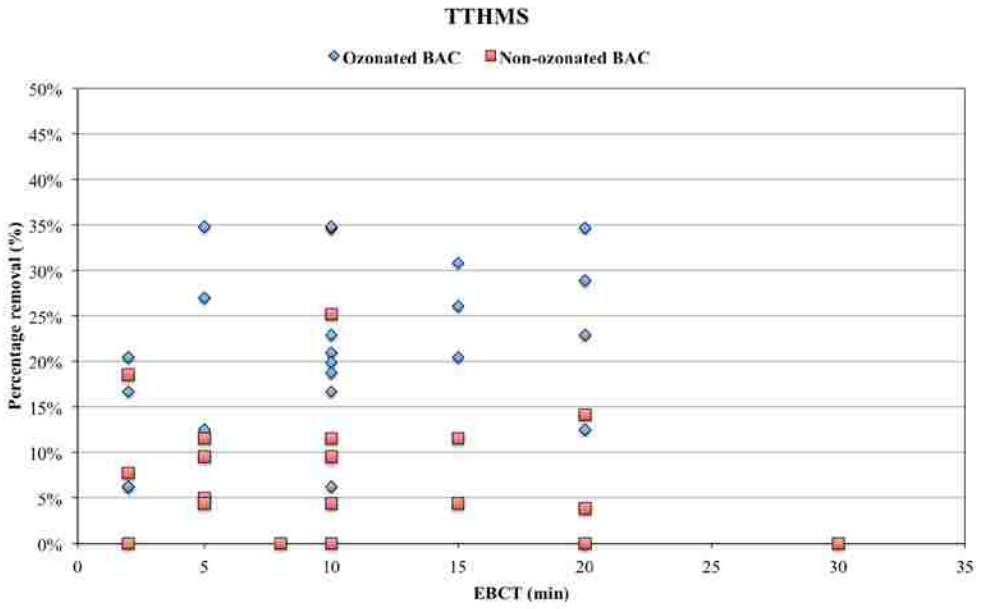

Figure 19 - TTHMs percentage removal attributable due to biofiltration only

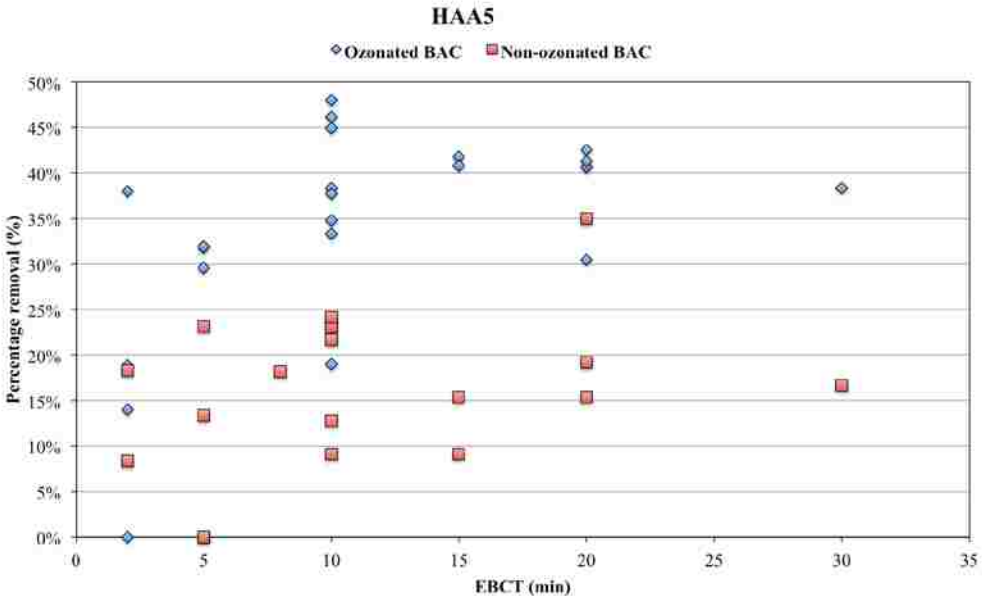

Figure 20 – HAA5 percentage removal attributable due to biofiltration only

4.3.4 – Speciation of DBPs

Chloroform was the major THM species formed during chlorination, with an average concentration of 143±43 µg/L in the MBR filtrate, which is consistent with other studies of THM formation in low bromide-containing waters (Chu et al., 2012). Chloroform precursors are often highly abundant in water matrices and generally consist of aromatic compounds such as phenols, β-ketones, and proteins (Weishaar et al., 2003). Brominated halogens were formed at lower concentrations. In the MBR filtrate, bromodichloromethane averaged 48±10 µg/L, dibromochloromethane averaged 14±3 µg/L, and bromoform was generally <0.7 µg/L. The formation of brominated DBPs is generally limited by the low initial bromide concentrations of many environmental waters (Farré et al., 2011).

In addition to being the most abundant species, chloroform also experienced a greater relative reduction in formation potential after ozonation, which suggests that chloroform

precursors are more likely to be hydrophobic, high molecular weight aromatics (Farré et al., 2011). Greater reductions in chloroform were also observed at higher ozone doses (Figure 22). The same trends were not observed for the more brominated compounds. In fact, the formation potentials of the brominated compounds often increased after pre-ozonation. For example, dibromochloromethane increased by 34%, and bromoform increased by 50%. Farré et al. (2011) also observed an increase in dibromochloromethane after ozonation from 11 µg/L to 15 µg/L. Nevertheless, the 30% reduction in the more abundant chloroform species yielded a net reduction in TTHMs following pre-ozonation and final chlorination. Although this is advantageous in terms of the regulatory framework for MCLs, the relative impacts on the toxicity of the final effluent should also be considered because brominated compounds have been shown to be more cytotoxic and genotoxic (Richarson, 2003).

Ozonation was able to considerably reduce all five of the regulated HAAs. Dichloroacetic acid and trichloroacetic acid were the most abundant species after chlorination. Bromoacetic acids and dibromoacetic acid were present at low concentrations in the MBR effluent (2±0.25 µg/L), but similar to the brominated THMs, their formation potentials actually increased after ozonation (bromoacetic acid = 19% increase and dibromoacetic acid = 35% increase), thereby suggesting that ozonation facilitates bromine substitution after chlorination (Figure 23). In the presence of bromide, hypochlorous acid oxidizes bromide to hypobromous acid, thus reacting with precursors in water to form brominated DBPs. Liang and Singer (2003) suggested that hypobromous acid is more reactive with lower molecular weight and more hydrophilic precursors, explaining the increase of formation of brominated DBPs after chlorinating ozonated samples. Supporting that, Xu et al. (2007) observed that smaller molecular weight compounds

(~1kDa) yielded higher formation of brominated THMs. Table 20 summarizes the concentration of DBPs in the MBR and ozonated effluent after chlorination.

Table 20 - Average DBP speciation of MBR effluent and ozonated effluent

Species	Average concentration Influent (ug/L)	Average concentration Ozonated Effluent (ug/L)
Bromoacetic Acid	2 ± 0.3	3 ± 0.4
Chloroacetic Acid	7 ± 2	10 ± 3
Dibromoacetic Acid	2 ± 0.2	3 ± 1
Trichloroacetic Acid	58 ± 30	27 ± 9
Dichloroacetic Acid	59 ± 18	54 ± 12
Haloacetic acids (HAA5)	123 ± 47	90 ± 22
Bromodichloromethane	48 ± 10	45 ± 9
Bromoform	0.7 ± 0.2	2 ± 0.8
Chloroform	143 ± 43	105 ± 36
Dibromochloromethane	14 ± 3	18 ± 6
Total Trihalomethanes (TTHMs)	207 ± 51	169 ± 34

Biofiltration without preozonation was able to decrease very little amount of chloroform (Figure 21). This can be related to chloroform precursors are higher molecular weight compounds that are not biodegradable. In the absence of ozone, the microbial community is not able to absorb and degrade these compounds. However, biofiltration following ozonation was able to further reduce chloroform formation, as well as formation of trichloromethane, dichloro- and trichloroacetic acid precursors as also observed by Farré et al. (2011). There was a notorious variation on HAA5s formation during chlorination of the influent (i.e., MBR effluent), as observed in Figure 23. This can be explained due the variation in water quality during the study.

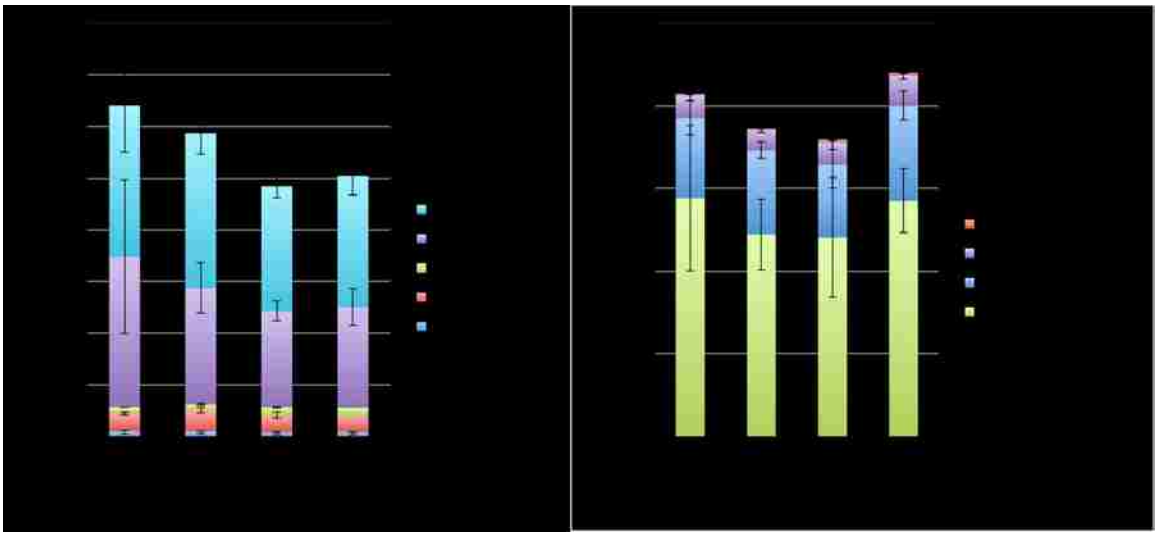

Figure 21 – Average speciation of DBPs in non-ozonated BAC effluent.

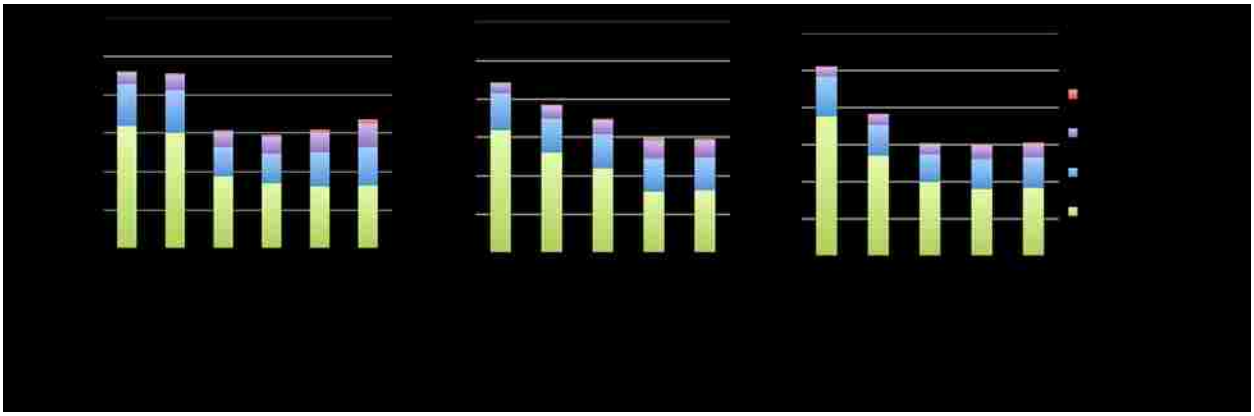

Figure 22- THM speciation of ozone-biofiltration samples

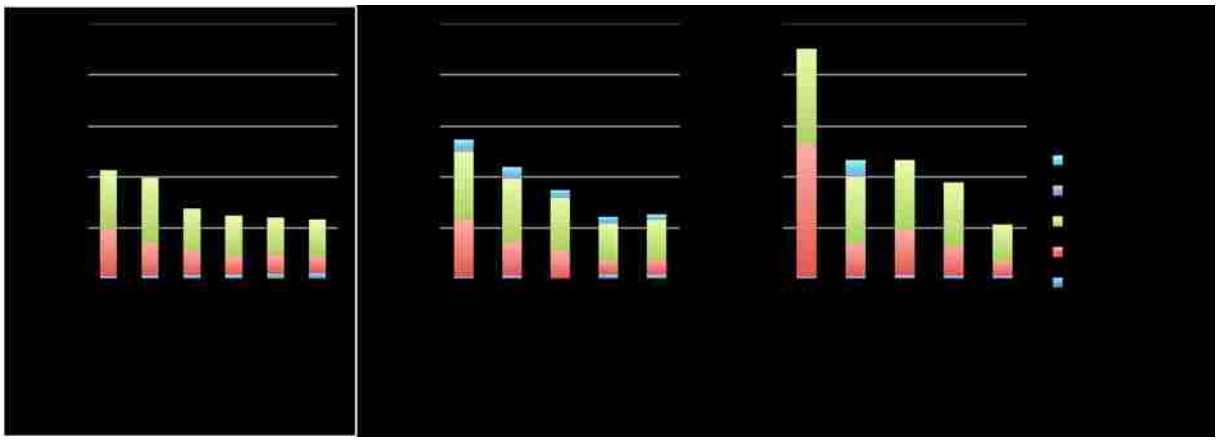

Figure 23 - HAA speciation of ozone-biofiltration samples

4.3.4 – Empirical Correlation between DBP Formation and TOC Concentrations

Table 21 represents a summary of DBP formation potentials and the corresponding removals after treatment in PR1. With the operational conditions employed in this study, which essentially capture the practical ranges for full-scale treatment, ozone-biofiltration was unable to achieve the USEPA MCLs for TTHMs and HAA5s. Therefore, additional TOC removal—or lower influent TOC concentrations to the ozone-biofiltration system—would have to be achieved in a DPR-type application. For PR2, in which the influent TOC concentrations were much lower (4.5 mg/L), the formation potentials of the effluent after ozone (O3/TOC = 0.7) and BAC (EBCT = 20 min) were 70.5 µg/L and 27 µg/L for TTHMs and HAA5s. Although this is still not ideal considering the TTHM concentration is just below the USEPA MCL, the results from PR2 demonstrate that some ozone-biofiltration systems may achieve adequate levels of TOC removal to satisfy DBP regulations. During the current study, in average, specific formation of DBPs (SFDBP; SFTTHM, SFHAA5) presented 26 µg-TTHMs/mg-DOC and 12 µg-HAA5s/mg-DOC,

which were similar to previous studies: 23 µg-TTHMs/mg-DOC and 21 µg-HAA5/mg-DOC (Sirivedhin and Gray, 2005; Liu et al., 201).

Table 21 - Average DBP concentration after Ozone-BAC treatment in PR1

	THMs (µg/L)	% Reduction	HAA5s (µg/L)	% Reduction
MBR Filtrate	226 ± 23	-	139 ± 38	-
BAC	206 ± 34	9%	102 ± 12	27%
Ozone	196 ± 34	13%	96 ± 15	31%
Ozone+ Anthracite	170 ± 33	25%	73 ± 18	48%
Ozone+BAC	156 ± 15	31%	61 ± 17	56%

Instead of focusing on potential correlations between ozone dose and/or EBCT, an alternative approach was used to exploit the fact that DBP formation is closely correlated with EfOM content (Farré et al., 2011). Specifically, correlations were developed between DBP formation potential and TOC concentration (Figure 24). The regression equation for TTHMs was developed based on a combination of the data from PR1, PR2, and independent data from Summers et al. (1996), whereas the regression equation for HAA5s was developed only with data from PR1 and PR2. All included data were consistent with the results from the current study.

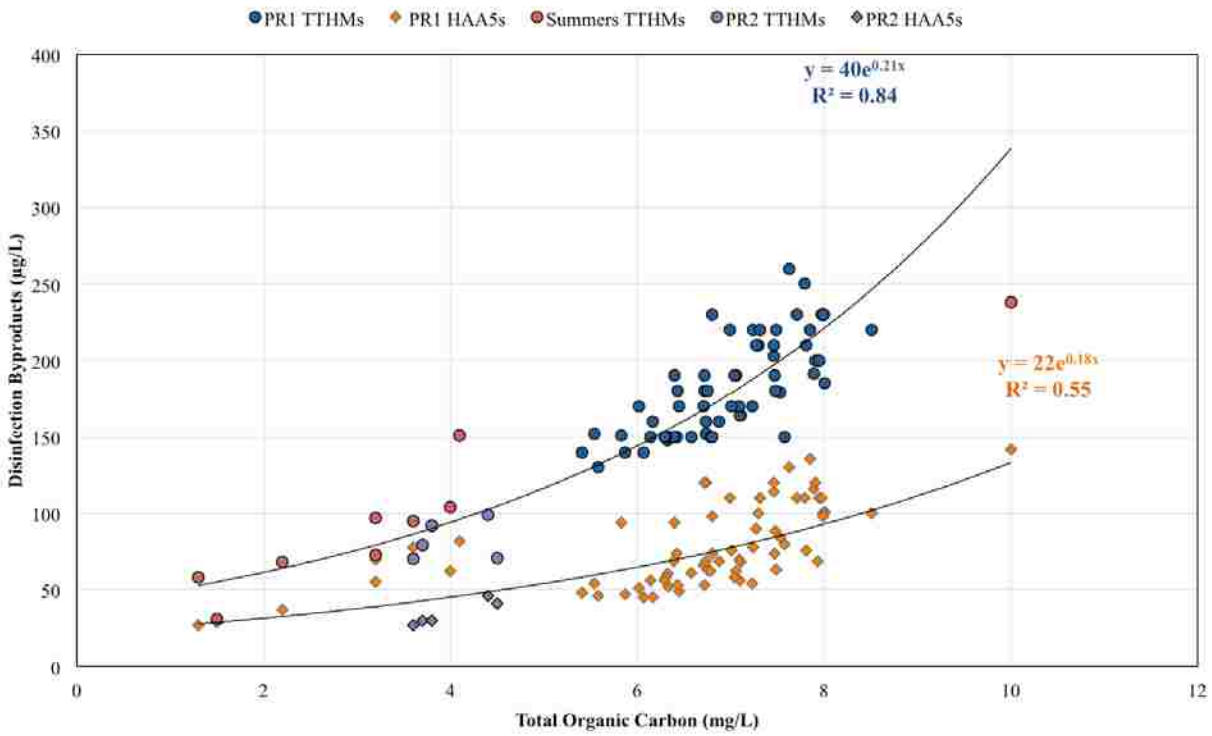

Figure 24 – Exponential correlation between DBP formation and TOC

For TTHMs correlation formation potential tests (UFC) from PR1, PR2 and Summers et al. (1996) were included. For HAA5 correlation data from PR1 and PR2 were included.

$$\text{TTHMs } (\mu g/L) = 40e^{0.21(TOC)} \qquad \textbf{Equation 3}$$

$$HAA5s\ (\mu g/L) = 22e^{0.18(TOC)} \qquad \textbf{Equation 4}$$

According to the exponential regression models illustrated in Figure 24, compliance with the USEPA MCLs for HAA5s and TTHMs would require TOC concentrations of 5.6 mg/L (**Equation 4**) and 3.3 mg/L (**Equation 3**), respectively. However, the inclusion of a safety factor is important for guaranteeing MCL compliance at all times. With a safety factor of 1.25 for the final DBP concentrations (i.e., TTHMs = 60 µg/L and HAA5s = 45 µg/L), a maximum TOC of

~2 mg/L would be required for the effluent from the ozone-biofiltration system, with THMs governing compliance.

4.4 – Conclusion

As a result of this assessment of THM and HAA formation in ozone-BAC effluents, the following can be concluded:

- A multivariate linear correlation was established between ammonia, TOC, and the applied chlorine dose necessary to achieve a free chlorine residual of 1 mg/L after 24 hours with the UFC approach.
- Ozonation (without biofiltration) was able to accomplish minimal TOC removal but significant TOC transformation, thereby reducing TTHMs and HAA5s by 13% and 31%, respectively.
- Biofiltration in the absence of pre-ozonation was able to reduce TTHM and HAA formation by 9% and 27% respectively.
- The combination of ozone and biofiltration reduced TTHMs by up to 31% and HAA5s by up to 56%. Also, BAC was superior to anthracite for TOC removal and DBP mitigation.
- Based on a relatively strong correlation between DBP formation and effluent TOC, a maximum TOC concentration of 3.3 mg/L was identified as the threshold for non-compliance with the TTHM MCL. With a safety factor of 1.25 on the TTHM concentration, a revised maximum TOC concentration of ~2 mg/L was identified.
- These TOC targets (i.e., 2-3.3 mg/L) are more achievable for ozone-biofiltration systems when compared to the 0.5-mg/L target in California. These revised targets are more

consistent with typical TOC concentrations in surface water and can also be justified in the context of public health impacts.

These results indicate that there is potential to develop regulations for potable reuse applications that are consistent with those intended for more conventional drinking water sources.

Chapter 5 - Microbial Community Analysis of Biofiltration Systems

5.1 – Introduction

Disinfection is an important process in water treatment for the inactivation of pathogenic organisms (e.g., protozoa, bacteria, and viruses), but not all microbes are pathogenic—some are beneficial for water and wastewater treatment processes. For example, biofiltration is a process often adopted in advanced wastewater treatment for removal of various contaminants (Gerrity et al., 2013). It relies on the activity of a biofilm attached to the media surface (e.g., exhausted granular activated carbon, sand, anthracite) for the biodegradation of organic matter, nutrients, and/or trace organic compounds (TOrCs) (Zhu et al., 2010).

The microbial community in the biofilm determines the fate of bulk organic matter and TOrCs present in the feed water. Certain factors, such as pH and redox conditions, may influence the development and stability of the microbial community and the amount of biomass in the biofilter (Zhu et al., 2010; Velten et al., 2011). The redox conditions are governed by the presence of one or more electron donors (e.g., organic matter, TOrCs, ammonia) and electron acceptors (e.g., oxygen, nitrate). Other operational conditions may also affect microbial community structure, such as pre-treatment (e.g., disinfection, coagulation, sedimentation, clarification, etc.), backwashing frequency, and contact time (Zhu et al., 2010; Jałowiecki et al., 2016).

Numerous methods are available for the quantification of biomass and microbial activity. One method that has been commercialized and has gained considerable attention in recent years is the measurement of adenosine triphosphate (ATP) as an indicator of total living biomass (LuminUltra, 2013). In addition, the emerging field of metagenomics allows for a greater understanding of microbial community structure and function and identification of factors

responsible for changes in different environments (Deutschbauer et al., 2006). Recently, a number of molecular tools have been developed and employed to accomplish this task (Spiegelman et al., 2005; Rittman and McCarty, 2001). For example, 16S rRNA gene sequencing consists of extracting DNA from cells, amplifying the DNA with target-specific primers (e.g., for all Bacteria), and then sequencing numerous fragments of the amplified DNA (e.g., >10,000 sequence reads) to identify microorganisms based on unique phylogenetic markers. Sequencing can be accomplished with several different approaches, including pyrosequencing ('second generation') and Illumina sequencing ('next generation') (Loman and Pallen, 2015). Using various bioinformatics tools (e.g., QIIME), the sequences compiled from the analysis are compared against known libraries to identify the various species present in the sample. The National Center of Biotechnology Information (NCBI) is a database commonly used to access biomedical and genomic information. The 16S rRNA gene contains 1,500 bases and provides enough genetic diversity to reliably differentiate one species from another (Rittman & McCarty, 2001). This molecular tool also carries the advantage of targeting both culturable and non-culturable microorganisms.

Once the sequences have been identified and assigned an 'operational taxonomic unit' (OTU), or effectively a species designation, additional statistical analyses can be performed to characterize the composition of the microbial community. For example, diversity and richness indices (e.g., Shannon-Weiner, Simpson, and evenness) can be calculated to provide a more objective characterization of the structure of the microbial community (Li et al., 2010). There are also different methodologies for estimating these indices. Rani et al. (2015) calculated indices using distance-based OTUs, while Jałowiecki et al. (2016) developed community-level physiological profiles (CLPPs) to characterize metabolic diversity in three different wastewater

treatment plants. Statistical analyses such as principal component analysis (PCA) can also be used to determine whether microbial communities are statistically different from each other and to identify which species are most closely linked to a particular sample or influenced by a particular experimental condition. In previous research, diversity indices and statistical analyses have been used to confirm similarities between microbial communities in different wastewater samples (Raychaudhuri et al., 2000, Jałowiecki et al., 2016).

The presence of certain microbes in biological treatment systems in wastewater treatment plants, such as the activated sludge process, depends largely on influent water quality (e.g., redox conditions) but also on operational parameters (e.g., solids retention time). Studies of pesticide and pharmaceutical wastewater treatment plants identified *Proteobacteria* (Onesios-Barry et al., 2014) and *Fermicutes* (Rani et al., 2008) to be the major phyla in both systems. However, the phylum *Actinobacteria* was only detected in samples from the pharmaceutical facility, thereby suggesting that influent water quality plays an important role in defining the microbial community. Different phyla have been identified in biofilters employed in drinking water treatment, such as *Acidobacteria* (natural soil bacteria) and *Nitrospirae* (Kielak et al., 2016). However, relatively little is known about the microbial community structure of biofilters in advanced wastewater treatment applications.

The goal of this phase of the study was to characterize the microbial community structure of three pilot-scale biofilters by sequencing 16S rRNA phylogenetic markers. One biofilter contained exhausted granular activated carbon [(otherwise known as biological activated carbon (BAC)] and was fed with ozonated membrane bioreactor (MBR) filtrate, the second biofilter contained anthracite and was fed with ozonated MBR filtrate, and the third biofilter also contained BAC but was fed with MBR filtrate without pre-ozonation.

5.2 – Materials and Methods

5.2.1 - Pilot Reactor and Media Sampling

The configuration of the pilot-scale ozone-biofiltration system was identical to the one described in the previous chapters. The full-scale treatment process prior to the pilot reactor included an MBR operated with a solids retention time (SRT) of 8-10 days, full nitrification and partial denitrification, and membranes with a 0.04-μm nominal pore size. In the pilot-scale reactor, during the course of the study, the MBR filtrate was ozonated with O_3/TOC ratios varying 0.1-2.5 and then fed to two biofilters, one containing 0.95-mm-diameter exhausted granular activated carbon (or BAC) (Norit 820, Cabot Corporation, Alpharetta, GA) and the other containing 1.2-mm-diameter anthracite media. A third column containing BAC received non-ozonated MBR filtrate and was used as a control. The BAC, which was provided by the F. Wayne Hill Water Resources Center in Gwinnett County, GA, was assumed to have no adsorptive capacity remaining because it had been used in full-scale wastewater biofilters for over 10 years. The San Jose Creek Water Reclamation Plant provided the anthracite media. Each biofilter had a bed height of 30 inches and a bed volume of 0.36 liters.

In order to avoid external contamination, media samples were collected from sampling ports in the biofiltration columns using autoclaved spatulas and transferred to sterile conical tubes. Samples were collected from two different depths (i.e., approximately 1 gram per sample): near the top of the biofiltration column (~5 in below the surface) and near the bottom of the biofiltration column (~19 inches below the surface). Samples were kept cool (<4°C) until further analysis. A total of 8 samples were collected for ATP analysis and the 16s rRNA analysis (Table 22) after all experiments delineated in previous chapters were performed (i.e., at the end of study). The layout of the biofilters is shown in Figure 25.

Table 22 - Total number of samples per point

Sample point	Number of samples	
	Top (5 inches from surface)	Bottom (19 inches from surface)
Ozonated-BAC	2	2
Ozonated-Anthracite	1	1
Non-Ozonated BAC	1	1

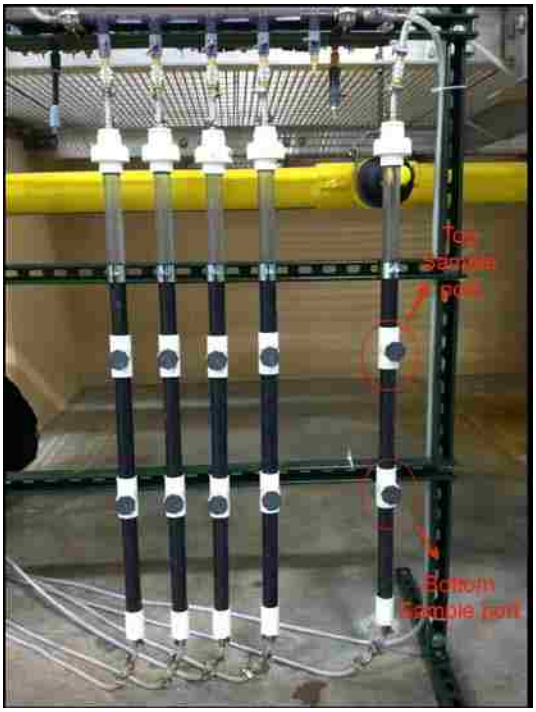

Figure 25 – Layout of biofilter and location of media sample ports

(Top = 5 in and Bottom = 19 in)

5.2.2 – Quantification of Microbial Activity through ATP

The concentration of ATP associated with attached growth (i.e., the biofilm on the media) was used as a surrogate for microbial community abundance and density on the biofiltration media. The deposit and surface analysis ATP test kit (Hach, Loveland, CO) was used to extract

ATP from living cells as well as released from dead cells and a PhotonMaster Luminometer (LuminUltra Technologies Ltd, New Brunswick, Canada) was used to measure the ATP concentration in each sample via luminescence. Dry BAC and anthracite media samples were also collected to compare the microbial community before and after the biofilter acclimation period.

5.2.3 – DNA Extraction and Sequencing

DNA extraction from the media and subsequent purification were performed using a PowerBiofilm™ DNA Isolation Kit (MoBIO, Carlsbad, CA, USA). This method is specifically designed to allow for high quality DNA isolation from several types of biofilms. The process involves the dissolution of polysaccharides to enhance lysis of organisms. Approximately 0.05-0.20 g of sample was placed in a 2-mL collection tube for extraction. The final 100 µL of extracted DNA for each sample was stored at -20°C until further analysis.

The extracted DNA was shipped to Research and Testing Laboratory (Lubbock, TX) where the 16S rRNA gene was amplified and sequenced using a MiSeq sequencer (Illumina, San Diego, CA). The contract laboratory used universal primers for bacteria (28F-388R) for initial amplification, as summarized in Table 23, which represents the variable regions (V1-V3) of the 16S rRNA. After PCR amplification and sequencing, denoising was performed using the USEARCH clustering algorithm to correct errors and remove noisy reads. Chimera checking was also performed after denoising using UCHIME chimera detection software. After denoising and chimera checks, the sequences were clustered into OTUs using a UPARSE algorithm. The centroid from each cluster was compared against high quality sequences derived from the NCBI database (Research and Testing Laboratory, 2016).

Table 23 - Bacteria primers used in amplification

#SampleID	Bar code Sequence	Linker Primer Sequence	Reverse Linker Primer Sequence	Description
Control BAC Top (CT)	AAAACAAA	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
Control BAC Bottom (CB)	AAAACAAC	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 Anthracite Top (AT)	AAAACAAG	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 Anthracite Bottom (AB)	AAAACAAT	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 BAC Top 1 (BT1)	AAAACACA	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 BAC Top 2 (BT2)	AAAACACC	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 BAC Bottom 1 (BB1)	AAAACACG	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R
O_3 BAC Bottom 2 (BB2)	AAAACACT	GAGTTTGATCNTGGCTCAG	TGCTGCCTCCCGTAGGAGT	MS28F-388R

5.2.4 - Statistical analysis

Diversity and richness indices were calculated to provide a numerical characterization of the microbial community for each media sample (Rani et al., 2008). The indices included the Shannon index (H) (**Equation 5**), which is a measure of diversity of the community, and the evenness index (E) (**Equation 6**), which is a measure of how they are distributed in the community. The total number of species (S), or richness, was determined by counting the total number of OTUs for each sample. In order to evaluate the similarity (or differences) between biofilters, a principal component analysis (PCA) was performed using the XLSTAT add-in (Addinsoft, New York, NY) for Microsoft Excel.

$$Shannon\ Index\ (H) = -\sum_{i=1}^{s} p_i \ln p_i \quad \textbf{Equation 5}$$

$$Evenness\ Index\ (E) = \frac{H}{ln\ S} \qquad \textbf{Equation 6}$$

5.3 – Results and Discussion

5.3.1 – Biomass Quantification

Prior to exposure to non-ozonated or ozonated MBR filtrate during the acclimation period, the media was analyzed for ATP to establish a baseline for comparing biofilm development over time. These initial ATP data are summarized in Table 24, along with similar data from Selvy (2015) (same pilot-scale system) and an independent study from the literature (Velten et al., 2011). The data from all three studies were similar. Table 25 shows the corresponding data collected at the same day (i.e., after five months since the acclimation period started) for the current study and data from the literature. In all studies, samples collected from the top of columns had higher ATP concentrations (10-80%), and the OTU counts were also higher at the top of the columns in the current study. This implies that microbial abundance and biofilm density were higher at the surface, although the values at the bottom of the columns were generally within an order of magnitude. Because biomass density decreases deeper in the biofilm, microbial activity and biodegradation potential might also decrease in the lower layers of the biofiltration media (Velten et al., 2011; Gibert et al., 2013; Selvy, 2015). As indicated in earlier chapters, there were no significant benefits in terms of TOC reduction or DBP mitigation with longer EBCTs. This implies that the biodegradable organics are rapidly consumed (i.e., in the top layers of the columns), potentially limiting the amount of biodegradable carbon available to bacteria deep in the biofilm. This might explain why there was less biomass at the lower depth in the current study.

Table 24 - ATP Concentration in dry media prior to acclimation period

Media Sample	Initial ATP (pg/g)		
	Current Study	Selvy (2015)	Velten et al. (2011)
Dry BAC	2.2E+04	-	~1E+04
Dry Anthracite	1.2E+02	0.6E+02	

Table 25 - Results for ATP from current study and literature

	Influent Characteristic	Media Sample	ATP (pg/g)		
			Top	Bottom	% difference
Current Study	Secondary Effluent (TOC ~8 mg/L)	Non-Ozonated BAC	4.8E+05	9.8E+04	79%
		Ozonated Anthracite	3.4E+05	2.3E+05	34%
		Ozonated BAC	1.5E+05	7.5E+04	50%
Selvy (2015)	Secondary Effluent (TOC ~8 mg/L)	Non-ozonated Anthracite	-	2.0E+05	-
		Ozonated Anthracite	6.3E+05	3.1E+05	50%
		Ozonated BAC	9.1E+05	2.2E+05	76%
Velten et al. (2011)	Drinking Water (TOC ~1 mg/L)	Ozonated BAC (90 days old)	1.83E+06	0.8E+06	30%
Magic-Knezev et al. (2004)	Drinking Water (DOC = 1.8-5.4 mg/L)	Average between Non- and Ozonated BAC	0.5E+04 to 2.5E+06		-
Gibert et al. (2013)	Drinking Water (DOC = 1.1-5.5 mg/L)	Ozonated GAC	3.3E+06	1.6E+06	52%

5.3.2 – Diversity Index Analysis

Diversity indices were calculated based on total OTU counts for each sample. Although total OTU counts and ATP concentrations were higher at the top of the biofilters, diversity (H) and evenness (E) indices were higher at the bottom of the biofilters, as observed in Table 26. This suggests the community towards the top of the biofilter was dominated by a small number of species and a more diverse community was present towards the bottom of the biofilter. The ozonated-BAC sample showed higher difference (30%) in microbial diversity between samples collected at the top and bottom than other biofilters (7-15%). Yang et al. (2011) also observed higher microbial diversity in lower parts of the biofilter and suggested that pre-ozonation decreases microbial diversity and evenness (i.e., distribution of species) at the top of the biofilter. According to Wu et al. (2014), ecosystems with greater evenness are generally more stable and have a higher probability of containing species tolerant to distress. Therefore, more favorable conditions may select for more dominant bacteria, while unfavorable or stressful conditions allow for the development of a more diverse community. Also, in biofilters, greater evenness can lead to greater removal of natural organic matter and DBP precursors (Wu et al., 2014).

Table 27 provides a comparison of diversity studies for different treatment applications. Samples from top of ozonated biofilters (BAC) showed similar diversity index (H) to those in wastewater biological treatment (Jałowiecki et al., 2016), whereas bottom of ozonated biofilters and non-ozonated biofilters showed similarity with diversity index from drinking water treatment (Wu et al., 2014).

Table 26 - Diversity index of biofilter samples

	Control BAC Top	Control BAC Bottom	O3 Anthracite Top	O3 Anthracite Bottom	O3 BAC Top	O3 BAC Bottom
Shannon Index (H)	2.53	3.10	2.78	3.01	1.95	2.72
Species Richness (S)	193	186	143	146	130	82
Evenness Index (E)	0.480	0.593	0.559	0.603	0.4	0.637

a. Shannon (H) Diversity Index: higher numbers represent greater richness and/or diversity (combined)
b. (S) Index: number of predicted taxa/OTUs
c. Evenness Index (E): 1 represents higher evenness and 0 lower evenness

Table 27 - Comparison of diversity indices with literature

	Analysis Type	H	S	E
Current Study	Ozonated and Non-ozonated Secondary Effluent	**2-3.1**	**82-193**	**0.4 – 0.64**
Rani et al. (2008)	Wastewater Biological Treatment Systems from Pharmaceutical and Pesticides Treatment Plant	**2.3-3.3**	**38-44**	**0.86 - 0.95**
Jałowiecki et al. (2016)[a]	Wastewater Biological Treatment Systems	**1.3-1.5**	**16-31**	**-**
Wu et al. (2014)	GAC Biofilter used in Surface Water Pretreatment	**2.55 ± 0.03**	**24.3 ± 1.4**	**0.78 ± 0.01**

5.3.3 – Microbial Characterization through 16S RNA Sequencing

The relative abundances of the top 20 species (after modification for the 0.5% cut-off) are shown in the heat map in Figure 26. The data indicate that Proteobacteria (α-, β-, γ-Proteobacteria) was the most abundant phylum in all biofilters. Studies have found that β- and α-Proteobacteria are generally the predominant bacteria in BAC systems and are responsible for most of the degradation of dissolved organic carbon and assimilable organic carbon (Yang et al., 2011; Wu et al., 2014).

In the current study, *Bradyrhizobium* was the dominant genus (relative abundance = 17 – 60%) in all samples, as shown in Figure 26. This has not been widely reported in previous biofiltration studies, although a similar genus—*Rhizobium*—which is also a member of the order *Rhizobiales* and phylum α-Proteobacteria, has been reported in biofilters inoculated with aerobic activated sludge from wastewater treatment plants (Zhai et al., 2017). *Bradyrhizobium sp.* is an aerobic Gram negative bacterium and belongs to the α-Proteobacteria phylum. They are naturally occurring in soil and induce the formation of nodules on legume roots (Bedmar et al., 2005). Within the nodules, the bacteria can produce nitrogenase, an enzyme responsible for the reduction of N_2 to ammonia (NH_4^+). As mentioned in Chapter 3, the concentration of ammonia sometimes increased after biofiltration, which is not typical of biofiltration systems (Basu et al., 2015). This also appeared to be associated with infrequent backwashing, thereby indicating that backwashing may reduce the prevalence and/or activity of *Bradyrhizobium sp.* within the biofilter.

A very large percentage (58%) of unknown and unclassified species comprised the subset of the microbial community that fell below the <0.5% cut-off on an individual basis. The 'unknown' designation indicates that the algorithm was unable to make a confident

determination regarding taxonomic classification, while 'unclassified' indicates that the taxonomic information retrieved from NCBI contains missing information at the specified level. Rani et al. (2008) also observed that almost 50% of the sequences referring culturable and unculturable bacteria were not found in available libraries/databases, suggesting that bacteria typically present in biofiltration systems have not been well characterized and documented.

In the control biofilter (CT and CB), *Nitrospira sp.* was the second most abundant species in the biofilter among known and classified species, as shown in the heat map in Figure 26. These bacteria are characterized by their ability to oxidize nitrite to nitrate, so they are also described as nitrite-oxidizing bacteria (NOB). They have been found in terrestrial habitats, marine waters, deep sea sediments, drinking water distribution systems, and wastewater treatment plants (Daims et al., 2015). The presence of these bacteria indicates the potential for nitrification in the biofilters. In fact, nitrification was observed in all biofilters after backwashing, as described in Chapter 3. However, the presence of *Nitrospira sp.* was very low in other biofilters (<0.4%). This might because the ammonia was oxidized by ozone, limiting ammonia in the ozonated biofilters.

Mycobacterium porcinum, a member of the *Actinobacteria* phylum, was detected in some biofilters (CT, CB, AT and AB) with relative abundance from 0.7-1.4%. This bacteria is considered a pathogen and has been identified as a high priority drinking water contaminant and public health concern. In fact, *Mycobacterium* is included on USEPA's Contaminant Candidate List. Clinical infections caused by the species include wound infections, intravascular catheter-related infections, and osteomyelitis (Brown-Elliot et al., 2011). This species have demonstrated an ability to degrade carbohydrates as a sole source of carbon in the presence of ammonia (Tsukamura et al., 1983). *Steroidobacter sp.* was also detected in the BAC filters (CT, CB, BT1,

BT2, BB1, BB2) with abundance ranging from 0.5-1.8%. These species belongs to *γ-Proteobacteria* and is characterized as a steroid-hormone-degrading bacterium (Fahrbach et al., 2008).

Class	Genus	Species	CT	CB	AT	AB	BT1	BT2	BB1	BB2
Actinobacteria	*Mycobacterium*	*Mycobacterium porcinum*	0.726%	1.160%	1.157%	1.350%	0.234%	0.125%	0.000%	0.211%
Nitrospira	*Nitrospira*	*Nitrospira sp.*	6.181%	9.065%	0.003%	0.017%	0.086%	0.083%	0.374%	0.376%
		Unknown	1.697%	2.153%	0.067%	0.416%	0.351%	0.297%	0.146%	1.855%
α-Proteobacteria	*Bradyrhizobium*	*Bradyrhizobium sp*	41.908%	22.451%	17.860%	16.928%	59.245%	61.397%	19.096%	40.501%
	Hyphomicrobium	*Hyphomicrobium sp*	1.407%	0.705%	0.506%	0.975%	1.107%	1.116%	0.489%	2.374%
	Pedomicrobium	Unknown	1.141%	0.639%	0.005%	0.020%	0.621%	0.378%	0.000%	0.479%
	Nordella	Unknown	0.937%	0.845%	0.263%	0.702%	0.267%	0.264%	2.036%	0.333%
	Unclassified	Unclassified	2.416%	3.750%	1.819%	1.403%	4.108%	3.727%	2.481%	4.088%
	Unknown	Unknown	1.939%	3.601%	0.836%	1.298%	1.763%	1.787%	3.807%	4.534%
	Unclassified	*Unclassified*	0.720%	0.970%	0.040%	0.033%	2.075%	1.453%	2.540%	2.040%
	Sphingobium	Unknown	0.425%	0.544%	0.008%	0.015%	0.000%	0.000%	0.000%	0.000%
	Unknown	Unknown	8.740%	7.582%	1.738%	3.371%	3.929%	5.234%	4.212%	6.622%
	Unclassified	Unclassified	3.400%	2.350%	0.181%	0.293%	2.394%	2.974%	10.111%	3.575%
β-Proteobacteria	*Nitrosomonas*	Unknown	1.012%	0.297%	0.406%	0.303%	0.299%	0.204%	0.000%	0.118%
	Unknown	Unknown	0.044%	0.122%	15.975%	3.917%	0.436%	0.628%	0.059%	0.169%
	Unknown	Unknown	0.775%	1.002%	2.625%	3.646%	0.771%	0.836%	0.676%	0.708%
	Unclassified	*Unclassified*	0.385%	1.378%	5.005%	3.902%	0.613%	0.642%	0.000%	0.407%
	Unknown	Unknown	1.973%	5.307%	16.143%	13.622%	2.456%	4.396%	4.424%	5.113%
γ-Proteobacteria	*Steroidobacter*	*Steroidobacter sp.*	1.654%	1.790%	0.028%	0.052%	1.307%	1.077%	0.529%	1.354%
Unknown	*Unknown*	Unknown	5.409%	4.247%	0.930%	1.988%	2.604%	3.311%	3.004%	5.040%
Others (<0.5% relative abundance)			17.110%	30.042%	34.406%	45.750%	15.335%	10.072%	46.015%	20.102%

Figure 26 - Heat map of the 20 most abundant species

Developed after 0.5% relative abundance cutoff was performed. Low abundance is represented with red shading while high abundance is represented with green shading. CT, CB = Control BAC top and bottom; AT, AB = Anthracite top and bottom; BT, BB = BAC top and bottom.

5.3.4 – Principal Component Analysis

Principal Component analysis was performed using the top 20 species to evaluate similarity between samples (Figure 27). Blue dots represent different classes and species, while red vectors represent each media sample. The two components identified by the PCA account for a total of 92% (81% + 11%) of the variability in the dataset. In general, the results demonstrated that the BAC samples had statistically similar microbial communities, at least among the most abundant species, regardless of whether the feed water was ozonated and regardless of the location. However, the microbial communities in the anthracite biofilters were significantly different from the BAC biofilters. This might be due to media was originated from two different wastewater treatment plant biofilters (please refer to section 5.2.1). One notable difference was that *Bradyrhizobium* was more closely linked to the BAC than the anthracite, although this bacterium was still relatively abundant in the anthracite samples. Unfortunately, it was not possible to identify the remaining species driving these differences because they are currently 'unknown' or 'unclassified'.

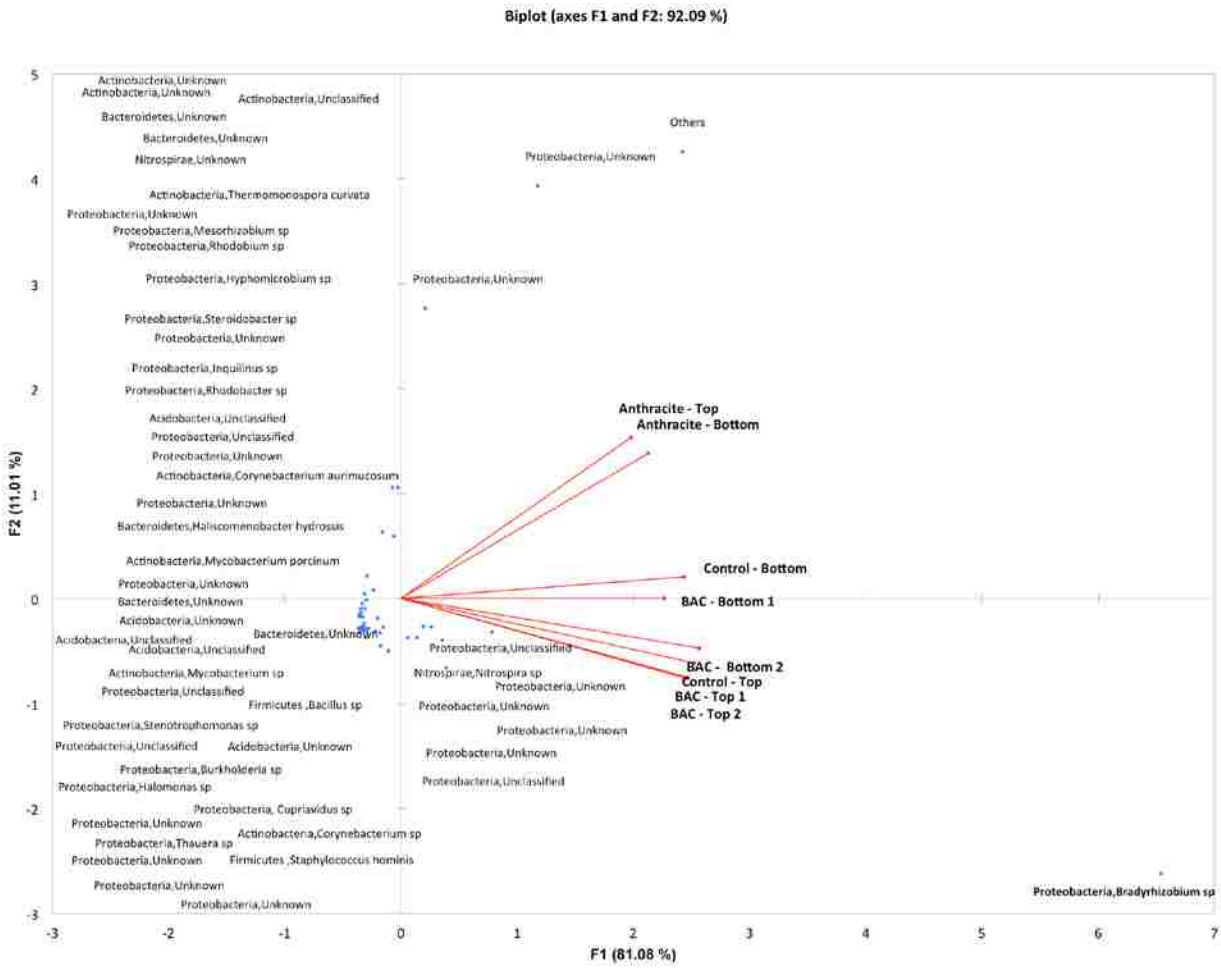

Figure 27 – Principal component analysis between media samples.

Two components account for a total of 92% (81% + 11%) of the variability in the dataset.

5.4 – Conclusions

The results from the ATP analyses and 16S rRNA gene sequencing yielded the following conclusions:

- ATP analyses revealed that microbial abundance was greater for samples collected at the top of the biofilter columns (i.e., 5 inches from the surface) compared to the bottom of biofilters (i.e., 19 inches from the surface).

- Index analyses demonstrated that microbial diversity (i.e., Shannon index) and evenness were greater at the bottom of the biofilters, thereby suggesting that the more favorable growth conditions (e.g., abundant carbon source) at the top of the biofilter columns allowed for certain bacteria to dominate the community.
- *Bradyrhizobium*, which is naturally prevalent in soil systems and has been shown to be an ammonifying organism, was dominant in all biofilters (abundance = 17-60%), although it was more closely linked to the BAC media based on the PCA analysis.
- The PCA analysis demonstrated that the microbial communities for all of the BAC samples were relatively similar, regardless of depth and feed water (i.e., non-ozonated vs. ozonated). Moreover, the microbial communities of the anthracite samples were different from the BAC samples.

Chapter 6 - Conclusions

6.1 - Findings confirming previous work

During the study, the following conclusions concurred with previous studies:

- Ozone alone achieves significant TOC transformation, as determined by reductions in absorbance and fluorescence, but it does not reduce the TOC concentration in the treated effluent (i.e., no mineralization of bulk organics). The transformation caused by ozone oxidation resulted in reductions of TTHMs and HAA5s by 13% and 31%, respectively (Hua and Reckhow, 2007; Linlin et al., 2011).
- Combination of ozone followed by biofiltration achieved up to 30% in TOC removal similarly to previous studies (Gerrity et al., 2011; Selvy, 2015; Knopp et al., 2016). This percentage removal was not yet sufficient to achieve wastewater-derived TOC concentrations as low as 0.5 mg/L, as required by CDDW with no blending, or even to achieve similar to typical drinking water concentrations (i.e., 3.2 mg/L). Therefore, a polishing process (e.g., adsorption with GAC, ion exchange) may still be required to achieve TOC concentrations in ozone-BAC effluents that are consistent with state requirements.
- Similar to Selvy (2015), TOC removal rapidly (EBCT < 10 minutes) plateaus at less than 10% for biofiltration without pre-ozonation, whereas with increase in pre-ozone dose, TOC removal also increased but then appeared to plateau at longer EBCTs. This plateau may indicate that limited bioavailable organics are completely consumed by microbial community at ‘optimum’ EBCT. TOC removal with ozone-biofiltration was up to 20% greater than with biofiltration alone. The combination of ozone and biofiltration reduced

TTHMs by up to 31% and HAA5s by up to 56%, while biofiltration was capable of reducing only by 9% and 27%, respectively.

- BAC was superior to anthracite with respect to TOC removal and DBP mitigation, presumably due to possible remaining adsorption capacity of the GAC, as ATP analysis shown similar microbial activity between bioflters. Further characterization is needed in order to quantify adsorption capacity in biofilters containing activated carbon.
- ATP analyses revealed that microbial activity was greater for samples collected at the top of the biofilter columns (i.e., 5 inches from the surface) compared to the bottom of biofilters (i.e., 19 inches from the surface), comparable to previous studies (Magic-Knezev et al., (2004); Velten et al., 2011; Gibert et al., 2013; Selvy, 2015).

6.2 - Significant findings

Current study resulted in the following significant findings:

- A multivariate linear correlation was established between ammonia, TOC, and the applied chlorine dose necessary to achieve a free chlorine residual of 1±0.4 mg/L after 24 hours with the uniform formation condition approach (UFC) approach used in DBP assessment.
- Based on a relatively strong correlation between DBP formation and effluent TOC, a maximum TOC concentration of 3.3 mg/L was identified as the threshold for non-compliance with the TTHM MCL. With a safety factor of 1.25 on the TTHM concentration, a revised maximum TOC concentration of 2 mg/L was identified. These TOC targets (i.e., 2-3.3 mg/L) are more achievable for ozone-biofiltration systems than the 0.5-mg/L target in California. These revised targets are more consistent with typical

TOC concentrations in surface water and can also be justified in the context of public health impacts.

- Index analyses demonstrated that microbial diversity (i.e., Shannon index) and evenness were greater at the bottom of the biofilters, thereby suggesting that the more favorable growth conditions (e.g., abundant carbon source) at the top of the biofilter columns allowed for certain bacteria to dominate the community.
- *Bradyrhizobium*, which is naturally prevalent in soil systems and has been shown to be an ammonifying organism, was dominant in all biofilters (abundance = 17-60%), although it was more closely linked to the BAC media based on the PCA analysis.
- The PCA analysis demonstrated that the microbial communities for all of the BAC samples were relatively similar, regardless of depth and feed water (i.e., non-ozonated vs. ozonated). Moreover, the microbial communities of the anthracite samples were different from the BAC samples.

6.3 – Implications

- The correlation between TOC concentration and DBP formation potential developed in this study also included results from DBP assessment in drinking water (Summers et al., 1996). The results indicated that DBP formation is closely linked to final TOC concentration in the water. Hence, there is a potential to develop regulations for potable reuse applications that are consistent with those intended for conventional drinking water sources.

- The effects of ozonation on the formation of DBPs not-covered in this study (i.e., NDMA and bromate) are also necessary to be analyzed when considering ozone-biofiltration applications.

- In waters with higher initial bulk organics concentration (i.e., TOC), it is important to consider a polishing treatment after ozone-biofiltration (e.g., GAC adsorption, ion exchange, ultrafiltration) in order to achieve TOC threshold and comply with MCLs established by EPA for TTHMs and HAA5s upon final chlorination.

6.4 - Future work

One of the significant findings of this work was the identification of a TOC threshold of 2 mg/L as sufficient to reliably achieve compliance with regulated disinfection by-products concentrations in drinking water (i.e., TTHMs = 80 μg/L and HAA5 = 60 μg/L) after chlorination. Other emerging disinfection by-products of toxicological interest (bromonitromethanes, iodo-thrihalomethanes, iodo-acids, bromate, NDMA) were not assessed during the current study. Therefore, another avenue for research is the need to further study the impacts of operational parameters (e.g., ozone dose, EBCT) on formation/mitigation of these emerging DBPs in ozone-biofiltration systems during ozonation (i.e. bromate, NDMA) and upon final chlorination (e.g. iodinated compounds).

It is also important to evaluate if TOC threshold established in this study yields significant formation of emerging DBPs upon final chlorination. Final determination of toxicity levels is important to be determined among the DBPs formed (i.e., regulated and emerging DBPs) in order to provide safe potable reuse water. Therefore, it would be possible to guarantee that TOC threshold does not imply public health concerns considering the formation of non-regulated DBPs formed upon final chlorination

It is also important to further characterize microbial community present in biofilters being employed in advanced treatment. The characterization could possibly contribute to enhancement of biofiltration process.

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