N-Nitrosodimethylamine Formation and Mitigation in Potable Reuse Treatment Trains Employing Ozone and Biofiltration

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N-NITROSODIMETHYLAMINE FORMATION AND MITIGATION IN POTABLE REUSE TREATMENT TRAINS EMPLOYING OZONE AND BIOFILTRATION

by

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Bachelor of Science in Environmental Engineering
São Paulo State University, Brazil
2016

A thesis submitted in partial fulfilment of the requirements for the

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Abstract

Potable reuse has been growing as a strategy to augment water supplies, especially in highly populated and water-scarce regions. Ozone and chloramines have emerged as important disinfectants and oxidants in potable reuse applications, but reactions with wastewater-derived constituents can lead to the formation of potentially carcinogenic disinfection byproducts (DBPs). One DBP that has received considerable attention is the nitrogenous DBP \textit{N}-nitrosodimethylamine (NDMA). NDMA is a potential human carcinogen and mutagen at trace concentrations — even at the sub-ng/L level. Several studies have reported successful attenuation of NDMA in biofiltration systems at wastewater treatment plants, but the associated mechanisms and design criteria are not well understood.

In the current study, a pilot-scale ozone-biofiltration system was used to treat membrane bioreactor (MBR) filtrate from a full-scale water reclamation plant to assess the role of various operational parameters, including ozone dose and empty bed contact time (EBCT), on NDMA removal. In the ozonated biological activated carbon (BAC) and anthracite columns, longer EBCTs (e.g., 10-20 minutes) achieved >90% NDMA removal, while shorter EBCTs (e.g., 2 min) achieved only 30-40% NDMA removal. In the non-ozonated BAC column, longer EBCTs were more important, with NDMA attenuation exhibiting a relatively steady increase toward ~45% for an EBCT of 20 min.

Pre-oxygenation of the MBR filtrate (i.e., instead of ozonation) also achieved ~90% removal in the BAC column, thereby suggesting that biodegradable dissolved organic carbon (BDOC) availability did not impact NDMA removal. Interestingly, when receiving ambient MBR filtrate (no pre-oxygenation or pre-ozonation), the typically ozonated column still achieved >90% NDMA removal, while the typically
non-ozone column only achieved 50% NDMA removal. In other words, NDMA removal was dependent on EBCT but did not necessarily require high concentrations of BDOC or dissolved oxygen. Instead, long-term exposure to ozonated MBR filtrate may have been critical in promoting the development of microbial taxa that were better adapted to NDMA biodegradation. The presence of monooxygenase genes responsible for NDMA biodegradation was confirmed by quantitative polymerase chain reaction (qPCR), although possible DNA extraction limitations for the BAC media prevented a reliable comparison by media type. Finally, this study confirmed the efficacy of ozone-biofiltration (but not biofiltration alone) for attenuating chloramine-reactive NDMA precursors. An overall reduction of 96% was observed, with a majority of that attenuation achieved by ozonation because of its ability to transform primary and secondary amines into nitrated intermediates and tertiary amines into N-oxides.

These data suggest that ozone-biofiltration is effective in achieving net reductions in NDMA in some potable reuse systems, particularly when chloramines are expected to be used as a final disinfectant. However, UV photolysis might still be necessary as a final polishing step to ensure compliance with relevant guidelines and regulations (e.g., 10-ng/L notification level in California). Also, additional studies are needed to better characterize microbial community structure and function in potable reuse systems.
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<tr>
<td>AOC</td>
<td>Assimilable organic carbon</td>
</tr>
<tr>
<td>AOP</td>
<td>Advanced oxidation process</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AWTF</td>
<td>Advanced water treatment facility</td>
</tr>
<tr>
<td>BAC</td>
<td>Biological activated carbon</td>
</tr>
<tr>
<td>BDOC</td>
<td>Biodegradable organic carbon</td>
</tr>
<tr>
<td>CCL</td>
<td>Candidate contaminant list</td>
</tr>
<tr>
<td>$C_q$</td>
<td>Quantification cycle</td>
</tr>
<tr>
<td>DBP</td>
<td>Disinfection byproduct</td>
</tr>
<tr>
<td>DDW</td>
<td>Division of Drinking Water</td>
</tr>
<tr>
<td>DEET</td>
<td>N,N-Diethyl-meta-toluamide</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DPR</td>
<td>Direct potable reuse</td>
</tr>
<tr>
<td>DRI</td>
<td>Desert Research Institute</td>
</tr>
<tr>
<td>DWTP</td>
<td>Drinking water treatment plant</td>
</tr>
<tr>
<td>EBCT</td>
<td>Empty bed contact time</td>
</tr>
<tr>
<td>eDNA</td>
<td>Extracellular DNA</td>
</tr>
<tr>
<td>EEM</td>
<td>Excitation-emission matrix</td>
</tr>
<tr>
<td>EfOM</td>
<td>Effluent organic matter</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
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<tr>
<td>EPS</td>
<td>Extracellular polymeric substance</td>
</tr>
<tr>
<td>FAT</td>
<td>Full advanced treatment</td>
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</table>
FP – Formation potential
GAC – Granular activated carbon
GC-MS/MS – Gas chromatography tandem mass spectrometry
HAA – Haloacetic acid
IDT – Integrated DNA Technologies
IESWTR – Interim Enhanced Surface Water Treatment Rule
IPR – Indirect potable reuse
LRV – Log removal value
MBR – Membrane bioreactor
MCL – Maximum contaminant level
MCLG – Maximum contaminant level goal
MF – Microfiltration
MGD – Million gallons per day
MRL – Method reporting limit
MV – Membrane vesicle
NCBI – National Center for Biotechnology Information
NDMA – N-Nitrosodimethylamine
NL – Notification Level
NPDES – National Pollutant Discharge Elimination System
NTC – Non-template control
NTDMA – N-nitrodimethylamine
NTMA – N-nitromethylamine
•OH – Hydroxyl radical
O&M – Operation and Maintenance
OCSD – Orange County Sanitation District
OCWD – Orange County Water District
PAC – Powdered activated carbon
PCR – Polymerase chain reaction
PFAA – Perfluoroalkyl acids
PFOA – Perfluorooctanoic acid
PFOS – Perfluorooctane sulfonic acid
PrMO – Propane monooxygenase
qPCR – Quantitative polymerase chain reaction
RO – Reverse osmosis
SDS – Simulated distribution system
SMP – Soluble microbial products
SNWA – Southern Nevada Water Authority
TAE – Tris/Acetate/EDTA
TCEP – Tris (2-carboxyethyl) phosphine
TDS – Total dissolved solids
TE – Tris/EDTA
THM – Trihalomethane
TOC – Total organic carbon
TOrC – Trace organic contaminant
UF – Ultrafiltration
UFC – Uniform formation condition
UNLV – University of Nevada, Las Vegas
U.S. – United States
UV – Ultraviolet

UV \textsubscript{254} – UV absorbance at a wavelength of 254 nm

WRF – Water reclamation facility

WWTP – Wastewater treatment plant
Chapter 1 – Introduction and Objectives

Water supply stressors such as climate change, population growth, and water pollution have been stimulating the consideration and adoption of water reuse throughout the world. Although non-potable reuse (e.g., for irrigation) has been practiced for decades, planned potable reuse—either indirect potable reuse (IPR) or direct potable reuse (DPR)—is a relatively new alternative for municipalities, in part because of past regulatory, technology, and public perception barriers to implementation.

IPR can be divided into unplanned (de facto) or planned systems. Unplanned IPR is the discharge of treated wastewater to a water body that is used by a downstream community as a drinking water source. On the other hand, a planned IPR system generally consists of a wastewater treatment plant (WWTP) coupled with an advanced water treatment facility (AWTF), and the purified water is discharged to an environmental buffer (e.g., lake, groundwater, etc.). The environmental buffer can act as (i) a natural treatment process to remove persistent organics, pathogens, and chemicals; (ii) a psychological barrier to disassociate the purified water from its wastewater origin; and (iii) a mechanism for providing response retention time in the case that failures are detected during treatment. Instead of an environmental buffer, the purified water in a DPR system can be discharged upstream of a drinking water treatment plant (DWTP), blended with finished water from the DWTP, or held in an engineered storage buffer prior to direct distribution.

To ensure public health safety, wastewater effluent should be disinfected regardless of whether the recycled water is intended for nonpotable or potable reuse. Common disinfectants include free chlorine, chloramines, chlorine dioxide, and ozone.
However, during these processes, the disinfectant can react with both organic and inorganic matter, thereby leading to the formation of disinfection byproducts (DBPs). For example, free chlorine can react with the complex effluent organic matter (EfOM), measured as total organic carbon (TOC), and form trihalomethanes (THMs) and haloacetic acids (HAAs), which are carcinogens regulated by the U.S. Environmental Protection Agency (U.S. EPA) with maximum contaminant levels (MCLs) of 80 and 60 µg/L, respectively (U.S. EPA, 2002). This discovery led to the use of chloramines, which are formed when ammonia reacts with free chlorine, as an alternative disinfectant with reduced THM and HAA formation potential. However, chloramines react with organic precursors to form the potential human carcinogen N-nitrosodimethylamine (NDMA) (Choi and Valentine, 2002), which is not currently regulated at the federal level in the United States (U.S.) but is regulated in some states at trace levels (e.g., 10 ng/L in California). NDMA has also been shown to form during ozone disinfection (Lee et al., 2007; Andrzejewski et al., 2008), and reactions between ozone and bromide can lead to the formation of another carcinogenic DBP known as bromate, which is regulated at 10 µg/L by the U.S. EPA (U.S. EPA, 2002). Therefore, potable reuse systems must weigh the benefits of various oxidants/disinfectants against their potential to form DBPs, which can sometimes vary considerably between systems.

NDMA is particularly concerning because concentrations as low as 0.69 ng/L correspond with a 10⁻⁶ lifetime cancer risk (U.S. EPA, 2014), which is a critical regulatory threshold. For reference, a concentration of 1 ng/L is equivalent to less than one drop of water in an Olympic-size swimming pool (TWDB, 2015). Due to its relatively recent discovery as a DBP, it has not been regulated at the federal level, but it is included on the U.S. EPA Contaminant Candidate List 4 (CCL4) and has a notification level (NL) in some states. As one of the more progressive regulatory states,
California has stipulated a NL of 10 ng/L (CDPH, 2014). For comparison, the
Australian Drinking Water Guidelines established a limit of 100 ng/L of NDMA, while
the Australian Guidelines for Water Recycling have a more stringent limit of 10 ng/L
(EPHC, 2008). In Canada, the allowable NDMA concentration in drinking water is 40
ng/L (Health Canada, 2011).

Numerous strategies to remove or prevent the formation of chemical
contaminants, including NDMA and other DBPs, have been evaluated for possible
implementation in potable reuse treatment trains. The most widely accepted treatment
paradigm is called full advanced treatment (FAT) by the California Division of
Drinking Water (DDW). An FAT system consists of microfiltration (MF) or
ultrafiltration (UF) as pre-treatment followed by reverse osmosis (RO) and an advanced
oxidation process (AOP), such as UV irradiation in the presence of hydrogen peroxide
(H2O2). This treatment train is prone to NDMA formation because chloramines are
typically used to mitigate biological membrane fouling. NDMA is partially removed by
RO, but the UV component of the AOP is the main process responsible for NDMA
destruction, although relatively high UV doses are generally needed to achieve the 10-
ng/L goal (Sharpless and Linden, 2003). In fact, UV doses required for NDMA
abatement are often an order of magnitude higher than those required for pathogen
inactivation (NWRI, 2012), thereby creating energy and cost issues (Gerrity et al.,
2014), especially for small facilities.

Due to concerns with the costs and energy consumption associated with FAT,
treatment trains employing ozone and biofiltration have been proposed as viable
alternatives to FAT (Gerrity et al., 2014). In ozone-biofiltration treatment trains, ozone
is responsible for the oxidation of trace organic compounds (Gerrity et al., 2011) and
transformation of complex EfOM into smaller, more assimilable compounds (von
Sonntag and von Gunten, 2012). The subsequent biofiltration process achieves significant removal of the transformed EfOM (Gerrity et al., 2011), which has been shown to reduce THM and HAA formation potential (Arnold et al., 2018) and NDMA concentrations (Farrè et al., 2011; Gerrity et al., 2015). However, some systems are prone to extremely high levels of NDMA formation during wastewater ozonation, which can overwhelm the downstream biofiltration process (Trussell et al., 2016). Therefore, there is a need to expand on the current knowledge base of NDMA biodegradation (Gunnison, 2000; Bradley, 2005; Sharp et al., 2005) and better understand the formation and removal of NDMA in ozone-biofiltration systems.

Considering these issues, the objectives, questions, and hypotheses for this research are as follows:

1. Investigate the operational conditions affecting NDMA formation and mitigation in ozone-biofiltration systems.

   Research question: Do MBR filtrate [low biodegradable dissolved organic carbon (BDOC) and low dissolved oxygen (DO) levels], pre-oxygenated MBR filtrate [low BDOC but high DO levels], and pre-ozonated MBR filtrate [high BDOC and high DO levels] exhibit different NDMA removal profiles during biofiltration, and do those removal profiles differ by EBCT?

   Hypothesis: Even though ozonation can potentially form NDMA, its ability to transform bulk organic matter, thereby creating more BDOC, and its ability to supersaturate water with dissolved oxygen will increase the rate and extent of biodegradation of NDMA in a biofiltration system.

2. Investigate NDMA formation potential during chloramination and the individual and synergistic impacts of ozone and biofiltration on NDMA formation potential.
**Research question:** Does NDMA formation during chloramination of MBR filtrate pose a significant concern in potable reuse applications, and if so, can these concerns be alleviated with upstream ozone-biofiltration?

**Hypothesis:** Even though chloramination will form NDMA when used as a final disinfectant, upstream ozone-biofiltration will reduce the concentrations of chloramine-reactive precursors, thereby achieving net reductions in NDMA concentration.

3. Assess biofilter resilience under extreme operational conditions.

**Research question:** Are biofilters sufficiently resilient to adapt to rapid changes in feedwater quality, specifically pH and dissolved oxygen concentration, without adverse impacts on performance?

**Hypothesis:** Biofilters are essentially biofilms composed of a complex matrix that enhances bacterial survival under various stresses, and these properties will offer sufficient resiliency to maintain nominal performance in the context of bulk organic removal under extreme pH and DO conditions.

4. Identify the presence of genes coding for monooxygenase enzymes that have been linked to NDMA biodegradation.

**Research question:** Are monooxygenase genes present in biofiltration systems, and do their relative abundances vary under different operational conditions?

**Hypothesis:** Quantitative polymerase chain reaction (qPCR) can be used to confirm that the genes coding for monooxygenase enzymes are more abundant in systems achieving greater removal of NDMA.
Chapter 2 – Literature Review

2.1 Background

Water is a natural resource and its lack has increasingly become a concern in many locations. In the U.S., arid areas such as the Southwest face water availability issues that have worsened in the past decades due to population growth, for example. Therefore, several strategies to overcome these issues have been proposed, such as importing water from areas with more availability, sea water desalination, water conservation measures, etc. Potable reuse was not considered a viable option 20 years ago (NRC, 1998), but due to compounding factors such as population growth, climate change, and water quality deterioration, potable reuse has recently emerged as a feasible, generally accepted, and sometimes more economical alternative to address these water issues (NRC, 2012).

Water reuse has been implemented for centuries in many parts of the globe, although mainly for nonpotable uses. There is evidence from over 4,000 years ago in locations such as Crete of sewage being used for irrigation (Angelakis and Gikas, 2014). Sewer farms applied raw sewage for irrigation of crops in Europe from the years 1500 to 1800. In the U.S., this sewer farm strategy was adopted at the end of the 19th and beginning of the 20th centuries to manage domestic sewage, especially in inland locations (NWRI, 2016). These sewer farms were producing edible crops such as corn, pumpkins, etc. from either raw sewage or sewage treated in septic tanks.

With advances in microbiology, concerns were raised about the safety of using raw sewage for irrigation, leading to the prohibition of this practice and to the creation of relevant guidelines for proper non-potable reuse implementation (California State Board of Health, 1918). With urban development, these sewer farms eventually evolved
into WWTPs that discharged biologically-treated wastewater effluent into rivers and streams (NWRI, 2016). Over time, beneficial reuse of the treated wastewater expanded to more direct applications with greater potential for human contact and adverse public health impacts (i.e., nonpotable to potable reuse). These new uses and a greater awareness of the potential microbial and chemical risks necessitated more advanced treatment consistent with the intended application and desired water quality. This concept became known as “fit for purpose” (U.S. EPA, 2012).

Figure 1 below illustrates and summarizes the reuse strategies.

Figure 1. Types of water reuse: wastewater effluent treated at the WWTP can be used for non-potable or potable reuse. Potable reuse can be either indirect (unplanned or planned) or direct.

2. 2 Potable Reuse

2.2.1. Indirect Potable Reuse

The discharge of treated wastewater effluents to a water body that is used as the drinking water source of a downstream community is considered unplanned IPR, or de
facto reuse, and many times it is practiced unintentionally, hence the name. Depending on seasonal fluctuations in stream flow, the wastewater effluent will comprise a varying fraction of the overall flow (i.e., recycled water contribution or dilution factor). Consequently, the drinking water characteristics of the downstream community can vary significantly, and a higher concentration of contaminants may be found during low flow periods. Figure 2 illustrates historical stream dilution factors in the U.S., with many water sources exhibiting >50% recycled water contributions (Rice and Westerhoff, 2017).

![Figure 2. U.S. map illustrating current dilution factors for wastewater discharges in U.S. streams (Rice and Westerhoff, 2017).](image)

Wastewater discharges to receiving bodies were particularly problematic in the U.S. prior to the 1970s, when there were no regulations controlling the practice. Wastewater-derived microbial and chemical contamination of surface water ultimately led to the development and implementation of the Clean Water Act in 1972. This act, among other measures, stipulated a minimum of secondary treatment in WWTPs, reduced chemical and microbial discharges to water bodies, imposed the National
Pollutant Discharge Elimination System (NPDES), and imposed industrial source control (Cotruvo, 2016). However, even today, only one-third of the WWTPs in the U.S. employ additional treatment processes, such as filtration and disinfection, to supplement secondary treatment (Rice and Westerhoff, 2017).

Although this de facto approach is still practiced in many places, it may not be adequately protective of public health (Amoueyan et al., 2017). On the other hand, planned IPR generally relies on processes beyond secondary biological treatment to achieve greater removal of chemicals and to inactivate pathogens prior to discharge into an environmental buffer. This additional treatment is sometimes employed in a separate AWTF. Moreover, the environmental buffer, which can be in the form of soil aquifer treatment, storage and travel time in a local aquifer, or retention and dilution in a surface water body, acts as an extra treatment process, as it is capable of reducing concentrations of bulk and trace organics, nutrients, pathogens, and other contaminants. The environmental buffer also provides response time in case of failure in the AWTF, and it works as a psychological barrier for the public, which aids in mitigating the purified water’s wastewater origin. This combination of processes (i.e., multi-barrier treatment) guarantees a higher safety level for human consumption (Pecson et al., 2015).

California is one of the pioneers of IPR in the U.S. In the past century, Orange County, which is located in semi-arid Southern California, faced drought events and a rapidly increasing population. These conditions led Orange County Water District (OCWD) to overdraft groundwater, and due to its proximity to the ocean, this continuous withdrawal of groundwater led to significant seawater intrusion into the aquifer. OCWD originally managed this issue by injecting imported freshwater to reduce seawater intrusion, but with the continuously growing population and rising
costs associated with water importation, this situation proved to be unsustainable (OCWD, 2013).

These conditions led to the development of several reuse projects in Orange County, including the most recent Groundwater Replenishment System—the world’s largest AWTF for potable reuse. This project, in partnership with Orange County Sanitation District (OCSD), generates 100 million gallons per day (MGD) of high quality water, with a capacity to be expanded to 130 MGD in the future. Consistent with the recently revised regulations for groundwater replenishment in California, OCWD now employs ‘full advanced treatment’ (FAT), which is the only treatment train accepted by California’s regulations for groundwater replenishment via direct injection. FAT specifically refers to RO and an AOP, but these processes are also preceded by secondary biological wastewater treatment and low-pressure membrane filtration (i.e., MF or UF). The OCWD advanced treatment train specifically consists of MF-RO-UV/H₂O₂. Figure 3 below illustrates the aim of the different processes in an FAT system (UV Trojan, 2017).

When employing spreading basins instead of direct injection of treated wastewater effluent, the California IPR regulations are less restrictive, and FAT is not necessarily needed, thereby allowing for alternative treatment trains. In other states, and in other countries as well, regulations regarding the treatment trains in IPR systems are less restrictive or non-existent. Some systems, such as Singapore’s NEWater project (Gerrity et al., 2013; Lee and Tan, 2016) and another in Perth, Australia (Seibert et al., 2014; Water Corporation, 2015), still employ RO-based treatment trains, but others rely on less costly treatment trains for IPR.
Figure 3. Goals of the different treatment processes in an FAT system (i.e., microfiltration, reverse osmosis, and UV/H₂O₂. (UV Trojan, 2017).

In Las Vegas, treatment prior to discharge to the environmental buffer (i.e., Lake Mead) ranges from typical tertiary treatment to a combination of UF and ozonation. There are no specific IPR regulations in Nevada for this application; instead, local utilities aim for compliance with their NPDES permits and to minimize the potential for eutrophication. The discharge of treated wastewater effluent to Lake Mead (i.e., ‘return flow credits’) is critically important because the water elevation at Lake Mead has been continuously decreasing in recent years. In fact, levels have reached historically low elevations in recent years (Figure 4).
Reno, NV, is currently investigating the possibility of constructing an IPR system for groundwater replenishment via direct injection. Previous pilot-scale studies in Reno have evaluated MF, ozone/H₂O₂, and biological activated carbon (BAC) and have shown positive results for pathogen inactivation and chemical removal and/or oxidation. This non-RO approach is beneficial for the location due to the potential for reduced costs (e.g., no brine disposal), and the use of direct injection instead of spreading (i.e., soil aquifer treatment) may reduce the potential for arsenic mobilization in the soil (Stantec, 2011).

2.2.2. Direct Potable Reuse

DPR also relies on treatment at an AWTF, but no environmental buffer is involved. Instead, the AWTF product water can be (i) discharged upstream of a drinking water treatment plant (DWTP), (ii) blended with finished water from the DWTP, or (iii)
held in an engineered storage buffer prior to direct distribution to the consumer. Eliminating the environmental buffer has potential economic benefits and is also attractive in areas with limited or no access to suitable environmental buffers (Lahnsteiner et al., 2017). Because this approach decreases the time between treatment and distribution, it also increases real (or perceived) public health risks due to the shortened duration in converting wastewater into drinking water. Therefore, the system needs to be reliable, ensuring redundancy, resiliency, and robustness (Pecson et al., 2015).

The first DPR system in the world was constructed in Windhoek, the capital of Namibia, in 1968. Severe drought conditions in the 1990s led to the development of a new DPR facility in 2002, at which point the original facility was converted to nonpotable purposes (von Rensburg, 2016). The New Goreangab Water Reclamation Plant employs a multi-barrier treatment approach, which includes powdered activated carbon (PAC), pre-ozonation, coagulation/flocculation, dissolved air flotation, dual granular media filtration, ozonation, BAC, granular activated carbon (GAC), UF, disinfection with chlorine, and stabilization with sodium hydroxide, as illustrated in Figure 5. The final product water is blended with other water sources, usually at a ratio of 25% recycled water to 75% source water and a maximum ratio of 35:65. The goal is to achieve an EfOM concentration of less than 1 mg/L, as stipulated by the City of Windhoek (Lahnsteiner et al., 2017).
In the U.S., even though DPR is not federally regulated yet, two DPR facilities were recently constructed and operated in Texas, and others are being implemented or investigated. In Big Spring, TX, tertiary dechlorinated effluent is directed to the AWTF, which employs a typical FAT treatment train consisting of MF-RO-UV/H₂O₂ (Figure 3). The product water is blended with surface water at a ratio of 15:85, and the blend is further treated at a conventional DWTP. Another example of DPR is Wichita Falls, which upgraded an existing facility originally intended to treat brackish lake water. Municipal secondary effluent was treated at the Wichita Falls AWTF by coagulation/flocculation, chloramination, sedimentation, MF, RO, and UV radiation (Figure 6). The final effluent was blended at a 50:50 ratio with surface water and further treated at a DWTP. However, the Wichita Falls AWPF was discontinued in 2015, after significant rainfall alleviated drought conditions (Lahnsteiner et al., 2017).
2.3 Regulations for Potable Reuse

2.3.1 Pathogens

The main driver of water reuse regulation is public health safety assurance. After several outbreaks of waterborne disease in the U.S., more stringent regulations were implemented for drinking water treatment. For example, the *Cryptosporidium* outbreak in Milwaukee in 1993 caused infections in more than 400,000 people and deaths of over 100 people. In the following year, a cryptosporidiosis outbreak also happened in Las Vegas, Nevada. Since those events, regulations were expanded to ensure greater public health protection and to reduce the probability of similar outbreaks in the future. These regulations included (i) the Interim Enhanced Surface Water Treatment Rule (IESWTR), which established a minimum 2-log removal requirement (99% removal) for *Cryptosporidium* oocysts for large public water systems; (ii) the Long Term 1 Enhanced Surface Water Treatment Rule, which extended the IESWTR to small public water systems as well; and (iii) the Long Term 2 Enhanced Surface Water Treatment Rule, which classified the surface water into different categories (or bins) based on oocyst concentration and required additional treatment depending on the bin classification. In response to these outbreaks and the subsequent regulations, many DWTPs impacted by wastewater effluent discharge implemented ozonation because it is a more robust unit process for the inactivation of *Cryptosporidium* oocysts (Gerrity et al., 2013).

Currently, for water reuse purposes in California, certain pathogen log removal values (LRVs) must be demonstrated before the water is used for nonpotable or potable applications. LRVs represent the percentage of pathogens inactivated: 90% for 1 LRV, 99% for 2 LRV, 99.9% for 3 LRV, and so on. For enteric viruses, the required LRV is 12, whereas for the protozoans *Cryptosporidium* and *Giardia*, the required LRV is 10.
Also, a single treatment process cannot be accredited less than 1 LRV or more than 6 LRVs, and a minimum of three treatment processes achieving at least 1 LRV must be employed (CDPH, 2014). These LRVs must be demonstrated in the treatment train between the receipt of raw sewage to the distribution of final product water. In the case of deep well injection, Cryptosporidium and Giardia LRVs must be accomplished before the well injection. For spreading basins, the total LRVs for all the mentioned pathogens should be accomplished from the raw sewage to the final water withdrawal point (CDPH, 2014). Nevada recently established regulations following the same framework as California regarding pathogen inactivation/removal: 12-10-10 LRVs for enteric viruses, Cryptosporidium, and Giardia, respectively (Nevada State of Environment Commission, 2016). The Australian Guidelines for Water Recycling established LRVs of 9.5, 8.1, and 8.0 for viruses, bacteria, and protozoa, respectively (EPHC, 2008). LRVs credited for treatment processes vary considerably for each of the target pathogens. A summary of the treatment processes and corresponding LRVs are presented in Table 1.

<table>
<thead>
<tr>
<th>Treatment Process</th>
<th>Viruses</th>
<th>Crypto</th>
<th>Giardia</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary Activated Sludge</td>
<td>1.9</td>
<td>1.2</td>
<td>0.8</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Filtered and disinfected tertiary effluent</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>MF/UF</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>RO</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Free chlorine post-RO</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>UV/H₂O₂</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Subsurface application</td>
<td>6*</td>
<td>0</td>
<td>0</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Spreading basins</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Ozone or ozone/ H₂O₂</td>
<td>6</td>
<td>1-2</td>
<td>3</td>
<td>NWRI, 2016</td>
</tr>
<tr>
<td>Ozone</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>TWDB, 2015</td>
</tr>
<tr>
<td>BAC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Trussell et al., 2016</td>
</tr>
<tr>
<td>Ozone-BAC</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>TWDB, 2015</td>
</tr>
</tbody>
</table>

*: 1.0 LRV for each month the water travels in the subsurface.
2.3.2. Chemicals

Differing sources of wastewater (e.g., industrial, domestic, etc.) make it a complex matrix composed of a wide variety of chemical constituents. These constituents include nutrients (nitrogen and phosphorus) that are not completely removed in the WWTPs, metals, total dissolved solids (TDS), and bulk and trace organic matter, among others. The organic matter present in wastewater is often described as effluent organic matter (EfOM) and is measured as total organic carbon (TOC) or dissolved organic carbon (DOC). The EfOM also consists of trace organic compounds (TOrCs), soluble microbial products (SMPs), disinfection byproducts (DBPs), etc.

In water reuse, as well as in drinking water, disinfection is a necessary step towards public health safety. However, the reactions between organic or inorganic matter and various disinfectants can lead to the formation of carcinogenic DBPs. Some DBPs, as well as other contaminants/chemicals, are regulated at the federal level in the U.S. For example, chlorine DBPs include the total trihalomethanes (TTHMs) and five regulated haloacetic acids (HAA5s), which have MCLs of 80 and 60 µg/L, respectively, and the ozone DBP bromate has an MCL of 10 µg/L (U.S. EPA, 2002).

With the advent of new treatment processes and analytical tools capable of lower detection limits, ‘new’ contaminants such as NDMA (Figure 7) have recently been discovered. NDMA is a DBP that can be formed by chloramination (Choi and Valentine, 2002), chlorination of ammonia-containing wastewaters (Mitch et al., 2003), or ozonation of wastewater (Lee et al., 2007). Although federal regulations regarding NDMA have not yet been established, NDMA is included on the EPA’s Contaminant Candidate List 4 (CCL4), and some states have established notification levels (NLs)
(e.g., 10 ng/L in California) (CDPH, 2014). A list of disinfectants and some of their DBPs and corresponding MCLs, NLs, or MCL goals (MCLGs), is provided in Table 2.

Table 2. Disinfectants and respective DBPs and reference values.

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>DBPs</th>
<th>MCL/NL (µg/L)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>THMs</td>
<td>MCL: 80</td>
<td>U.S. EPA, 2002</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HAAs</td>
<td>MCL: 60</td>
<td>U.S. EPA, 2002</td>
<td>-</td>
</tr>
<tr>
<td>Chloramine</td>
<td>NDMA</td>
<td>NL (CA): 0.01</td>
<td>CDPH, 2014</td>
<td>CCL4</td>
</tr>
<tr>
<td>Chlorine Dioxide</td>
<td>Chlorite</td>
<td>MCL: 1,000</td>
<td>U.S. EPA, 2002</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chlorate</td>
<td>MCLG: 0.21</td>
<td>U.S. EPA, 2016a</td>
<td>CCL4</td>
</tr>
<tr>
<td>Ozone</td>
<td>NDMA</td>
<td>NL (CA): 0.01</td>
<td>CDPH, 2014</td>
<td>CCL4</td>
</tr>
<tr>
<td></td>
<td>Bromate</td>
<td>MCL: 10</td>
<td>U.S. EPA, 2002</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 7. NDMA molecule structure.

NDMA removal can be accomplished by UV photolysis, which is one of the primary reasons UV was originally included in the FAT train. Supplementing high-dose UV with hydrogen peroxide results in the generation of hydroxyl radicals [i.e., an advanced oxidation process (AOP)] capable of oxidizing a wide variety of chemical compounds, including ibuprofen, carbamazepine (Lee et al., 2016), and 1,4-dioxane (McCurry et al., 2017).

TOrCs from different origins are commonly found in wastewaters. Numerous pharmaceuticals, personal care products, endocrine disrupting compounds, etc. reach the wastewater treatment plants, where they have varying susceptibility to treatment. Although not all of these contaminants pose risks to human health at the concentrations found in wastewaters, facilities sometimes monitor TOrCs as indicators of treatment.
train performance. Some TOrCs such as ibuprofen and acetaminophen are susceptible to biological treatment (activated sludge) and are found in low concentrations in final effluents. However, many compounds are resistant to biodegradation (e.g., antibiotic agents) and need further treatment for their removal from water. Some TOrCs are well oxidized by ozone and/or ozone/H₂O₂ (e.g., naproxen, carbamazepine, sulfamethoxazole), others are susceptible to UV photolysis (e.g., NDMA, diclofenac), or by UV AOP (e.g., 1,4-dioxane). Some compounds, such as the flame retardant TCEP, are resistant to all of these treatments (Lee et al., 2013; Lee et al., 2016).

Perfluorinated compounds such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been gaining more attention recently, since they are persistent in the environment. These compounds are potentially toxic and have been found in wastewater and water reuse systems (Inyang and Dickenson, 2017). EPA recommends a concentration (i.e., health advisory level) no higher than 70 ng/L for the combination of these two compounds (U.S. EPA, 2016b). Since these substances are difficult to remove from wastewaters, source control plays an important role in controlling their concentrations and for compliance with regulations.

2.4. Treatment Alternatives

2.4.1. FAT

Although California established FAT as the mandatory treatment train for IPR via groundwater injection or surface water augmentation, experts believe that the processes chosen for one site might not be the best option in other places due to the complexity and site-specificity of some wastewaters (Pecson et al., 2015).
The primary drawbacks of FAT systems are the high energy consumption, high capital and O&M costs, and need for brine (i.e., RO concentrate) disposal. For coastal cities, the brine is sometimes discharged into the ocean. However, in inland cities, additional treatment for the brine must be considered when implementing RO in the AWTF. Brine treatment alternatives are currently being investigated with several different technologies, such as BAC, ozone-BAC, UV/AOP-BAC (Justo et al., 2015), membrane distillation (Yan et al., 2017), wetlands (Chakraborti et al., 2015), etc. Regardless of the treatment adopted, it adds costs for the facilities. After FAT, the water needs stabilization by lime addition, for example, which further increases costs (Bell et al., 2016). This stabilization is required to avoid corrosion in the pipes that could lead to leaching of heavy metals. Also, if used for groundwater replenishment, lack of stabilization will promote mineral leaching, such as arsenic (Stantec, 2011).

The main advantages for the employment of FAT, specifically RO, are (i) removal of TDS, (ii) elimination of most TOrCs, (iii) low TOC concentrations in the product water (usually less than 0.5 mg/L), and (iv) consistency in product water quality for a wide range of feed water qualities. In areas where the source water is known to have high TDS concentrations, TDS levels will ultimately increase even further in closed-loop potable reuse systems, thereby necessitating RO-based treatment. RO is also common in California because the state’s IPR regulations specify a maximum wastewater-derived TOC concentration of 0.5 mg/L. If the AWTF product water concentration is higher than this limit, blending is needed (CDPH, 2014). As noted earlier, one of the justifications for TOC removal is that bulk organic matter is a known precursor for THMs and HAAs, so it must be removed to some degree prior to chlorination. Therefore, TOC removal is justifiable for public health protection, but the 0.5-mg/L target in California may be too conservative, particularly considering that the
median TOC concentration for drinking water in the U.S. is 3.2 mg/L (Trussell et al., 2013). Arnold et al. (2018) suggested a TOC target of 2 mg/L for reliable compliance with the TTHM and HAA5 MCLs in the U.S., which is consistent with EPA’s recommendations for water reuse (U.S. EPA, 2012).

Plumlee et al. (2014) estimated the costs of FAT and non-RO based treatment trains. A 10-MGD facility would cost $69 million for capital costs and $5.1 million annually for operation and maintenance (O&M) costs. In comparison, ozone-BAC systems with the same capacity were estimated at $16 million to $38 million for capital costs and $0.6 million to $2.4 million annually in O&M costs, depending on EBCT and supplemental treatment processes (Plumlee et al., 2014). Therefore, there is a significant economic benefit related to implementation of ozone-biofiltration alternatives if the target design and public health criteria can be achieved.

2.4.2. Ozone-Biofiltration

Ozone has been largely used in DWTPs in the U.S. and Europe since last century. In the U.S., the use of ozone-biofiltration has been increasing, particularly in reuse applications, in part because it is a more sustainable and energy-efficient alternative to FAT. This synergistic combination relies on the transformation of EfOM by ozone, which generates more biodegradable substrate for microbial communities in downstream biological processes. Therefore, it is important to understand the role of each process (i.e., ozone vs. biofiltration) and the synergism achieved by their combination.

Ozone is an effective disinfectant, being efficient against viruses, bacteria, and protozoa. It acts by damaging the nucleic acids and carbon-nitrogen bonds of DNA and
by destroying the cell wall of microorganisms. However, it decomposes rapidly and does not leave a residual (U.S. EPA, 1999). Ozone is also effective for the removal of taste, odor, and color. It is also a great oxidant, responsible for the transformation of numerous TOrCs present in wastewater (Gerrity et al., 2011; 2014; Lee et al., 2013). Ozone’s success in oxidizing these trace organics, as well as other contaminants, comes from its oxidative contributions from both molecular ozone and hydroxyl radicals (•OH) (von Sonntag and von Gunten, 2012). Hydroxyl radicals are non-selective and react with most wastewater compounds, even if poorly. On the other hand, molecular ozone is more selective and reacts with only some moieties, such as aromatic rings and double bonds (Michael-Kordatou et al., 2015). Therefore, some compounds are effectively oxidized by molecular ozone, others by hydroxyl radicals, and some by both.

The pH of the water also plays an important role in ozone decomposition. At high pH, the decomposition of molecular ozone towards hydroxyl radicals is favored, while lower pH values favor molecular ozone formation. The presence of hydroxyl radical scavengers, such as carbonate, bicarbonate, and EfOM, can decrease ozone efficiency when targeting pathogen inactivation and/or trace organic oxidation. Because of differences in wastewater composition and the complexity of wastewater constituents, ozone doses are usually standardized to TOC or DOC concentration and expressed as O₃/TOC or O₃/DOC (Lee et al., 2013).

As previously mentioned, NDMA can be formed from the reaction of ozone with EfOM, and the concentrations formed can vary considerably. Gerrity et al. (2014) reported NDMA concentrations of 160-180 ng/L in secondary effluent after ozonation. Pisarenko et al. (2015) reported a range of 7 to 77 ng/L of NDMA formation in different wastewaters during bench-scale ozonation. Pisarenko et al. (2015) also applied the same TOC-standardized ozone dose (i.e., O₃/TOC ratio) to the different wastewaters and
observed different levels of NDMA formation, thereby indicating the site-specificity of ozone-reactive NDMA precursor concentrations. Other studies have identified molecular ozone as the oxidant species responsible for NDMA formation (Lee et al., 2007; Marti et al., 2015), and not the hydroxyl radicals formed during ozone decay. Therefore, manipulating the pH in order to favor hydroxyl radicals might be an interesting strategy to reduce NDMA formation upon ozonation in reuse applications.

In biofilms, as well as in other biological processes, electron donors and electron acceptors are needed for oxidation-reduction (redox) reactions to occur. Limited availability of redox constituents (e.g., inadequate concentrations, mass transfer limitations) will adversely impact the thermodynamic favorability and/or kinetics of the target reactions. Ozone is responsible for the transformation of more complex molecules such as aromatic compounds into smaller, more assimilable organic material. For example, Linlin et al. (2011) reported a shift in molecular weight towards smaller compounds when ozonating treated wastewater effluent. Although DOC concentrations did not decrease, they observed a significant reduction in aromaticity (Linlin et al., 2011). This transformation ultimately increases the concentration of available electron donors for the microbial community in the biofiltration system. Terry and Summers (2018) summarized several studies reporting TOC and the BDOC fraction in systems employing ozonation or not. Overall, the BDOC fraction of TOC in ozonated systems is higher than in non-ozonated ones (Terry and Summers, 2018). Because the decomposition of ozone also leads to supersaturation with dissolved oxygen, ozonation simultaneously increases the concentration of the critical electron acceptor.

Studies have shown that toxicity may actually increase after ozonation due to this EfOM transformation (Macova et al., 2010). Fortunately, downstream biofiltration has been shown to mitigate any increase in toxicity via biodegradation and assimilation.
of the ozone transformation products, specifically ketones and aldehydes that are easily consumed by microorganisms (von Sonntag and von Gunten, 2012). Ozone-biofiltration has also been shown to remove nutrients (Kalkan et al., 2011), SMPs originating from the upstream activated sludge process, nitrogenous compounds, and other dissolved compounds (Chu et al., 2015). Ozone-biofiltration can eliminate some DBPs such as NDMA, reduce DBP precursor concentrations, and achieve significant TOrC attenuation, either via ozone oxidation and/or subsequent biodegradation (Gerrity et al., 2011; Reungoat et al., 2012; Arnold et al., 2018). This is particularly important for DBP control (e.g., THMs and HAAs) and to prevent microbial regrowth in distribution systems by reducing substrate sources.

Exhausted GAC, which is typically described as biological activated carbon (BAC) due to its lack of adsorption capacity, is often used to support microbial growth in ozone-biofiltration systems. In contrast, GAC is often used in water and wastewater applications for removal of bulk and trace organic compounds, but GAC needs to be regenerated or even replaced to restore the adsorptive capacity of the system as contaminant breakthrough is reached. In contrast, BAC does not need regeneration since its main mechanism of contaminant removal is via biodegradation. Media loss that occurs during backwashing of the biofilters may necessitate periodic media addition, however. Other media types, such as anthracite or sand, can be also used in biofiltration, but BAC has been shown to be superior with respect to some treatment targets, such as TOC removal (Arnold et al., 2018).

Biofiltration is often employed downstream of an ozonation process. Microbial attachment and growth onto the media can be promoted by eliminating any residual disinfectant that might persist through the biofiltration system (Zearley and Summers, 2012), either by not adding a disinfectant or by quenching the disinfectant through
reactions with the media (e.g., by using GAC). For ozone-biofiltration, ozone typically reacts or decomposes rapidly in the preceding contactors, thereby transforming the EfOM but not acting as a disinfectant in the biofiltration system. When residual disinfectants are used, studies have documented significant differences in microbial community structure and reductions in biological activity (de Vera et al., 2018), which may adversely impact the TOC removal goal in potable reuse applications.

Despite the benefits of ozone-biofiltration with respect to cost savings and energy consumption when compared with FAT, potential drawbacks include practical limits on TOC removal or other refractory compounds, particularly in low temperatures. Terry and Summers (2018) evaluated biofiltration performance in DWTPs and concluded that lower temperatures generally result in less removal of bulk organics, although the temperature limitation can potentially be overcome by employing longer EBCTs. Hallé et al. (2015) assessed removal of a few trace organics (naproxen, ibuprofen, etc.) and noticed that less biodegradable compounds required longer EBCTs in lower temperatures, and they concluded that temperature coefficients must be taken into account when estimating removal of those compounds. With respect to TOC removal in potable reuse applications, optimized systems often achieve effluent TOC concentrations of ~4 mg/L, which is eight times higher than the limit required by California. Effluents from these systems may require final polishing or blending.

Another drawback of ozone-biofiltration is its potential variability in product water quality. With FAT, the product water is consistently of high quality, although operational performance (e.g., membrane fouling) may vary considerably depending on feed water quality. On the other hand, operational performance (e.g., backwashing frequency) and effluent water quality (e.g., TOC concentration) may both suffer from poor feed water quality (Bull et al., 2016). Therefore, pilot-scale studies are always
encouraged to predict system performance and finalize design criteria. Additionally, an acclimation period for the microbial community is necessary to obtain relatively consistent values, and this period can vary from weeks to months, depending on capacity, climate, etc. (Hallé et al., 2015; Marti et al., 2017).

Ozone-BAC is currently employed in the DPR facility in Namibia, as shown in Figure 5. Other facilities relying on this combination are the F. Wayne Hill Water Resources Center in Gwinnet County, Georgia; the Fred Hervey Water Reclamation Plant in El Paso, Texas; and Landsborough, Gerringong, and Caboolture in Australia, although the Australian facilities have since been decommissioned (Gerrity et al., 2013). The GAC process at the Upper Occoquan Service Authority in Fairfax County, Virginia, is currently being upgraded with pre-ozonation to convert the existing adsorptive process to a biofiltration system, with the final effluent being discharged to local surface water. Another ozone-biofiltration facility is currently being designed and constructed in Hampton Roads, Virginia, with the final effluent being recharged into local groundwater.

2.4.2.4. Pre- and Post-Treatments

In potable reuse systems, MF or UF is sometimes employed before ozone or after biofiltration to reduce solids loading and aid in achieving pathogen LRVs. The use of these low-pressure membranes in the pre-treatment configuration increases ozone efficiency due to slight reductions in TOC concentration. Membrane bioreactors (MBR), used for separation of solids and liquids in an activated sludge process, can also be placed ahead of ozone instead of independent secondary clarifiers and MF or UF membranes. If placed post-biofiltration, MF or UF can reduce the loading of SMPs expelled by microorganisms during biofiltration and remove solids originating from the
biofiltration process (perhaps due to biomass sloughing). Ozone-biofiltration has also been shown to significantly improve the operational performance of low-pressure membranes (Trussell et al., 2016).

In DPR applications, a final disinfectant with a stable residual, such as free chlorine, chloramines, or chlorine dioxide, is needed before discharging the water into a distribution system. The potential formation of DBPs, such as THMs, HAAs, or NDMA, must be considered to determine whether additional mitigation strategies might be needed. In addition to its role in providing pathogen LRVs, high dose UV irradiation can also be used for further reductions of NDMA, which might persist through the biofiltration process. By supplementing the UV process with \( \text{H}_2\text{O}_2 \), the resulting advanced oxidation process could achieve further TOrC attenuation (Gerrity et al., 2016). However, some studies have shown that advanced oxidation prior to final chlorination may actually increase THM formation potential (Gerrity et al., 2009), so the need for post-treatment must be balanced with its potential unintended consequences.

In Texas, the DPR Guidelines suggest three types of non-RO-based treatment trains (Figure 8). Each of these treatment trains also indicates the use of an engineered storage buffer, which replaces the environmental buffer when employing DPR instead of IPR (TWDB, 2015).
2.5. NDMA

2.5.1. NDMA Properties and Formation

The properties of NDMA are described in Table 3, and its molecular structure was presented previously in Figure 7. Before its discovery as a DBP of ozonation (Figure 9) or chloramination (Figure 10), NDMA occurrence was principally linked to water contamination by rocket fuel, antioxidants manufacturing, and other industrial applications. Today, it is only produced intentionally for research purposes (U.S. EPA, 2014) because of its demonstrated role as a carcinogen (Sedlak and Kavanaugh, 2006).
Table 3. NDMA basic properties. Modified from U.S. EPA, 2014.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value/Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Formula</td>
<td>C_2H_6N_2O</td>
</tr>
<tr>
<td>Color</td>
<td>Yellow</td>
</tr>
<tr>
<td>Molecular Weight (g/mol)</td>
<td>74.08</td>
</tr>
<tr>
<td>Boiling Point (°C)</td>
<td>152</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>-25</td>
</tr>
<tr>
<td>Density at 20 °C (g/mL)</td>
<td>1.0059</td>
</tr>
<tr>
<td>Water solubility at 25°C</td>
<td>Miscible</td>
</tr>
</tbody>
</table>

Figure 9. NDMA formation due to oxidation of an ozone-reactive precursor. Modified from Lim et al. (2016).

Figure 10. NDMA formation due to oxidation of a chloramine-reactive tertiary amine precursor. Mono- and dichloramines are represented. Modified from Selbes et al. (2013).

Besides wastewater, NDMA has been reported in drinking water (Sedlak and Kavanaugh, 2006), surface water (Schreiber and Mitch, 2006; Liao et al., 2017), and groundwater (Kaplan and Kaplan, 1975; Gunnison et al., 2000; Bradley et al., 2005). NDMA has also been found in beer, cured meat, and other consumables (Gunnison et al., 2000). In wastewaters with industrial effluent contributions, NDMA concentrations tend to be higher when subjected to disinfection due to the presence of certain precursors (Kosaka et al., 2009; 2014), which differ in their reactions with ozone and chloramines (Marti et al., 2015). NDMA precursors involve dyes and detergents used for laundry (Oya et al., 2008; Zeng and Mitch, 2015), certain polymer coagulants used
in DWTPs (Mitch and Sedlak, 2004), certain pesticides (Asami et al., 2009), SMPs (Bukhari et al., 2017), etc.

Regarding ozonation, it has been demonstrated that full nitrification, which is achieved with longer solids retention times in the activated sludge process, sometimes leads to a decrease in NDMA formation during ozonation (Gerrity et al., 2015), but that does not apply to all facilities (Gerrity et al., 2014). There also appears to be a positive correlation between NDMA formation and ozone dose, but the level of formation seems to plateau at a certain point (i.e., $O_3/\text{DOC} > 0.5 \, \text{mg}O_3/\text{mgTOC}$) (Gerrity et al., 2015; Pisarenko et al., 2015). Pisarenko et al. (2015) also found that the main driver of NDMA formation is molecular ozone instead of hydroxyl radicals, while Marti et al. (2015) found that tertiary amines with good leaving groups (e.g., $-\text{SO}_2$, $-\text{CO}_2$) are good ozone-reactive precursors (Figure 9).

NDMA formation from chloramination has not been completely elucidated, although several mechanisms have been proposed. Some studies indicate that the main chloramine specie responsible for NDMA formation is monochloramines (Choi and Valentine, 2002; Chen and Valentine, 2006; LeRoux et al., 2011), whereas others point to dichloramines (Mitch et al., 2005; Schreiber and Mitch, 2006; McCurry et al., 2016a). It has been previously believed that monochloramine was the main driver of NDMA formation, but a recent study points to dichloramine as the main disinfectant specie to be concerned (Huang et al., 2018). Favoring of monochloramines and dichloramines in water is pH-dependent and/or due to the chlorine to nitrogen (ammonia) ratio ($\text{Cl}:\text{N}$, as $\text{Cl}_2:\text{NH}_3$). Monochloramine is the main specie between pH values of 6.5 and 9.0 or in a $\text{Cl}:\text{N}$ ratio less than 5:1 at 25°C. Dichloramine is present in water under pH values of 4 to 7 or when the $\text{Cl}:\text{N}$ ratio is 5-7:1. Trichloramine, or nitrogen trichloride, starts being formed in pH values lower than 4.4 or under excess
amount of chlorine (high Cl:N ratios), and it will become the main species of this disinfectant under pH values around 2 (Kirmeyer et al., 2004). The speciation of chloramines and pH relationship can be seen below in Figure 11.

![Figure 11. Chloramine speciation under different pH values. Modified from University of Cincinnati.](image)

The same uncertainty is valid for the classification of the precursors – secondary (Schreiber and Mitch, 2006; Choi and Valentine, 2002), tertiary (Mitch and Sedlak, 2002, 2004; Selbes et al., 2013), or quaternary amines (Kempner et al., 2010). Discrepancies regarding the molecular weight of those precursors have also been reported. Mitch and Sedlak (2004) found that the chloramine-reactive precursors were mostly low molecular weight compounds, and Pehlivanoglu-Mantas and Sedlak (2008) added that these low molecular weight compounds were hydrophilic, similar to NDMA itself. Krauss et al. (2009) found that the majority of NDMA precursors in one WWTP studied was dissolved low molecular weight compounds, while they were mainly hydrophobic colloidal or particulate compounds in another WWTP.
2.5.2. NDMA Removal

2.5.2.1. Membrane Rejection

In an FAT system, the MF or UF process has no significant impact on NDMA removal because of NDMA’s hydrophilic nature and low molecular weight (74 g/mol; Table 3). In fact, the practice of chloramination to reduce MF/UF membrane biofouling is one of the principle reasons for NDMA formation and occurrence in an FAT system (Filloux et al., 2016; Plumlee et al., 2008; Zeng et al., 2016). Since these membranes lose treatment efficiency when fouled, thereby compromising RO treatment performance, the addition of a disinfectant is a necessary measure (Michael-Kordatou et al., 2015), and since they are not as destructive to the membranes as free chlorine, chloramines are usually the preferred disinfectant.

The RO process achieves variable, but mostly moderate, rejection rates for NDMA, again because it is a hydrophilic and uncharged molecule. Sgroi et al. (2015) reported 50% rejection, Plumlee et al. (2008) obtained 50-65% rejection, Zeng et al. (2016) showed 65-100% rejection, and Fujioka et al. (2013) reported highly variable rejection ranging from 8-82%. Recent studies associate this variability in NDMA rejection with RO to several operational factors such as membrane material, temperature of the RO feed water, and degree of fouling (Fujioka et al., 2017). Fujioka et al. (2017) identified a positive correlation between secondary effluent and fulvic-like acids with NDMA rejection by RO membranes. Both secondary effluents and fulvic-like acids contain low molecular weight compounds that can create a dense fouling layer, thereby blocking the passage of NDMA and other low molecular weight compounds. On other hand, fouling by large molecular compounds (e.g., humic-like substances) may allow for a “cake enhanced polarization concentration phenomenon” that can actually increase NDMA passage (Fujioka et al., 2017). Furthermore, a recent study reported the
possibility of NDMA reformation after RO treatment, with concentrations varying based on how pH adjustment (i.e., neutralization of acidic RO permeate) was implemented (McCurry et al., 2017).

2.5.2.2. Photolysis
Due to formation during chloramination and then variable rejection by RO, additional treatment is required in FAT trains for NDMA attenuation. California potable reuse regulations require the inclusion of an advanced oxidation process (AOP) in FAT trains, and because of the need to address NDMA to ensure compliance with the 10-ng/L notification level, UV/H$_2$O$_2$ is generally selected as the AOP. This is because UV photolysis is relatively effective for NDMA destruction (Figure 12; Lee et al., 2005), with a rate constant of $4.5 \times 10^{-3}$ mJ$^{-1}$cm$^2$ (Lee et al., 2016), and the addition of H$_2$O$_2$ allows for •OH generation for oxidation of other TOrCs. In contrast with the vast majority of TOrCs, NDMA is susceptible to photolysis but highly resistant to oxidation by •OH. Its rate constant for oxidation with hydroxyl radicals is estimated at $4 \times 10^8$ M$^{-1}$s$^{-1}$ (Lee et al., 2016). Compounds susceptible to UV photolysis exhibit rate constants equal to or greater than $1.4 \times 10^{-3}$ mJ$^{-1}$cm$^2$, whereas compounds susceptible to hydroxyl radical oxidation exhibit second order rate constants on the order of $10^9$ to $10^{10}$ M$^{-1}$s$^{-1}$ (Gerrity et al., 2012).

Sharpless and Linden (2003) investigated low-pressure and medium-pressure UV lamps, with and without the addition of hydrogen peroxide, to determine the rate constants involved in the photolysis mechanism. Both lamp types are suitable for NDMA destruction, since this compound greatly absorbs light at a wavelength of 254 nm (Sharpless and Linden, 2003), but low-pressure lamps are usually employed in AWTPs. However, high UV doses (i.e., ~1,000 mJ/cm$^2$) are necessary to obtain ~99% destruction of NDMA (Sharpless and Linden, 2003), which can be cost-prohibitive in
reuse applications (Gerrity et al., 2014) considering that the required UV dose is several times or even orders of magnitude higher than the doses typically used for pathogen inactivation.

Figure 12. NDMA photolysis pathways (Lee et al., 2005).
2.5.2.3. Biodegradation

Alternatively, NDMA can be biodegraded. The first report of NDMA biodegradation was provided by Kaplan and Kaplan (1975) and then Gunnison et al. (2000). Kaplan and Kaplan (1975) reported the disappearance of NDMA in groundwater and attributed it to biodegradation, once sterilized samples of the same soil showed no decrease in NDMA concentration. Gunnison et al. (2000) reported similar findings in a different groundwater system. Although NDMA was biodegraded to a better extent under aerobic conditions, a small degree of biodegradation was also observed under anaerobic conditions, thereby suggesting that facultative anaerobes might be responsible for NDMA co-metabolism (Gunnison et al., 2000). Padhye et al. (2009) used anaerobic digester mixed liquor samples from three different WWTPs to assess NDMA biodegradation under anaerobic conditions. While in two plants there was moderate NDMA biodegradation (<50%), there was no significant change in concentration in another plant, reaffirming the complexity of NDMA biodegradation, particularly under anaerobic conditions (Padhye et al., 2009). In all these cases, the attempts of isolating the microorganisms responsible for NDMA biodegradation failed or were not attempted.

NDMA biodegradation in eukaryotes has also been studied. Tulip bulbs (Stiborova et al., 2000) and mammals (Tu and Yang, 1985) were used for the studies, and researchers attributed NDMA degradation in these higher organisms to a cytochrome P-450 enzyme (a type of monooxygenase). In order to find microorganisms capable of degrading NDMA, and based on these past studies in higher organisms, Sharp et al. (2005) proposed the investigation of NDMA degradation by monooxygenase enzymes in prokaryotes.
In prokaryotes, monooxygenases have the capability of splitting molecular oxygen into two atoms. One of these oxygen atoms binds to an electron donor that activates the enzyme. This process requires reduction of NAD\(^+\) to NADH. These electron donors can be propane, methane, ammonia, and toluene, for example, and the monooxygenases are usually specific for each donor (Sharp et al., 2005). Monooxygenase activities can be inhibited by acetylene (Pham et al., 2015; Sharp et al., 2010).

Sharp et al. (2005) relied on different bacterial strains containing different monooxygenases to test their ability to degrade NDMA under controlled conditions (i.e., pure culture of each strain in minimal basal salts media containing a primary substrate equivalent to the monooxygenase type, such as propane, toluene, and methane). Bacterial strains containing toluene 2-monooxygenase, particulate methane monooxygenases, dioxygenases, or no oxygenases at all did not exhibit NDMA removal, even in the presence of primary substrates. In contrast, bacterial strains containing propane monooxygenases, toluene 4-monooxygenase, and soluble methane monooxygenases did exhibit NDMA removal in the presence of primary substrates. This variable degradation by different monooxygenases suggests that there might be enzymatic and transportation differences between them. Also, since NDMA was not degraded when added to bacterial cultures without a primary substrate, it was proposed that this compound is co-metabolized (Sharp et al., 2005). Strains capable of degrading NDMA in the presence of a primary substrate are presented in Table 4.
Table 4. Bacterial strains capable of degrading NDMA and their respective enzymes.

<table>
<thead>
<tr>
<th>Monooxygenase Gene</th>
<th>Bacterial strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>soluble methane</td>
<td>sMMO * Methylosinus trichosporium OB3b</td>
<td>Sharp et al. 2005</td>
</tr>
<tr>
<td>propane</td>
<td>PMO * Mycobacterium vaccae JOB5</td>
<td>Sharp et al. 2010</td>
</tr>
<tr>
<td>toluene 4-</td>
<td>T4MO * Pseudomonas mendocina KR1</td>
<td>Sharp et al. 2005</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Rhodococcus sp. RR1</td>
<td>Sharp et al. 2005, 2010</td>
</tr>
<tr>
<td>toluene 4-</td>
<td>T4MO * Ralstonia pickettii PKO1 *</td>
<td>Sharp et al. 2005</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Rhodococcus ruber ENV 425</td>
<td>Streger et al. 2003</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Rhodococcus sp. RHA1</td>
<td>Sharp et al. 2007</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Mycobacterium smegmatis MC2155</td>
<td>Sharp et al. 2007</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Gordonia sp. TY-5</td>
<td>Sharp et al. 2007</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Mycobacterium TY-6</td>
<td>Sharp et al. 2010</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Pseudonocardia TY-7</td>
<td>Sharp et al. 2010</td>
</tr>
<tr>
<td>propane</td>
<td>PrMO * Methylibium petroleiphilum PM1</td>
<td>Sharp et al. 2010</td>
</tr>
<tr>
<td>unknown</td>
<td>PrMO * Rhodococcus cercidiphylly A41 AS1</td>
<td>Wang et al. 2015</td>
</tr>
</tbody>
</table>

*partial degradation

Among the strains found, *Rhodococcus sp. RR1* is an intriguing one because its main carbon source or type of monooxygenase was not defined in the study, it was capable of degrading NDMA without an isolate specific primary substrate (i.e., in the presence of soy broth), and it was not inhibited by acetylene. This last finding suggests that the monooxygenase hydroxylates different regions of the substrate (Sharp et al., 2005). In wastewater, the substrate would be the biodegradable portion of TOC.

Fournier et al. (2006) was the first to propose a specific pathway for NDMA degradation by prokaryotes. They used the strain *Pseudomonas mendocina* KR1 identified previously by Sharp et al. (2005) to study the degradation mechanism in further detail. The study was conducted in the presence of labelled NDMA (i.e., $^{14}$C) and labelled $^{18}$O in a closed atmosphere. After several hours, the NDMA was degraded to $N$-nitrodimethylamine (NTDMA), which has an extra oxygen atom than the original NDMA molecule. Due to the use of labelled $^{18}$O, they found that the incorporation of the oxygen was from the atmosphere, thereby ruling out anaerobic mechanisms. The NTDMA was then co-metabolized by this bacterial strain to $N$-nitromethylamine.

37
(NTMA) and formaldehyde. The main metabolic pathway they identified is presented in Figure 13 below. Finally, since 100% of the labelled NDMA was not recovered (89% and 94%), they proposed a minor secondary pathway similar to demethylation by eukaryotes (Figure 14) (Fournier et al., 2006).

Figure 13. Metabolic pathway proposed for NDMA biodegradation by Pseudomonas mendocina KR1. Modified from Fournier et al. (2006). T4MO represents the activity of toluene 4-monoxygenase, which is the first enzyme used in the breakdown of NDMA.
Figure 14. Demethylation pathway of NDMA biodegradation in mammalian. Modified from Fournier et al. (2006). This pathway is also suggested as a minor one during NDMA biodegradation by *Pseudomonas mendocina* KR1

A follow-up study by Sharp et al. (2007) further investigated monooxygenases in the *Rhodococcus* sp. RHA1 strain (or *Rhodococcus jostii* RHA1), which was also found to degrade NDMA. *Rhodococcus* RHA1 has a large genome with different catabolic enzymes, and their natural presence in soil environments (phylum Actinobacteria) can make them a powerful tool for bioremediation (Sharp et al., 2007; McLeod et al., 2006). A propane and an alkane monooxygenase were identified on the
genome of this bacterial strain. The propane operon (PrMO) studied contains 13 genes, as shown in Figure 15.

![Propane monooxygenase operon in Rhodococcus sp. RHA1. Hatched lines indicate upregulation when in the presence of propane (Sharp et al., 2007). pmA and prmC are hydroxylases; prmB is a reductase; prmD encodes for a coupling protein; prmE is an alcohol dehydrogenase.](image)

When searching for these same propane monooxygenase (prm) genes in other Actinobacteria, the authors observed similarities within the first 8 genes of the PrMO operon in the strains *Gordonia sp* TY-5, *Mycobacterium smegmatis* MC 2155 (Sharp et al., 2007), *Mycobacterium TY*-6, *Pseudonocardia TY*-7, and *Methylibium petroliphilum* PM1 (Sharp et al., 2010). The *prmA* gene encodes for a hydroxylase large subunit of the operon; *prmB* results in a reductase protein; *prmC* is the small subunit of the hydroxylase; *prmD* encodes for a coupling protein; and *prmE* results in an alcohol dehydrogenase. The genes *prmB* and *prmE* were both related to the catabolism of propane (Sharp et al., 2007).

In *Rhodococcus sp*. RHA1, NDMA degradation and gene expression (PrMO genes) on propane was hundreds of folds higher than without propane (Figure 14). The knockout of *prmA* completely removed the ability of the bacterium to degrade NDMA, strongly suggesting this large subunit of the PrMO is related to this chemical’s degradation (Sharp et al., 2007). Using the *Rhodococcus* strains RHA1 and RR1, Sharp et al. (2010) found that propane and NDMA fight for the active monooxygenase
enzyme, and propane actually serves as an enzyme inducer. RR1 exhibited a preference for NDMA over propane for the enzyme (Sharp et al., 2010).

Fournier et al. (2009) found another pathway for NDMA metabolization by *Rhodococcus ruber* ENV 45, involving a denitrosation similar to the one achieved by mammals with the P-450 enzyme. The byproducts detected when experimenting with labeled $^{14}$C and propane were mainly carbon dioxide (mineralization), formate, formaldehyde, nitrite, nitrate, methylamine, and dimethylamine. Since NTDMA and NTMA were not found during NDMA degradation by *Rhodococcus ruber* ENV 425, different pathways were proposed (Fournier et al., 2009; Figure 16).

This difference in pathways might be related to the difference in enzymes. *Pseudomonas mendocina* KR1 degraded NDMA through a toluene-4-monooxygenase (Fournier et al., 2006), while the suggested pathway by Fournier et al. (2009) is due to a propane monooxygenase similar in structure to the one found by Sharp et al. (2007). Even though the pathways are different for the different bacterial species, NDMA is not used for cell growth in either of them.

Although these studies have proposed mechanisms for NDMA biodegradation, there are still several knowledge gaps requiring further investigation. In ozone-biofiltration systems in which numerous compounds can serve as growth substrate by countless microorganisms, NDMA biodegradation may be significantly more complex. Since ozone-biofiltration systems have recently been attracting attention due to their cost benefits for potable reuse applications, a better understanding of NDMA biodegradation in this context is needed.
Figure 16. Denitrosation pathway proposed for NDMA degradation by *Rhodococcus ruber* ENV45 accomplished by a propane monooxygenase (Fournier et al., 2009).
2.6. Conclusions

Because conventional water sources are becoming increasingly compromised, many agencies are pursuing potable reuse to augment their water resource portfolios. It is imperative that the treatment trains used to transform wastewater into a finished drinking water be properly designed to ensure adequate public health protection and optimized to reduce the associated costs.

The use of FAT reliably achieves all current requirements by California’s DDW. However, this system can be cost-prohibitive to many agencies, thereby highlighting the need for alternative treatment trains. Ozone-biofiltration is currently employed in several facilities throughout the world, and this treatment train has been shown to be nearly ‘equivalent’ with respect to pathogen reduction and the attenuation of many chemicals. Nevertheless, the potential formation of NDMA and other DBPs, as well as the parameters and design criteria that govern their removal, must be investigated to ensure public health preservation and sustainability while augmenting water supplies.
3.1. Introduction

As mentioned earlier, ozonation of wastewater can lead to the formation of \(N\)-nitrosodimethylamine (NDMA), which is a potential human carcinogen with a notification level (NL) of 10 ng/L in California. When ozonation is combined with downstream biofiltration, studies have demonstrated attenuation of NDMA to concentrations less than the corresponding method reporting limits (MRLs), which are typically ~2 ng/L (Zeng et al., 2016). Gerrity et al. (2015) studied several WWTPs and AWTFs employing ozone-BAC, as well as other treatment processes, and reported the ability of BAC to consistently remove NDMA formed during ozonation. However, the ozone-induced formation of NDMA was typically low in these systems, thereby limiting the NDMA load to the downstream biological process. In contrast, Trussell et al. (2016) reported significant formation of NDMA during ozonation at one facility (up to 400 ng/L in one sampling event), which overloaded the downstream biofiltration process and resulted in detectable NDMA in the ozone-biofiltration effluent.

Although some of the pathways for NDMA removal through biological metabolism are known (Fournier et al., 2006; 2009), studies have generally focused on individual bacterial strains and their respective monooxygenase enzymes in controlled laboratory experiments (Sharp et al., 2005; Webster et al., 2013; Fournier et al., 2006). Although these studies are extremely useful to understand the co-metabolism processes, the mechanisms might be different in more complex environments (e.g., during wastewater treatment) due to the presence of other microorganisms and compounds (e.g., inhibitory substances). The removal rates may differ as well. In other words, the operational parameters that impact these processes within more complex systems and at larger scales are not yet fully understood. With efforts to understand the role of these
parameters in NDMA formation and subsequent biodegradation, the findings can ultimately be applied to minimize NDMA formation during ozonation and maximize NDMA attenuation during downstream biofiltration, perhaps by enhancing, stimulating, or selecting for favorable microorganisms.

Within this context, the aim of this phase of the research was to investigate different operational parameters [e.g., empty bed contact time (EBCT), dissolved oxygen (DO) levels, biodegradable dissolved organic carbon (BDOC) levels, media type, etc.] in a pilot-scale ozone-biofiltration system fed with membrane bioreactor (MBR) filtrate from a water reclamation facility (WRF) to understand the major variables that control NDMA levels in potable reuse applications.

### 3.2. Materials and Methods

#### 3.2.1 Study Site

A 1-liter-per-minute pilot-scale ozone-biofiltration system was constructed and operated at a full-scale WRF in Las Vegas, NV. The full-scale plant has a capacity of 10 MGD but currently operates at an average of 5 MGD. Coarse screens, grit chambers, and fine screens (2 mm) are placed before the activated sludge process, which achieves full nitrification with a solids retention time of 8 to 10 days and relies on a membrane (nominal pore size = 0.04 µm) to separate the solids from the treated liquid. This treatment configuration is known as a membrane bioreactor (MBR). The MBR filtrate serves as the influent to the pilot-scale ozone-biofiltration system. The plant uses chlorammines (sodium hypochlorite followed by aqueous ammonia addition) to disinfect the final effluent, which is used for nonpotable reuse purposes, and the sludge produced is returned to the sewers for further treatment in a separate treatment facility, thereby characterizing the facility as a scalping plant.
3.2.2. Pilot-Scale Ozone-Biofiltration

The pilot-scale ozone-biofiltration system consisted of an oxygen concentrator (AirSep, Denver, CO), an air dehumidifier (Magnum 600, Ozone Solutions Inc., Hull, IA), and an ozone generator (Nano dielectric, Absolute Ozone, Edmonton, AB, Canada) to apply the desired ozone dose to the MBR filtrate. The equipment can be seen in Figure 17. A Venturi injector (Mazzei, Bakersfield, CA) was installed to achieve ozone gas injection. The ozonated water then passed through twelve 4-ft-tall ozone contactors to allow for complete ozone decay before reaching the biofilters (Figure 18). The first four contactors were 1 inch in diameter, and the final eight contactors were 2 inches in diameter. Teflon tubing was installed at the top of each ozone contactor for ozone off gassing, and the off gas was then passed through a manganese dioxide ozone destruct system (Ozone Solutions Inc, Hull, IA).

As shown in Figure 19, the ozonated water was collected in a small water tank and pumped with two peristaltic pumps (Cole Palmer, Vernon Hills, IL) to two parallel columns, one containing anthracite (1.2 mm in diameter) and another containing biological activated carbon (BAC) (0.95 mm in diameter). The BAC was exhausted granular activated carbon (GAC) (Norit 820, Cabot Corporation, Alpharetta, GA) from the F. Wayne Hill Water Resources Center in Gwinnet County, GA, and had been previously used for over 10 years in a full-scale wastewater treatment plant (WWTP). Since the media was exhausted, which was later confirmed by experimental testing, biodegradation was considered as the main mechanism for organics removal. The exhausted anthracite was provided by San Jose Creek Water Reclamation Plant, in Los Angeles, CA. An additional BAC column was fed with ambient MBR filtrate as a control. The PVC biofiltration columns were 1 inch in diameter, and the height of the media was approximately 27.6 inches. Media lost during backwashing was replaced to
maintain a consistent media height during the study. The effluent flow rate from the biofilters was controlled by the peristaltic pumps and needle valves. In addition to the tubing that allowed for collection of biofiltration effluent, the biofilter columns also had two sampling ports, one at a media depth of 3 in and another at a media depth of 16.5 in from the surface, for media collection.

Figure 17. Ozone generator (red), oxygen dehumidifier (brown), and oxygen concentrator (grey). Oxygen from the ambient air is concentrated and it passes through an air dryer before being sent to the ozone generator to remove moisture content from the air.
Figure 18. One liter per minute pilot-scale ozone-biofiltration system. Ozone destructor (left), ozone contactors (transparent PVC pipes), BAC column, anthracite column, and control column (left).

Figure 19. One liter per minute pilot-scale ozone-biofiltration system seen from the opposite side as in Figure 18. Peristaltic pumps (bottom left) to the biofilters and tank with ozonated water with no residual ozone.

### 3.2.3. Water Quality Tests

#### 3.2.3.1. Bulk Organic Matter Quantification and Characterization

Weekly monitoring tests were performed to track the performance of the pilot-scale system. TOC levels were measured by a TOC Analyzer (TOC V-csn, Shimadzu,
Kyoto, Japan) equipped with an autosampler. Prior to analysis, the samples were collected in 40 mL amber glass vials and acidified with 400 µL of 2 N hydrochloric acid (HCl) to reach a pH<2, which inhibited microbial activity and allowed for the conversion of the inorganic carbon to carbon dioxide (CO₂) by the TOC analyzer method (non-purgeable organic carbon). The CO₂ generated by acidification was sparged by the carrier gas (carbon-free compressed air) inside the analyzer. The remaining carbon in the sample was then combusted by a platinum-catalyzed furnace inside the analyzer, and the final CO₂ measured was quantified by a nondispersive infrared detector. Calibration standards were prepared each day of testing before sample measurement to ensure accuracy.

Several tests were also performed to differentiate TOC and DOC. Samples were collected in the TOC vials, and a portion of the sample was filtered through 0.7-µm glass microfiber syringe filters (GE Healthcare, Buckinghamshire, UK). The filters were preconditioned with approximately 20 mL of deionized water and then 5-10 mL of sample. The samples were then analyzed as described previously: unfiltered samples were reported as TOC and filtered samples as DOC.

UV absorbance and fluorescence were tested using an Aqualog spectrofluorometer (Horiba, Edison, NJ). Sample corrections included blanks in each day of testing with deionized water. Differential UV_{254} absorbance was used to estimate the applied ozone dose with Equation 1, according to the method developed by Gerrity et al. (2012).

\[
UV_{254} \text{ Absorbance Reduction (\%)} = 0.1863 \ln \left( \frac{\text{Applied O}_3 \text{ dose}}{\text{TOC}} \right) + 0.5066
\]

Equation 1. UV absorbance change correlation with ozone dose.
3.2.3.2. Nutrients

Nutrients such as ammonia, nitrate, nitrite, and phosphate were analyzed by Hach (Loveland, CO) colorimetric methods (handheld DR 900 for nitrogen species and DR 5000 for phosphate). Low-range ammonia (0.02 to 2.5 mg/L as N) was analyzed with the salicylate method (Hach Method 10023), high-range nitrate (0.3 to 30 mg/L as N) was analyzed with the cadmium reduction method (Hach Method 8039), low range nitrite (0.005 to 0.350 mg/L as N) was analyzed with the diazotization method (Hach Method 8507), and phosphate (0.02 to 2.50 mg/L as phosphate) was measured with the ascorbic acid method (Hach method 8048). Since the full-scale plant does not employ phosphorus removal, the phosphate concentrations in the pilot-scale ozone-biofiltration systems were relatively high (~5-9 mg/L as phosphate). Therefore, samples were diluted by a factor of 4 times with deionized water prior to analysis.

3.2.3.3. Adenosine Triphosphate (ATP)

Attached adenosine triphosphate (ATP) tests were performed to monitor biological activity in the biofilters. Since ATP is an essential molecule in cell growth, it can be used as a surrogate for the activity of the microorganisms in the biofilter media (Justo et al., 2015). A deposit and surface test kit (LuminUltra Technologies Ltd, New Brunswick, Canada) was used to extract ATP from the cells colonizing the biofilters, and a PhotonMaster Luminometer (LuminUltra Technologies Ltd, New Brunswick, Canada) was used to quantify the ATP in each sample. The method relies on the reaction between the ATP and luciferase enzymes to quantify the luminescence, and blanks are prepared using the luciferase with an ultracheck solution and ran immediately before the samples readings. Media samples were collected using autoclaved spatulas from the upper and lower sampling ports of the biofiltration columns.
3.2.4. NDMA tests

Besides the weekly monitoring parameters, the water was analyzed for NDMA concentrations during specific experiments. 1-L samples were collected in amber glass bottles containing sodium azide and sodium thiosulfate to inhibit microbial activity and to quench any chlorine or chloramines residual, respectively. The glass bottles containing the preservatives were provided by the Southern Nevada Water Authority (SNWA), who also analyzed the samples with solid phase extraction followed by gas chromatography tandem mass spectrometry (GC-MS/MS) using a modified version of U.S. EPA Method 521 (Holady et al., 2012). The MRL ranged from 2.0 to 2.9 ng/L, depending on the sample event.

3.2.4.1. NDMA biodegradation under different EBCTs

The first NDMA test was performed in March 2017 with a constant ozone dose (O₃/TOC ~ 1.0) and different EBCTs to evaluate the role of longer contact time in the biodegradation process. This experiment also allowed for an investigation of ozone’s role in NDMA formation/mitigation (i.e., ozonated BAC column vs. the control BAC column) and the contribution of different media types (i.e., BAC vs. anthracite).

Since the ambient NDMA levels were low (~7 ng/L in the non-ozonated MBR filtrate and ~30 ng/L in the ozonated MBR filtrate), an NDMA solution was prepared and spiked into the water tank to target an initial NDMA concentration of ~300 ng/L in the feed to the biofiltration columns. Separate spiking experiments were performed with non-ozonated and ozonated MBR filtrate. Before spiking, the tank was emptied and wiped to remove any possible microbial growth that could potentially degrade NDMA during the storage period. The water at the tank was sampled before and after the
experiment was completed to ensure that the concentration was relatively constant, i.e.,
well mixed during the whole test and not biodegraded prior to biofiltration.

Once the NDMA solution was well mixed in the tank, the samples were
collected only after a period of 3 times the EBCT to flush the ‘old’ water. For all
columns, three EBCTs were tested: 2, 10 and 20 minutes. pH and temperature were
measured on-site for all samples. The effluent water from the biofilters was collected in
1-L amber glass bottles in duplicate. After collection, the samples were refrigerated at 4
°C prior to delivery to SNWA for processing and analysis.

3.2.4.2. NDMA biodegradation under different redox conditions for BAC columns

The second NDMA test was applied only to the ozonated and non-ozonated
BAC columns, with a constant EBCT of 10 minutes. In this experiment, different
operational conditions were evaluated: (1) ozonation (i.e., high BDOC and high DO),
(2) oxygenation (i.e., low BDOC and high DO), and (3) no pre-treatment (i.e., low
BDOC and low DO). The experiment was performed as described in the previous
section (i.e., with experimental water samples spiked with ~300 ng/L of NDMA), but
the same water was fed to the parallel BAC columns (i.e., typically ozonated BAC
column and typically non-ozonated BAC column) for each test.

The ozonation test was performed with ozonated MBR filtrate ($O_3$/TOC = 1.4)
spiked with NDMA and fed to the BAC columns in parallel. For the oxygenation test,
the ozone generator was shut off, so only concentrated oxygen was being fed into the
MBR filtrate. For the MBR filtrate test, both the oxygen concentrator and the ozone
generator were shut off, and the MBR filtrate was then fed to the parallel BAC columns.
Effluent samples were collected in triplicate in 1-L amber glass bottles provided by
SNWA. Besides temperature and pH, DO levels were also measured (on-site) using a Sension + DO6 Portable DO Meter (Hach, Loveland, CO). The samples were brought to the laboratory and refrigerated 4°C prior to delivery to SNWA for processing and analysis.

3.2.4.3. NDMA biodegradation and formation potential upon final chloramination

Since a chlorine or chloramine residual is necessary in water distribution systems to prevent microbial regrowth, the effects of final chloramine addition were studied in the MBR filtrate, ozone effluent, and biofilter effluents to assess chloramine-reactive precursors and NDMA formation potential. In this experiment, no NDMA was spiked into the water tank since the aim was to simulate real treatment train and distribution system conditions. The EBCT was fixed at 10 minutes for all columns, and the ozone dose was fixed at $O_3/TOC = 1.5$. The pilot-scale samples were collected in triplicate, such that the ambient NDMA could be measured in one sample and the formation potential, under uniform formation conditions (UFC), could be assessed in duplicate.

For the NDMA UFC tests, a similar approach described by Zeng and Mitch (2015) was used to simulate final chloramination. The UFC test involved the addition of 5 mL of a borate buffer (0.8 M) and the addition of 3.7 mL of freshly prepared chloramines solution at a concentration of 1.32 g/L as Cl$_2$. This led to a pH of approximately 8.0 and a chlorine to ammonia ratio (Cl:N) of 3.5:1. The initial concentration targeted was 5 mg/L as Cl$_2$ of pre-formed chloramines. The samples were then stored in the dark for 3 days at 20°C until quenching with ascorbic acid. After quenching, the samples were sent to SNWA for NDMA analysis, while the other water quality parameters were tested at the UNLV laboratory.
3.2.5. Trace Organic Contaminants

The presence of typical TOrCs and perfluorinated compounds was also evaluated in the ozone-biofiltration pilot plant. The following samples were analyzed during a single sample event: MBR filtrate, ozonated MBR filtrate, MBR+biofiltration alone (i.e., BAC control column), and MBR+ozone+biofiltration (i.e., ozonated BAC column). The target compounds included acetaminophen, atenolol, caffeine, carbamazepine, \textit{N,N}-Diethyl-meta-toluamide (DEET), fluoxetine, gemfibrozil, ibuprofen, meprobamate, naproxen, primidone, sucralose, sulfamethoxazole, \textit{tris(2-chloroethyl) phosphate (TCEP)}, triclocarban, triclosan, and trimethoprim. The perfluorinated compounds were PFOS, PFOA, perfluorobutane sulfonate, perfluorobutanoic acid, perfluorodecane sulfonate, perfluorodecanoic acid, perfluoroheptanoic acid, perfluorohexane sulfonate, perfluorohexanoic acid, perfluorononanoic acid, and perfluoropentanoic acid. This experiment was performed under ambient conditions with no spiking of any target compounds.

A constant \textit{O}_3/TOC ratio of 1.3 was applied during this experiment, and the EBCT was fixed at 10 minutes. The effluent samples were collected in 1-L high density polyethylene bottles (for perfluorinated compounds) and in 1-L amber glass bottles (for other TOrCs) with sodium azide for preservation and sodium thiosulfate for oxidant quenching. All bottles were prepared and provided by SNWA. After collection, the samples were refrigerated at 4°C prior to delivery to SNWA for processing and analysis.
3.3. Results and Discussion

3.3.1. Water Quality Tests

3.3.1.1. Nutrients

The collected data can be divided into two phases. Phase 1 focused on NDMA spiking/removal in the ozone-biofiltration system, and Phase 2 focused on NDMA UFC formation potential testing. Before these NDMA tests were performed, weekly testing of general water quality parameters was conducted to establish a baseline level of performance and assess system stability and acclimation. Table 5 below summarizes the resulting water quality data from four months of monitoring.

Table 5. Average (± one standard deviation) results of four months of weekly sampling of typical water quality parameters.

<table>
<thead>
<tr>
<th></th>
<th>UVA\textsubscript{254} (cm\textsuperscript{-1})</th>
<th>PO\textsubscript{4}\textsuperscript{3-} (mg/L)</th>
<th>NO\textsubscript{3}\textsuperscript{-} (mg/L as N)</th>
<th>NO\textsubscript{2}\textsuperscript{-} (mg/L as N)</th>
<th>NH\textsubscript{3} (mg/L as N)</th>
<th>TOC (mg/L)</th>
<th>TOC removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBR</td>
<td>0.14±0.01</td>
<td>5.9±1.1</td>
<td>6.7±1.9</td>
<td>0.03±0.01</td>
<td>0.01±0.02</td>
<td>7.5±0.5</td>
<td>N/A</td>
</tr>
<tr>
<td>MBR+O\textsubscript{3}</td>
<td>0.07±0.02</td>
<td>5.5±1.5</td>
<td>5.6±2.0</td>
<td>0.0</td>
<td>0.01±0.01</td>
<td>7.1±1.1</td>
<td>N/A</td>
</tr>
<tr>
<td>MBR+O\textsubscript{3}+BAC</td>
<td>0.07±0.02</td>
<td>5.8±1.2</td>
<td>5.2±1.2</td>
<td>0.01</td>
<td>0.02±0.02</td>
<td>5.1±0.9</td>
<td>21.5±4.6</td>
</tr>
<tr>
<td>MBR+O\textsubscript{3}+Ant</td>
<td>0.07±0.01</td>
<td>5.3±1.6</td>
<td>5.6±2.0</td>
<td>0.02±0.01</td>
<td>0.02±0.02</td>
<td>6.2±0.5</td>
<td>15.4±7.1</td>
</tr>
<tr>
<td>MBR+BAC</td>
<td>0.13±0.01</td>
<td>5.1±1.3</td>
<td>5.7±2.0</td>
<td>&lt;0.02</td>
<td>6.3±0.3</td>
<td>14.4±5.1</td>
<td></td>
</tr>
</tbody>
</table>

3.3.1.2. ATP

In order to observe the development of the biofilters, media was extracted from each column and analyzed for ATP. The data points for ATP data are represented in Figure 20 below. Since the original data was collected as pg/g of wet media, moisture content (measured for each media type in triplicate) was used to convert wet mass to dry mass and to convert pg/g of dry media to pg/cm\textsuperscript{3} based on bulk density. These calculations can be seen in Appendix 1.
Magic-Knezev and van der Kooij (2004) reported a range from $5 \times 10^4$ to $1 \times 10^7$ pg/g in BAC filters, and Velten et al. (2011) reported a range of $8 \times 10^5$ to $6 \times 10^6$ pg/g in BAC filters, with ATP varying by depth. Therefore, biofilter ATP values reported in the literature vary significantly, but the data presented here are generally in accordance with other studies.

From Figure 20 it is possible to see that, except for the anthracite filter, the samples from the bottom of the filter (low) had lower microbiological activity (i.e., ATP) than the top of the columns (high). These results are in accordance with other studies attributing a higher amount of assimilable organic carbon (AOC) or BDOC closer to the influent water feed, which promotes greater biological activity, followed by a reduction with filter depth, which results in less biological activity deeper in the column (Hallé, 2009; Han et al., 2013; Peldszus et al., 2012; Gerrity et al., 2018; Velten et al., 2011). Despite these general trends, the differences in depth for each media type were statistically insignificant ($p=0.15$ for ozonated BAC, $p=0.27$ for non-ozonated BAC, and $p=0.08$ for ozonated anthracite).
Some researchers argue that biomass quantity, as measured by ATP, does not exhibit a reliable correlation with biofilter performance (Pharand et al., 2015) and that other metrics such as TOC removal might be a better representation. From Table 5, it is possible to see that the ozonated BAC filter generally exhibited superior performance with respect to TOC removal than the anthracite, with the ozonated BAC and ozonated anthracite media exhibiting similar ATP levels. In contrast, the non-ozonated BAC column had significantly higher ATP concentrations (p=0.01) but lower TOC removal, which supports the observation from Pharand et al. (2015).

3.3.1.3. Bulk Organic Matter Quantification and Characterization

Since the pilot-scale system received full-scale MBR filtrate (pore size of 0.04 µm) and the DOC procedure requires filtering samples with 0.7-µm filters, DOC concentrations were expected to be similar to TOC concentrations in the pilot effluents. To confirm, samples were collected for comparison of DOC and TOC values. The average results are shown in Table 6. Since DOC and TOC samples showed less than 4% difference, only TOC samples were collected and analyzed going forward. The ozone doses (O₃/TOC = 1.1) and EBCTs (~5 minutes) were the same for all tests summarized in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>TOC, mg/L</th>
<th>DOC, mg/L</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBR</td>
<td>7.1</td>
<td>7.1</td>
<td>0.8</td>
</tr>
<tr>
<td>MBR+O₃</td>
<td>7.1</td>
<td>6.9</td>
<td>3.2</td>
</tr>
<tr>
<td>MBR+O₃+BAC</td>
<td>5.9</td>
<td>5.7</td>
<td>2.9</td>
</tr>
<tr>
<td>MBR+O₃+Ant</td>
<td>6.6</td>
<td>6.5</td>
<td>2.0</td>
</tr>
<tr>
<td>MBR +BAC</td>
<td>5.9</td>
<td>5.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 6. Average (n=4) TOC and DOC results comparison.
TOC removal was monitored weekly and throughout the various experimental phases. Figure 21 illustrates the TOC removals observed as a function of media type and EBCT for a constant O$_3$/TOC of 1.0.

![Graph showing TOC removal as a function of EBCT](image)

**Figure 21.** Average (n=4) TOC percentage removal by the three columns using EBCTs of 2, 10, and 20 minutes. Error bars represent standard deviations.

Ozonated columns outperformed the non-ozonated column with respect to TOC removal. In this experiment, a decrease in TOC removal was observed in both ozonated columns for the 20-minute EBCT when compared to the 10-minute EBCT. The explanation for this is not entirely clear, but it might be due to an increase in the release of soluble microbial products (SMPs) due to the longer EBCT. SMPs are organics linked to microbial metabolism or biomass decay that are released into the water during biological treatment processes (Barker and Stuckey, 1999).

The fact that the ozonated anthracite column did not reach the same removal as the BAC (p=0.11) might be because the anthracite grains are bigger than the GAC and, therefore, have a lower surface area (Appendix 2). As a result, greater biomass can theoretically attach to the GAC grains to develop a biofilm, and a greater quantity of
bacteria can colonize the overall BAC column. The ATP data in Figure 20 can corroborate this hypothesis, illustrating that there was slightly more biological activity (i.e., ATP) in the BAC column than the anthracite column. Using scanning electron microscopy, Shen et al. (2016) analyzed the biomass developed in biofilters with different media types operating for the same amount of time. They concluded that there was more biomass in a GAC biofilter than in filters with flaky media (e.g., anthracite) (Shen et al., 2016).

The BAC control column (\(O_3/TOC = 0\)) presented a more linear correlation with increasing time, but even at longer EBCTs, the removal is still poor when compared to the ozonated columns. This can be explained by the formation of BDOC (or AOC) during the ozonation process, and it agrees with previous research. Lee et al. (2012) studied the biodegradable and nonbiodegradable fractions of DOC from treated wastewater with ozonation. They observed that with higher ozone doses, the percentage of nonbiodegradable DOC decreased and, consequently, the biodegradable fraction increased. Therefore, the ozonation step transforms the organic compounds present in the wastewater into compounds that are easier for the microbial community to biodegrade (Lee et al., 2012).

Even though the adsorption capacity of the media in the columns is assumed to be exhausted, it is important to add that these EBCT values may be compound-specific due to adsorption. The different compounds comprising the TOC will interact differently with the column and the microbial community and therefore they will present different residence times.

Excitation emission matrices (EEMs) have been used to characterize the organic matter in various water matrices, including SMPs, humic-like substances, and fulvic-
like substances (Chen et al., 2003; Gerrity et al., 2011). Figure 22-a (Gerrity et al., 2011) exemplifies the three regions (1: SMPs, 2: fulvic-like substances, and 3: humic-like substances), while Figure 22 b-e illustrates the ‘fingerprints’ of the water samples from the current study. While the MBR filtrate and BAC Control EEMs show typical wastewater fingerprints, the ozone completely transforms the water. Even though there is a slight increase in fluorescence in the BAC effluent, it is still far from the typical wastewater features of the MBR filtrate.

The EEMs in Figure 22 demonstrate that characterization of effluent water quality requires simultaneous evaluation of multiple water quality parameters. Although ozonation achieves significant transformation of the bulk organic matter, as demonstrated by the reduction in fluorescence, there is not a considerable reduction in overall TOC during ozonation. On the other hand, the post-ozone biofiltration process exhibits an increase in fluorescence in some regions despite an overall reduction of TOC. Therefore, surrogate water quality parameters such as UV$_{254}$ absorbance and fluorescence are valuable for demonstrating bulk organic matter transformation, while other quantitative measures such as TOC are useful for showing removal of bulk organic matter.
Figure 22. Excitation-emission matrices of water samples. a) Typical secondary effluent EEM. *Modified from Gerrity et al. (2011); b) MBR filtrate; c) Non-ozonated BAC Control column effluent (biofiltration alone); d) ozonated effluent; e) ozonated BAC effluent.
TOC removal is critical in terms of regulatory compliance in some jurisdictions. For example, California established a 0.5-mg/L limit on wastewater-derived TOC in potable reuse applications. Waters with a TOC concentration higher than 0.5 mg/L, which can really only be accomplished with RO treatment, must be blended with conventional source waters (e.g., groundwater in the environmental buffer). Interestingly, the median TOC concentration of drinking water in the U.S. is 3 mg/L (Trussell et al., 2013), which raises questions about the legitimacy of the 0.5-mg/L benchmark. Therefore, the 0.5-mg/L target might be useful as an indicator of treatment performance in FAT trains (i.e., RO product water), but it may not be a justifiable target on the basis of public health protection.

Arnold et al. (2018) identified a TOC limit of ~3 mg/L for strict compliance with the TTHM MCL in the U.S. and a 2-mg/L target when considering a 25% safety factor for increased reliability. This is in agreement with the 2012 U.S. EPA Guidelines for Water Reuse, which recommends a 2-mg/L TOC limit for potable reuse applications (U.S. EPA, 2012). This less stringent target allows for different treatment train alternatives (e.g., ozone-biofiltration) that have still been shown to be “equivalent” to FAT and adequately protective of public health (Trussell et al., 2016).

### 3.3.2. NDMA Biodegradation

#### 3.3.2.1. NDMA biodegradation under different EBCTs

The first NDMA test was performed after the pilot-scale ozone-biofiltration system was running for about 2 months in order to make sure the microbial community was acclimated. This period of time was chosen based on consistent TOC and nutrients data and extensive previous use of the media as part of another study. During this
testing phase, a constant O₃/TOC ratio of ~1.0 was administered, and EBCT was varied between 2 and 20 minutes in the three biofiltration columns.

The ambient NDMA concentration in the MBR filtrate was 6.9 ng/L, and this concentration increased to 33 ng/L after ozonation, which is a moderate/typical level of NDMA formation during wastewater ozonation (Gerrity et al., 2015). NDMA formation varies considerably between wastewaters because it depends on the presence of ozone-reactive precursors, which are very site-specific. Gerrity et al. (2015) investigated NDMA formation with ozone in several WWTPs in the U.S. and Australia and reported a wide range of values. In some WWTPs, NDMA formation varied from around 30 to around 140 ng/L, whereas in other places ozonation caused minimal or no NDMA formation (Gerrity et al., 2015). Kosaka et al. (2009) reported NDMA concentrations of 14-16 ng/L rising to 280-290 ng/L after ozonation. Zeng et al. (2016) reported even higher NDMA concentrations formed after ozonation: from a range of <2-21 ng/L to 250-470 ng/L. In many of the systems with high levels of NDMA formation, the WWTPs received considerable industrial discharges.

Because ozone-reactive precursors need to be present in order to react with ozone and form NDMA, the moderate level of NDMA formation upon ozonation suggests that ozone-reactive precursors are present in relatively low concentrations in the MBR filtrate. This is likely related to the fact that the full-scale WWTP receives primarily domestic wastewater from the local community (i.e., minimal industrial contributions).

Due to the relatively low ambient concentration in the non-ozonated and ozonated feed waters, an NDMA solution was spiked to target concentrations of ~300 ng/L in the non-ozonated and ozonated feed waters to the anthracite and BAC columns. After analysis, the concentrations in the ozonated and non-ozonated feed waters were
determined to be 285 and 255 ng/L, respectively, with the difference explained by the NDMA formed during ozonation of the MBR filtrate. Subsequent NDMA removal by the biofiltration columns is shown in Figure 23.

![Figure 23](image-url)

Figure 23. Average (n=2) NDMA percentage removal in the different columns using EBCTs of 2, 10, and 20 minutes. Error bars represent standard deviation.

As seen from Figure 23, all columns achieved greater NDMA removal with longer EBCTs, but the removal profile did not appear to be linear, at least for the ozonated columns. Previous studies have also reported a non-linear correlation between EBCT and the removal of bulk organic matter or DBP precursors. Arnold et al. (2018) reported minimal reduction in HAA5 and TTHMs formation potential when increasing the EBCT from 15 to 20 minutes in the same pilot-scale ozone-biofiltration system using an O$_3$/TOC ratio of 0.8. When the EBCT was increased from 10 to 15 minutes, a significant decrease in HAA5 formation potential (~40%) was observed when using a higher ozone dose (O$_3$/TOC ratio of 2.25), and almost no removal was observed when using a lower ozone dose (O$_3$/TOC ratio of 0.13). Wu and Xie (2005) observed that longer EBCTs only affected the removal of HAA5s in low temperatures (4 to 10 °C)
and that the removal percentage plateaued faster (i.e., shorter EBCTs) in higher temperatures.

Because the removal found was found to be non-linear in the ozonated columns, a first-order reaction was assumed to allow calculation of the corresponding biodegradation rate constants. The rate constants (Table 7) were calculated based on linear regression of the data generated with Equation 2.

\[
\ln \frac{NDMA_f}{NDMA_0} = -k \times EBCT
\]

Equation 2. Rate constant calculation for NDMA.

<table>
<thead>
<tr>
<th>First Order Rate Constant ( (k_{NDMA}), \text{ min}^{-1} )</th>
<th>BAC</th>
<th>Anthracite</th>
<th>BAC Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.197</td>
<td>0.158</td>
<td>0.029</td>
<td></td>
</tr>
</tbody>
</table>

Ozonated BAC showed the highest rate constant, and the rate constants for both ozonated columns were higher than that of the non-ozonated BAC column. In the ozonated columns, increasing the EBCT from 2 to 10 minutes had a significant impact on NDMA removal (30-37% vs. ~90%), but when the EBCT was increased to 20 minutes, there was only a nominal additional increase (from ~90% to ~96%). Therefore, in the selection of design/operational criteria for biofiltration systems, the need for maximum removal must be balanced against the point of diminishing return. Longer EBCTs can impact full-scale facilities significantly. Longer EBCTs necessitate biofiltration columns with larger structural footprints, which require more land area and higher capital costs.
With an initial NDMA concentration of ~285 ng/L, the ozonated BAC column achieved an average effluent NDMA concentration of 6.5 ng/L with a 20-min EBCT, which would comply with California’s NL, whereas the anthracite column exceeded the NL with an average NDMA concentration of 18.6 ng/L with a 20-min EBCT. In order to reliably comply with the regulations, a final polishing strategy, such as UV photolysis, might be needed in this case. However, the UV dose needed to achieve the target concentration would be considerably lower than without the biofiltration step, thereby contributing to a potential reduction in costs. It is also important to note that ozonation of this particular wastewater generated <50 ng/L, so assuming the same relative removal was achieved without spiking, the system would easily be able to comply with the NL with either media type. Moreover, in a DPR configuration, UV would likely be required for pathogen LRVs, although the applied UV dose could probably be reduced significantly when targeting pathogen inactivation instead of NDMA photolysis.

Even with a 20-min EBCT, the non-ozonated BAC control column achieved only moderate NDMA removal, with an average of 45.1% and a final NDMA concentration of 140 ng/L. However, the non-ozonated BAC column would only be receiving the ambient NDMA concentration in the MBR filtrate in a normal treatment configuration, so the NL would likely not be an issue unless the facility experienced periodic spikes in NDMA.

However, the more important observation from this initial phase of testing was the significant difference in performance between the ozonated and non-ozonated biofiltration systems when receiving similar feed water NDMA concentrations. The better performance for NDMA removal by the ozonated columns suggests the ozone might be the major factor impacting the removal. However, the reason for this
difference was unclear from these experiments. Potential reasons include the fact that ozone transforms the bulk organic matter into more BDOC (or AOC), thereby creating a more favorable environment for co-metabolism; ozone leaves a higher dissolved oxygen (DO) concentration in the water, thereby providing a more abundant electron acceptor for biochemical reactions; or ozone (or DO) may be responsible for shaping the microbial community colonizing the biofilters (Gerrity et al., 2018). The following phase of testing evaluated these hypotheses in greater detail.

### 3.3.2.2. NDMA biodegradation under different redox conditions for BAC column

The role of different redox conditions was investigated in the BAC columns in an attempt to understand which ozone-related effects play major roles in determining the efficacy of ozone-biofiltration systems. The typically ozonated BAC column and the typically non-ozonated BAC column each received three different waters within a short timeframe: ozonated MBR filtrate (high BDOC and high DO levels); oxygenated MBR filtrate (low BDOC content and high DO levels); and untreated MBR filtrate (low BDOC and low DO levels). The O$_3$/TOC ratio was held constant at around 1.4, and the EBCT was fixed at 10 minutes. NDMA was spiked to target an initial concentration of ~300 ng/L. For the typically ozonated BAC column, the actual NDMA concentrations were 280, 270, and 270 ng/L for ozonation, oxygenation, and untreated MBR filtrate, respectively. For the typically non-ozonated BAC column, the actual NDMA concentrations were 280, 250, and 270 ng/L for ozonation, oxygenation, and untreated MBR filtrate, respectively. The DO levels during the experiment can be seen in Table 8.
Table 8. Influent and effluent dissolved oxygen concentrations for the BAC columns (triplicate experiments) under different experimental conditions.

<table>
<thead>
<tr>
<th></th>
<th>Dissolved oxygen concentration, mg/L</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ozonation</td>
<td>Oxygenation</td>
<td>MBR Filtrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAC</td>
<td>BAC Control</td>
<td>BAC</td>
<td>BAC Control</td>
<td>BAC</td>
</tr>
<tr>
<td>Initial DO concentration (Influent for replicate 1)</td>
<td>18.11</td>
<td>18.88</td>
<td>20.73</td>
<td>19.94</td>
<td>3.72</td>
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<tr>
<td>Effluent for replicate 1</td>
<td>8.82</td>
<td>8.64</td>
<td>10.76</td>
<td>8.54</td>
<td>2.31</td>
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<tr>
<td>DO consumed</td>
<td>9.29</td>
<td>10.24</td>
<td>9.97</td>
<td>11.4</td>
<td>1.41</td>
</tr>
<tr>
<td>Initial DO concentration (Influent for replicate 2)</td>
<td>14.72</td>
<td>15.14</td>
<td>14.88</td>
<td>14.42</td>
<td>3.83</td>
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<tr>
<td>Effluent for replicate 2</td>
<td>8.77</td>
<td>7.97</td>
<td>10.07</td>
<td>8.59</td>
<td>2.63</td>
</tr>
<tr>
<td>DO consumed</td>
<td>5.95</td>
<td>7.17</td>
<td>4.81</td>
<td>5.83</td>
<td>1.2</td>
</tr>
<tr>
<td>Initial DO concentration (Influent for replicate 3)</td>
<td>13.23</td>
<td>13.46</td>
<td>14.13</td>
<td>13.94</td>
<td>3.91</td>
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<td>Effluent for replicate 3</td>
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<td>7.36</td>
<td>8.87</td>
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<td>2.37</td>
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<tr>
<td>DO consumed</td>
<td>5.00</td>
<td>6.10</td>
<td>5.26</td>
<td>5.67</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Since the experiment consisted of spiking a known NDMA concentration into a fixed volume of water in the feed tank, no water was added to the tank while the experiment was running. Therefore, DO levels tended to naturally decrease to reach the saturation concentration since no more oxygen was being provided to the water (either by ozonation or oxygenation). In order to ensure that the NDMA concentration was kept constant during the experiment, the water was mixed every 5 minutes. This mixing could be the responsible for the slight increase in DO concentration during the untreated MBR filtrate experiment.

Figure 24 below illustrates the findings for TOC removal, in percentage, by the BAC columns under the different conditions.
Ozonation achieved greater TOC removal than the other two conditions. Therefore, the fact that ozonation transforms the bulk organic matter appears to have a more important role in TOC removal than increasing the DO concentration. Again, this is likely attributable to the generation of BDOC, which is more easily assimilable by the microbiota colonizing the BAC column (von Sonntag and von Gunten, 2012). In the absence of pre-ozonation (i.e., with pre-oxygenation or ambient MBR filtrate), less TOC removal was observed in both columns, although TOC removal was particularly low in the typically non-ozonated BAC column fed with pre-oxygenated MBR filtrate. Additional experiments would be necessary to determine whether high DO concentrations (i.e., 14-20 mg/L) may have inhibited the microbial community that had been previously acclimated to MBR filtrate with 3-4 mg/L of DO.

NDMA removal was also investigated under these experimental conditions, and the findings are presented in Figure 25.
Contrary to TOC, the different redox conditions appeared to have no immediate effect on NDMA degradation by the microbiota in the typically ozonated BAC column, as indicated by the minimal difference in NDMA removal (<5%) for the different conditions. Even though good removal rates were achieved for NDMA under all of these conditions, the final target of 10 ng/L was not accomplished in any of the cases. Again, this would only be a concern in systems experiencing NDMA formation of ~300 ng/L during ozonation, and those systems would likely have additional polishing steps downstream of the biofiltration process.

Surprisingly, for the typically non-ozonated column, the highest removal rate observed among the three conditions was for the MBR filtrate (i.e., low BDOC and low DO), and the lowest rate was observed in the ozonation experiment (i.e., high BDOC and high DO). Again, this might be explained by the long-term exposure of the microbial community to the MBR filtrate water.
Wang et al. (2015) investigated changes in microbial community structure in BAC filters before and after continuous addition of NDMA. The study showed significant changes to the microbial communities after 60 days of continuous exposure to nitrosamines, indicating that there was a further acclimation of the microbial community when continuously exposed to the substrate of interest (Wang, et al. 2015). Trussell et al. (2018) also showed improvement of NDMA and TOC removal rates in a soil aquifer treatment system treating dechlorinated secondary effluent over time. Both NDMA and TOC removal rates increased over time and the authors attributed this improvement in degradation to the biofilters’ acclimation (Trussell et al., 2018). Therefore, the long-term exposure of the microbial community colonizing the BAC control column to the MBR filtrate can be linked to the enhanced TOC and NDMA removal rates under that type of water.

More evidence that short-term increases in DO level might not significantly impact NDMA biodegradation can be seen in Table 8. The DO consumed during the 10-minute EBCT in both the ozonation and oxygenation tests were similar, as well as the initial and final concentrations. For the MBR filtrate, the oxygen consumption was significantly lower, and, yet, the NDMA degradation was not significantly improved or inhibited by this factor.

The amount of DO consumed during the MBR filtrate experiment is actually in accordance with other biological treatment processes in WWTPs, such as activated sludge (Metcalf and Eddy, 2013). Most activated sludge processes target a dissolved oxygen concentration of 2 to 3 mg/L to be applied in the aeration basins since the aeration process is costly and is characterized by poor oxygen transfer efficiency (Rittman and McCarty, 2001). On the contrary, the artificially high DO concentration also raises questions related to the tolerance of the microbial community to
supersaturation conditions (e.g., the lower TOC removal achieved by the non-ozonated control BAC column during the pre-oxygenation test). Stress conditions and their effects on biofilter performance are investigated in the next chapter.

Another potential explanation for the minimal differences among the test conditions might be attributable to the microbial community colonizing the ozonated BAC filter. In other words, long-term exposure to ozonated MBR filtrate (or elevated DO levels) might be responsible for shaping that community. Since short-term changes in BDOC and/or DO levels had minimal impact on NDMA removal, it is hypothesized that the microbial community colonizing the ozonated biofilter offers some degree of resiliency once acclimation has been achieved.

3.3.2.3. NDMA biodegradation and formation potential upon final chloramination

In a full-scale DPR system, as well as in more conventional drinking water systems, chlorine or chloramines must be added for final disinfection and to achieve a residual in the distribution system to prevent bacterial regrowth. Although NDMA might be partially or completely (i.e., below the detection limit) removed during the biofiltration process, its precursors might not experience the same fate. The previous experiments only investigated the effects of ozonation on NDMA formation, whereas NDMA might also form upon chloramination (Schreiber and Mitch, 2006). Moreover, the precursors are often different, so low levels of ozone-induced NDMA formation do not necessarily mean there will also be low levels of chloramine-induced NDMA formation (Marti et al., 2015).

In this study, NDMA UFC tests were performed with the pilot-scale effluents. During this phase of testing, the O₃/TOC ratio was maintained at 1.5, and the EBCT
was fixed at 10 minutes. Since this test was performed as part of a separate phase and during a different time period, a new summary of general water quality parameters was prepared (Table 9).

Table 9. Average of weekly water quality parameters during Fall 2017.

<table>
<thead>
<tr>
<th></th>
<th>UVA₂₅₄ (cm⁻¹)</th>
<th>PO₄³⁻ (mg/L)</th>
<th>NO₃⁻ (mg/L as N)</th>
<th>NO₂⁻ (mg/L as N)</th>
<th>NH₃ (mg/L as N)</th>
<th>TOC (mg/L)</th>
<th>TOC removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBR</td>
<td>0.14</td>
<td>8.9±1.1</td>
<td>7.7±1.1</td>
<td>0.09±0.04</td>
<td>0.1±0.1</td>
<td>6.7±0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>MBR+ O₃</td>
<td>0.06±0.01</td>
<td>8.4±1.3</td>
<td>6.6±1.0</td>
<td>0.00</td>
<td>0.1</td>
<td>6.6±0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>MBR+ O₃+BAC</td>
<td>0.06±0.05</td>
<td>8.0±1.1</td>
<td>6.4±1.9</td>
<td>0.01±0.01</td>
<td>0.0±0.1</td>
<td>5.1±0.1</td>
<td>23.3±5.6</td>
</tr>
<tr>
<td>MBR+ O₃+Ant</td>
<td>0.06±0.01</td>
<td>8.4±0.9</td>
<td>6.5±0.4</td>
<td>0.01±0.01</td>
<td>0.0</td>
<td>5.3±0.1</td>
<td>21.0±4.9</td>
</tr>
<tr>
<td>MBR+ BAC</td>
<td>0.12±0.02</td>
<td>7.6±0.5</td>
<td>6.6±0.2</td>
<td>0.03±0.02</td>
<td>0.0</td>
<td>5.6±0.1</td>
<td>15.5±1.4</td>
</tr>
</tbody>
</table>

The TOC results for this experiment are plotted below in Figure 26.

Figure 26. Average (n=6) TOC removal by the different columns during the UFC test. Error bars are standard deviations.

The BAC column continued to outperform the anthracite column, as the TOC percentage removal from Figure 26 shows. The non-ozonated BAC control column exhibited limited TOC removal, as expected. These data are similar to the first NDMA
experiment under the 10-minute EBCT condition. Even though this experiment
employed a higher O₃/TOC ratio (1.5 vs. 1.0), TOC removal was relatively similar to
the previous experimental phase.

Ambient NDMA concentrations were tested in single samples, and NDMA
formation potential using the UFC approach was tested in duplicate for all samples. The
averages of the results are shown in Figure 27.

![Figure 27. NDMA concentrations: ambient and upon chloramination (UFC approach). Ambient conditions were sampled once, and the values reported were below the MRL. For the UFC approaches, samples were collected in duplicates and the values reported are the average of those values. Error bars represent standard deviations.](image)

Consistent with the Phase 1 testing, direct NDMA formation during ozonation
was low (i.e., increased from <2.9 to 20 ng/L; blue/left column for ozone effluent in
Figure 27), but formation due to chloramination was significantly greater. For the MBR
filtrate, the addition of chloramines increased the NDMA concentration from <2.9 to
960 ng/L. As explained previously, ozone-reactive precursors differ from chloramine-
reactive precursors, and the reaction mechanisms also differ. Marti et al. (2017) also
reported a much higher NDMA formation upon chloramination than upon ozonation of
tertiary effluent, indicating a major presence of chloramine-reactive NDMA precursors.
Interestingly, biofiltration alone was unable to remove NDMA precursors. As presented previously, the non-ozonated BAC control column achieved ~20% NDMA removal with a 10-min EBCT in the first experiment (Figure 23). For NDMA precursors, however, the removal was only 3% with the same EBCT (Figure 27). This is in accordance with a previous study that added a model chloramine-reactive NDMA precursor (ranitidine) to assess NDMA FP after biofiltration of tertiary effluent (Marti et al., 2017). Without pre-ozonation, even long EBCTs of 20 minutes did not remove ranitidine significantly. This precursor has been studied previously and has been shown to have an NDMA molar yield higher than 50% (Shen and Andrews, 2011). The current study did not quantify ranitidine specifically, but it is assumed that the MBR filtrate contained similarly biologically-recalcitrant chloramine-reactive NDMA precursors.

Despite the small increase in NDMA due to ozonation, NDMA formation upon chloramination was considerably lower than for the non-ozonated MBR filtrate. Specifically, upon chloramination, NDMA increased from 20 to 41 ng/L in the ozonated MBR filtrate and from <2.9 to 960 ng/L in the non-ozonated MBR filtrate. This indicates that ozone was effective in oxidizing the chloramine-reactive NDMA precursors. Oxidation of NDMA precursors can actually be accomplished by chlorine, ozone, chlorine dioxide, permanganate, hydrogen peroxide, or ferrate (Krasner et al., 2013), but it is important to balance the formation of various DBPs in each pre-treatment scenario (e.g., NDMA, THMs, HAAs, etc.) with the net reduction in NDMA. Liao et al. (2017) performed true formation potential (FP) tests, which differ from the UFC test because they target a higher chloramine dose (20 mg/L) and a longer holding time (7 days). In that study, the ozonation process in the DWTP reduced the NDMA formation potential by 40%. Ozone is particularly effective for amine oxidation (Shah et
al., 2013). It can oxidize primary and secondary amines into nitrated byproducts such as aldehydes, amides, and oximes, and tertiary amines into N-oxides. These reactions happen rapidly, particularly with high O₃/TOC ratios, thereby reducing NDMA formation upon chloramine addition (McCurry et al., 2016b).

For ozone-biofiltration, the biofiltration step was able to eliminate the NDMA that had formed during ozonation, regardless of media type (i.e., blue/left column for both ozonated biofiltration effluents in Figure 27). Upon chloramination, the NDMA concentrations increased to 23 and 17 ng/L for anthracite and BAC, respectively. Although these concentrations are above the California NL, they are both significantly lower than the other treatment scenarios, thereby highlighting the importance and synergism of ozone-biofiltration. Liao et al. (2017) also performed NDMA FP in ozone-BAC effluent and reported similar results (i.e., 82% reduction in chloramine-reactive precursors) (Liao et al., 2017). Again, a DPR system would likely include UV or UV/H₂O₂ as a final polishing step, which might further reduce the concentration of chloramine-reactive precursors and allow for full compliance with the California NL. This was not the goal of the current study, however.

Besides UFC and FP tests, simulated distribution system (SDS) tests have also been reported in the literature (Zeng et al., 2016). As the name suggests, this test simulates the actual conditions in a system-specific distribution system. Zeng et al. (2016) added 2.5 mg/L as Cl₂ of pre-formed monochloramines and incubated the final effluents after FAT treatment in the dark at room temperature for 3 days. The ambient NDMA concentrations after UV AOP were below the detection limit (2 ng/L), and in all treatment trains investigated, NDMA concentrations increased during the SDS assay but stayed below the 10-ng/L NL required by California (Zeng et al., 2016). These data suggest that even though NDMA was removed during UV photolysis, its precursors
were not completely removed. Sgroi et al. (2015) investigated NDMA formation potential after each treatment process in an FAT train by the addition of 4 mg/L of chlorine. Since the WWTP feeding the AWTF did not employ nitrification, the ambient ammonia concentrations were high (i.e., RO feed concentration of 39 mg/L as N; average final effluent concentration of 2.9 mg/L as N), and the addition of chlorine resulted in chloramine formation. They used different holding times for the formation potential tests (1, 4, 7, 14, and 28 days), and they also analyzed the ambient conditions to calculate sample-specific formation. The average final effluent concentration was 10 ng/L, and, when subjected to chloramination, the samples surpassed the NL of 10 ng/L (13-16 ng/L), also indicating that not all precursors are removed during FAT. It is worth noting that except for the RO feed water (influent to the AWTF), the samples did not show a significant increase in NDMA concentration after day 1, thereby indicating that the precursors react relatively rapidly with the disinfectant (Sgroi et al., 2015).

Furthermore, SMPs generated after biological processes are known to be chloramine-reactive precursors (Bukhari et al., 2017). That might imply that even though the ambient chloramine-reactive NDMA precursors were oxidized by ozonation and then possibly biodegraded, some ‘new’ precursors might have been released during biofiltration, thereby increasing the final NDMA concentration upon chloramination. Also, as seen in Figure 22e, a slight increase in fluorescence takes place after biofiltration, likely due to organics released during biofiltration. If polishing strategies are employed after biofiltration, TOC, NDMA, and DBP precursors might be further reduced, and reliable compliance with regulations might be possible.
3.3.3. Trace Organic Contaminants

A suite of indicator TOrCs was also analyzed in the effluents from the pilot-scale ozone-biofiltration system. For this test, the O₃/TOC ratio was held constant at 1.3, and the EBCT was fixed at 10 minutes for both columns tested (i.e., non-ozonated BAC Control and ozonated BAC). Table 10 below shows the concentrations found throughout the system for the different compounds.

Table 10. Trace organic compounds concentrations in the ozone-biofiltration system.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MBR filtrate, ng/L</th>
<th>BAC Control, ng/L</th>
<th>Ozone effluent, ng/L</th>
<th>Ozone-BAC effluent, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Atenolol</td>
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<td>160</td>
<td>&lt;20</td>
<td>&lt;20</td>
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<td>&lt;5</td>
<td>&lt;100</td>
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<td>Carbamazepine</td>
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<td>DEET</td>
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<td>Fluoxetine</td>
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<td>32</td>
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<td>&lt;1</td>
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<tr>
<td>Gemfibrozil</td>
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<td>16</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<tr>
<td>Ibuprofen</td>
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<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<td>Meprobamate</td>
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<tr>
<td>Naproxen</td>
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<td>&lt;1</td>
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<td>61,000</td>
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<tr>
<td><strong>Perfluoroalkyl Acids</strong></td>
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<td>Perfluoroheptanoic acid</td>
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<td>Perfluorohexanoic acid</td>
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<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Perfluoropentanoic acid</td>
<td>48</td>
<td>39</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>
As expected, compounds susceptible to biological treatment, such as acetaminophen and ibuprofen (over-the-counter medications), were present in low concentrations in the MBR filtrate (<5 ng/L), while biologically recalcitrant compounds, such as sucralose (artificial sweetener) and sulfamethoxazole (antibiotic), were found at higher concentrations (>1 µg/L).

Ozonation has been shown to be an effective oxidant for many TOrCs (e.g., carbamazepine, naproxen, sulfamethoxazole, triclosan) and is capable of reducing ambient concentrations to analytical method reporting limits (MRL). Some TOrCs (e.g., atenolol, meprobamate) experienced significant attenuation during ozonation, but they were not completely removed in this process, thereby suggesting that hydroxyl radicals might be the main oxidizing factor for these more recalcitrant compounds (Gerrity et al, 2011). These results are consistent with Lee et al. (2013), which also demonstrated consistency in TOrC oxidation across a wide range of WWTPs when the ozone dose was standardized to the TOC concentration.

In this study, the concentrations of some TOrCs (e.g., caffeine, sucralose, TCEP) actually increased after biofiltration, suggesting desorption might be taking place in the system. In activated carbon, desorption can happen under two circumstances: (i) when stronger adsorbing compounds are present (i.e., chromatographic effect) or (ii) when there is a concentration gradient in the water and the adsorbed compound desorbs into the water instead of being adsorbed (Corwin and Summers, 2011) in an attempt to re-establish equilibrium (To et al., 2008).

Greenstein et al. (2018) fed pilot-scale BAC and anthracite columns in a DWTP with several TOrCs in high concentrations for over 200 days and noticed biodegradation/adsorption during (bio)filtration for some of the compounds. Then, they decreased the TOrCs concentrations in the feed water and noticed an increase in the
effluent concentrations for those TOrCs, suggesting desorption. In the case of this pilot-scale system, the media in the BAC columns was exhausted due to over 10 years of use in a full-scale WWTP in Georgia. Desorption in the pilot-scale system suggests that the concentrations of the TOrCs in the previous water were higher than during the current study, thereby creating a desorption gradient. Therefore, the higher concentrations in the effluent waters could be due to restoration of chemical equilibrium during the biofiltration process. In general, the desorption process under longer EBCTs might increase the concentrations, once more time is allowed for the equilibrium to be achieved (To et al., 2008).

These results are somewhat different from other ozone-biofiltration systems that show greater removal of TOrCs after ozone-BAC. Gerrity et al. (2011) investigated the abatement of several TOrCs in another pilot-scale ozone-BAC system (EBCT = 30 minutes) and noticed minimal (e.g., musk ketone, atrazine) or further removal (e.g., TCEP, benzophenone) of some contaminants after ozone but did not observe desorption. However, in that study, the BAC media had been used exclusively at that plant for just under 2 years, and the carbon may have still had some adsorption capacity remaining (Gerrity et al., 2011), particularly when considering the adsorption of compounds like TCEP. Reungoat et al. (2012) also reported increased abatement of some TOrCs after ozone-BAC when compared to just ozone. In this case, there was likely no adsorption capacity left, and the media was used exclusively in the studied plants.

Perfluorinated compounds had minimal or no removal by ozonation or biofiltration. However, due to limited industrial inputs to the full-scale facility, the concentrations of the critical perfluoroalkyl acids (PFAAs), specifically PFOS and PFOA, were less than the U.S. EPA Health Advisory Level of 70 ng/L for the combined
concentrations of the two compounds. If necessary, these compounds can be removed by non-exhausted GAC (Kucharzyk et al., 2017), but this treatment train requires regeneration and even media replacement after long-term operation. Also, because wastewater contains a considerable amount of bulk and trace organic matter, GAC used in potable reuse applications will likely have a shorter lifespan than in drinking water applications, thereby increasing costs even further. There are currently several studies investigating methods to treat perfluorinated compounds from wastewater and drinking water (Inyang and Dickenson, 2017; Zhao et al., 2013; McCleaf et al., 2017), but in general, the best practice is source control.

3.4. Conclusions

The data gathered in this chapter shows that NDMA is biodegradable, but that certain conditions control the level of biodegradation in ozone-biofiltration systems. Empty bed contact time is an important factor in NDMA biodegradation. The longer the EBCT, the greater the removal achieved. The experiments indicated that NDMA follows pseudo first order kinetics with rate constants of 0.197 min⁻¹, 0.158 min⁻¹, and 0.029 min⁻¹ for an ozonated BAC column, an ozonated anthracite column, and a non-ozonated BAC column, respectively. Due to the fact that NDMA removal will plateau at some point (e.g., EBCT > 10 minutes in ozonated columns), one must balance the additional removal achieved with longer EBCTs with the point of diminishing return in order to adequately protect public health while controlling capital costs.

Although BAC receiving ozonated MBR filtrate generally achieved greater TOC removal than the ozonated anthracite, the removal of NDMA was relatively similar for both media types. Despite the fact that ozonation can result in NDMA formation, ozone-induced formation was relatively low in this facility, and both ozonated biofiltration
columns were far superior to the non-ozonated column with respect to both TOC and NDMA removal.

The effects of ozonation on microbial community structure appear to have the most significant impact on acclimation and NDMA removal efficiency, as opposed to ozone-induced transformation of bulk organic matter (i.e., higher BDOC concentrations) or elevated DO concentrations. Since NDMA is aerobically biodegraded (Fournier et al., 2006) and is assumed to be co-metabolized, the high levels of BDOC provided by ozonation and the high levels of DO provided by ozonation or oxygenation were expected to improve the performance of biofiltration with respect to NDMA removal. However, acclimation to ozonated MBR filtrate appeared to be more important, so it is hypothesized that this altered the structure and function of the microbial community by selecting for microbes that are better suited for NDMA biodegradation. In order to investigate if the microbial community was indeed different among the columns and how those differences might impact TOC and NDMA removal, molecular biology experiments were performed, as explained in the following chapter.
Chapter 4 – Biofilm Assessment

4.1. Introduction

Filtration systems, such as rapid or even slow sand filtration, date back centuries in water treatment applications. In WWTPs, filtration with dual-media anthracite and sand filters is also a common component of tertiary treatment for polishing and to achieve target water quality metrics, such as total suspended solids and biochemical oxygen demand. When a disinfectant is not added ahead of these filtration processes, the microorganisms present in the water attach to the surface of the media grains and develop a biofilm (Zearley and Summers, 2012).

Biofilms are defined as a community of microorganisms embedded in a matrix formed by extracellular polymeric substances (EPS). These aggregates can be either attached to a stationary surface – where there is a direct layer of cells in contact with the surface – or to suspended substrate – where they move in flocs. This form of life is found all over the world and it drives a series of reactions. In the environmental engineering field, biofilms are responsible for the biodegradation of organic compounds in WWTPs (e.g., activated sludge systems and trickling filters), in composting processes, in drinking water filters, etc. Biofilms can also cause fouling on membranes used in drinking water treatment or advanced treatment for water reuse applications, and these biofilms have been shown to both compromise and improve treatment (Flemming et. al., 2016). Besides a variety of microorganisms and EPS, several other compounds can be found entrapped in biofilms, especially when they are found in wastewater processes. Using electron microscopy, Gibert et al. (2013) identified diatom skeletons, detritus, fungal hyphae, etc.
Biofilm development consists of four steps. The first step, called conditioning film, includes adsorption of water and small molecules to a surface, such as GAC or other filter media, until a monolayer is established. These conditions create an attractive environment for bacteria, which already have a natural tendency to attach to surfaces. Initially, this adsorption of bacteria to the surface is reversible, and depends on the quality of the conditioning film, and the extent of bacterial attachment. The subsequent production of EPS by the attached bacteria then creates an irreversible aggregation of these organisms. Finally, the biofilm develops as the cells grow and further attach to other layers of cells (Zhu et al., 2010). A schematic of this development can be seen below in Figure 28.

![Figure 28. Biofilm development starts with attachment of microorganisms in a surface. Microbial activity generates EPS matrix. Once mature, biofilms can start dispersion to further inhabit new environments. Modified from Passos da Silva et al. (2017).](image)

Biofilm formation depends on several factors such as the type of media (GAC, anthracite, sand, etc.), backwashing frequency, temperature, water quality (i.e., the presence of nutrients and substrate), hydraulic loading, etc. Besides these parameters, biodegradation also depends on EBCT, pre-treatment such as ozonation and chlorination, etc. (Gibert et al., 2013).

In general, biofilms are more robust than free-living cells. These living forms count on physical and social interactions and augmented rates of gene exchange, which
confers greater resistance to antibiotics, for example. The proximity among the cells and the presence of EPS allows extracellular DNA (eDNA) to be present in this environment, which can then be taken up by the cells. Besides eDNA, polysaccharides, proteins, and lipids are also found in EPS and comprises most of the biomass in biofilms (Flemming et al., 2016).

Biofilms are able to entrap particles present in water (i.e., similar to filtration) or act as an adsorptive site for target compounds (Crittenden et al., 2012; Flemming et al., 2016), but biodegradation is often the primary mechanisms of treatment achieved by the microbial community. As a result, biofilters can remove the biodegradable part of the TOC concentration in water (i.e., the BDOC), nutrients such as ammonia and phosphate, pathogens, DBPs and DBP precursors (Liao et al., 2015; Basu et al., 2015), TOrCs (Zearley and Summers, 2012; Lee et al., 2012), odor-causing compounds (Crittenden et al., 2012), etc. Biofilters also help to reduce the risk of bacterial regrowth in distribution systems, once they consume most of the BDOC present in the water, thereby leaving almost no substrate for other microorganisms (Crittenden et al., 2012). Biofiltration can also decrease the formation of DBPs if placed ahead of disinfection by consuming DBP precursors. For example, THM and HAA formation by chlorination are dependent on TOC levels. If biofiltration is placed before chlorination, TOC levels are reduced and, therefore, THM and HAA formation can be reduced as well (Wu and Xie, 2005; Arnold et al., 2018).

Although biofilters have been employed for decades in water treatment in the U.S., the concept has been gaining more attention recently (Zhu et al., 2010). Biofiltration has become critically important in potable reuse applications, particularly when coupled with pre-ozonation, because of its ability to remove TOC and NDMA when it is present. As noted earlier, previous studies identified pure culture bacterial
strains capable of degrading NDMA, especially when in the presence of certain primary substrates (Sharp et al., 2005; 2007; 2010). These findings led to the discovery of certain monooxygenases as the primary enzymes responsible for co-metabolism of NDMA by certain bacterial species. However, in WWTPs, due to the presence of innumerable compounds (including inhibitory substances) and a wide variety of microorganisms, the mechanisms of NDMA biodegradation are more complex and, as of yet, not completely characterized. Therefore, studies expanding the knowledge base of NDMA biodegradation in potable reuse systems are needed. Ultimately, these findings can lead to the optimization of biofiltration systems for NDMA removal, further supporting the legitimacy of ozone-biofiltration as an alternative to FAT for potable reuse.

4.2. Methods

4.2.1. Pilot-Scale Ozone-Biofiltration and Full-Scale Water Reclamation Plant

The pilot-scale ozone-biofilters employed for the following tests were the same as previously described at Section 3.2.1 and 3.2.2.

4.2.2. Biofilm Stress Conditions

Challenge tests in the biofilters were performed by creating stress conditions for the biofilms, such as pH and dissolved oxygen changes. Both challenge experiments were performed twice.
4.2.2.1. pH Changes

The pH of the feed water to the ozonated BAC (O₃/TOC = 1.2) and non-
ozonated BAC columns was adjusted by adding either 1 M sodium hydroxide (NaOH) or 2 N hydrochloric acid (HCl). The target pH values were 3, 5, 7 (ambient condition), 9, and 11. The pH was measured by a YSI Model 63 pH meter (Yellow Springs, OH) on-site.

For both biofilters, the pH was adjusted in the feed tank. Acid or base was added slowly, mixed, and measured to reach the target pH. During operation of the system, the EBCT was fixed at 10 minutes for both columns. Between two different pH values, a total of 3 times the EBCT (i.e., 30 minutes) elapsed before collecting samples for analysis.

Biofilter resilience toward rapid changes in pH was monitored via TOC removal and compared against TOC removal under normal conditions. Nutrients and effluent pH were also monitored.

4.2.2.2. Inhibitory Substances and Dissolved Oxygen Changes

As high DO concentrations in the water are typical for ozone processes, it was hypothesized that drastic changes in this parameter could cause a stress condition in the biofilm, thereby affecting biofilter performance. In practice, this could occur during an operational upset caused by equipment malfunction at a WWTP or due to unexpected industrial discharges. To simulate these conditions, the experimental waters were spiked with sodium sulfite (Na₂SO₃), which along with other sulfites and bisulfites, serves as a DO scavenger. Sulfite acts as a scavenger by reacting with oxygen to form sulfate. 8.12 parts of Na₂SO₃ per part of oxygen are required to reduce the DO level (Cavano, 2007). Additionally, sulfites are used in the food industry as antioxidants and preservatives.
(Ramis-Ramos, 2003), thereby potentially inhibiting microbiological activity within the biofilters.

Ambient DO concentrations were measured with a Sension + DO6 Portable DO Meter (Hach, Loveland, CO). Na$_2$SO$_3$ was slowly added to the water tank, mixed, and the concentrations were measured again in order to reach the following target DO levels: 15 mg/L, 8 mg/L, and 0.5 mg/L – the latter being below the essential level for aerobic biological treatment. Since the high DO levels only occur after ozonation (i.e., not in the MBR effluent), this experiment was performed only with the ozonated BAC column. The O$_3$/TOC was 1.3 and the EBCT was 10 minutes. A total of three times the EBCT (i.e., 30 minutes) elapsed prior to sample collection. Again, biofilter performance was monitored by effluent TOC concentration and the corresponding TOC removal.

4.2.3. NDMA Molecular Biology Tests

Molecular biology tests were also performed to characterize the microbial community inhabiting the columns, as well as the genes involved in NDMA degradation. Since NDMA biodegradation was observed in the biofilters, it was hypothesized that monooxygenase genes might be present in the biofilters. Therefore, polymerase chain reaction (PCR) and quantitative PCR (qPCR) assays were performed.

In order to prepare for these molecular assays, media samples were collected from the top and bottom sample ports of each biofilter column, similar to the aforementioned ATP assays. DNA was extracted from the media particles with a DNeasy PowerBiofilm DNA extraction kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions, and with the addition of a quick heat-thaw step. In this step, the bead tubes with the lysis solution were stored in the freezer, and as soon as the
solution froze, the tubes were quickly heated to 60°C. The combination of the lysis solution with the sudden change in temperature can help break open the cells, thereby improving DNA extraction and increasing DNA yields. The DNA concentrations in the samples were quantified using a NanoDrop ND-1000 Spectrophotometer (ThermoFischer Scientific, Waltham, MA) and a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA) using the double strand DNA high sensitivity method.

The selected primers targeted the monooxygenase genes *prmA*, *prmB*, and *prmE* from the *Rhodococcus* sp. RHA1 PrMO operon (Figure 15). The DNA sequences for the primers (both forward and reverse) targeting *prmA* and *prmB* were found in Sharp et al. (2007). The primers targeting *prmE* were designed using the BLAST tool from the National Center for Biotechnology Information (NCBI) and the gene sequence provided by Sharp et al. (2007), which is registered in the NCBI gene database. The primer sequences are shown in Table 11 below, and the total fragment length is around 100 base pairs. The primers were designed and purchased from Integrated DNA Technologies (IDT, Coralville, IA) and diluted with DNase free water to reach a final concentration of 10 µM.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Sequence (5' to 3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>prmA</em> - forward</td>
<td>CGCGGCACACATCTACCT</td>
<td>Sharp et al. (2007)</td>
</tr>
<tr>
<td><em>prmA</em> - reverse</td>
<td>TGGTACGACACGGGTGTG</td>
<td></td>
</tr>
<tr>
<td><em>prmB</em> - forward</td>
<td>GGACGAGGATTGACGGATTTC</td>
<td>Sharp et al. (2007)</td>
</tr>
<tr>
<td><em>prmB</em> - reverse</td>
<td>CGCGGTCGACTCGAT</td>
<td></td>
</tr>
<tr>
<td><em>prmE</em> - forward</td>
<td>GGAAGCTACGTCGTCGGG</td>
<td>NCBI BLAST Primer using sequence by Sharp et al. (2007)</td>
</tr>
<tr>
<td><em>prmE</em> - reverse</td>
<td>GACCCGACGAGATTTCGAT</td>
<td></td>
</tr>
</tbody>
</table>

DNase free water and a 2X master mix GoTaq solution were purchased from Promega (Madison, WI). The PCR reactions were conducted in a manual Mastercycler personal thermocycler (Eppendorf, Hamburg, Germany). Since low DNA
concentrations were found in the extracted samples, 5 µL of extracted DNA were added to a 0.2 mL tube along with 4 µL of sterile and DNase free water, 12.5 µL of master mix solution, and 1.75 µL of 10 µM primers (forward and reverse). Each 0.2 µL tube had only one set of primer added. The PCR conditions were as follows: 2 minutes of initial denaturation at 95°C; 40 cycles of 15 seconds of denaturation at 95°C followed by 30 seconds of annealing at 55°C and 30 seconds of extension at 72°C; 5 minutes of final extension at 72°C; and a hold step at 4°C until the sample products were taken from the thermocycler.

The PCR products were then separated using gel electrophoresis in order to identify the presence or absence of the monooxygenases. The gel was prepared using 0.4 g of agarose, 40 mL of 1x Tris/Acetate/EDTA (TAE) buffer solution, and 1.5 µL of ethidium bromide. After the solution solidified, a TAE solution was added to conduct the electric current through the gel. A mixture of 5 µL of sample (PCR product) along with 2 µL of a blue-orange dye (Bio-Rad, Hercules, CA) were added to each well in the gel. An electric current was used for 20 minutes to run the PCR products towards the positive pole. The gel was visualized in a UV-light chamber to assess the results.

Once the presence of the monooxygenase genes was confirmed, qPCR tests were performed in a CFX96 TouchReal-Time PCR Detection System (Bio-Rad, Hercules, CA) to quantify the monooxygenases in the samples. For these tests, the same primers and same samples were used, but a different master mix solution was used: iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA).

Since qPCR quantifies the target gene, standard curves need to be prepared. Standards for the specific primer sets were purchased from IDT. The standards included the primer sequences and product sequences, which resulted in a length of 129 base
pairs for each gene (i.e., \textit{prm}A, \textit{prm}B, and \textit{prm}E). The standards were resuspended following the manufacturer’s instructions using a Tris/EDTA (TE) buffer to bring the solution to a concentration of 10 ng/µL. Eight curve points for the standard curve were created. The “DNA Copy Number and Dilution Calculator” from the Thermo Fisher Scientific website was used to calculate the amount of initial standard, named stock, and the amount of TE buffer needed to start at an amount of $10^8$ copies/µL. 3 µL of the stock solution were diluted into 27 µL of water to create a curve point of $10^7$ copies/µL. These serial dilutions continued until reaching 7 points (i.e., $10^2$ copies/µL as the last standard curve), and the last point served as the no-template control (NTC).

The samples and standards were loaded into 96 well plates as follows. For each well, a total volume of 15 µL was added, in which 5.9 µL was sterile water, 7.5 µL was the master mix solution containing SYBR Green, 0.3 µL was forward primer, 0.3 µL was reverse primer, and 1 µL was DNA extract. The samples and the standards were run in triplicate.

The qPCR conditions were the same as used for PCR: 2 minutes of initial denaturation at 95°C; 40 cycles of 15 seconds of denaturation at 95°C followed by 30 seconds of annealing at 55°C and 30 seconds of extension at 72°C; and 5 minutes of final extension at 72°C. For the \textit{prm}E primers, 50 cycles were performed instead of 40. This number of cycles was chosen after preliminary tests with the standards showed late quantification cycles for this set of primers and standards.
4.3. Results and Discussion

4.3.1. Challenge Tests

Stress conditions were created in the biofilters by changing either the pH or the DO levels, as described previously. Shock conditions can happen in WWTPs due to several factors. For example, Orange County noticed TOC concentrations spikes in their final water after an FAT system, that achieved above 0.5 mg/L, which is the established limit in California. That situation was created by dampening of acetone in a manhole that led to the treatment facility. Since situations similar to this and other events can happen, the resiliency of the columns towards shock conditions was tested. Since in WWTPs and/or AWTFs, these shock conditions are usually time-limited, the shock conditions tested did not evaluate the long-term effect of upsetting conditions, but the impacts of these shocks on the biofilters right away.

4.3.1.1. pH

The average results based on two replicate experiments with varying pH are summarized in Table 12.

<table>
<thead>
<tr>
<th>pH targeted</th>
<th>pH measured in the field</th>
<th>pH effluent</th>
<th>TOC, mg/L</th>
<th>TOC removal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBR Filtrate</td>
<td>7.0 ± 0.2</td>
<td>-</td>
<td>7.2 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>Ozone Effluent</td>
<td>7.0 ± 0.1</td>
<td>-</td>
<td>6.7 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>7.3 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>21.6 ± 15.2</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>7.4 ± 0.3</td>
<td>5.4 ± 0.4</td>
<td>23.4 ± 13.7</td>
</tr>
<tr>
<td>7 (original)</td>
<td>7.0 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>5.3 ± 1.0</td>
<td>24.0 ± 11.6</td>
</tr>
<tr>
<td>9</td>
<td>9.1 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>5.2 ± 0.9</td>
<td>25.8 ± 10.7</td>
</tr>
<tr>
<td>11</td>
<td>11.0 ± 0.1</td>
<td>7.3 ± 0.2</td>
<td>5.5 ± 1.0</td>
<td>21.4 ± 13.4</td>
</tr>
</tbody>
</table>
Interestingly, pH changes appeared to have no discernible impact on the performance of the columns with respect to TOC removal. Drastic pH changes, as well as other changes in other factors such as temperature, salts concentration, etc., are known to cause inactivation of enzymes. The presence of more hydrogen or hydroxyl ions disturbs the composition of the amino acids as well as the bonds of the amino acids on the enzymes, causing them to alter their shapes. Conformation alteration also causes enzymes to lose their functional capacity by inactivating them.

The resiliency towards changes in the feed water suggests that the biofilm within the BAC column is in the latter stage of biofilm formation, as introduced previously. In this latter phase of biofilm formation, the EPS is well established among the cells. This is expected once this column has been receiving the same feed water for around 3 months since it was once brought up online and, therefore, acclimated. Besides, before being employed in this study, the media had been used over 10 years in a full-scale WWTP. As described in Table 12, the effluent pH values were neutral even for the extreme pH conditions in the feed water. Therefore, the activated carbon might play a role in neutralizing the pH of the water.

Biofilm resistance towards inhibitory agents is still an extensive area of research. In natural environments, biofilms consisting of different microbial species present several positive interactions that confer an ability to respond to environmental/operational upsets. Examples of these mechanisms are selective enrichment, enzyme regulation, metabolic cooperation, sensing systems, and incorporation and transference of genetic material, either via plasmid or DNA fragments in the environment due to cell lysis (Rittman and McCarty, 2001; Roilides et al., 2015; Crittenden et al., 2012). This diversity of species has different levels of gene expression.
over time, conferring distinct characteristics in space and time for the biofilm (Kumar et al., 2017).

Stress conditions caused by the addition of certain compounds can select for organisms that are able to survive and grow under those conditions, and the relative abundance of the critical microbial taxa will represent a larger portion of the community structure. Metabolites can also serve as substrate to less tolerant species. Changes in microbial communities due to stress conditions might not be necessary: activation of enzymes already present in the community might be triggered because of the new situation as a response to survival (Rittman and McCarty, 2001).

Biofilms have developed response mechanisms towards heavy metals by sequestration of metal complexes, by reducing them to a less toxic species, or by rejecting them out of the bacterial cells (Teitzel and Parsek, 2003). In WWTPs, microbial communities (such as in activated sludge) can quickly adapt to shock loadings of nutrients in the water and still perform similarly as compared to when they are under normal conditions (Purohit et al., 2016). With respect to antibiotic resistance, the EPS plays an important role. It confers resistance characteristics to the microbial community embedded in this matrix by blocking the transport of these antibiotics or by causing adsorption of the antibiotics onto the EPS (Donlan et al., 2002).

The EPS is a complex matrix composed of a variety of compounds that interact within each other and with external components, and this matrix varies significantly depending on environment, species present, etc. This matrix is “activated” by the release of membrane vesicles (MV). These MVs can bind to foreign compounds and deactivate them, and they can also behave as lytic enzymes to those compounds (i.e., causing death) increasing the resistance of biofilms (Flemming et al., 2007). In this
study, the EPS matrix might be contributing to the neutralization of the feed water pH, thereby protecting the biofilm from the potentially harmful effects of extreme pH.

During the pH resiliency experiment, small solids were detected in the effluent under the pH 11 condition. The solids were collected and observed under the microscope under a magnification factor of 1,000 times, as seen below in Figure 29.

The release of biofilm particles could be associated with this particular stress condition. Detachment of biofilms is a part of the life cycle of biofilms. Once the biofilms are mature, they can detach and move to colonize other areas, spreading the biofilm to other environments. Biofilm dispersion or detachment can be divided into active and passive, where active means natural detachment and passive detachment is mainly caused by external forces (Kaplan, 2010). Therefore, the biomass seen in Figure 29 could indicate an acceleration of detachment of biofilm due to external forces in the increased pH condition.
Figure 29. Microscopic observation of biomass eluted from BAC Control column at challenge experiment with pH 11. Microscopic magnification factor of 1000 times.

4.3.1.2. Inhibitory Substances and Dissolved Oxygen Changes

The average results based on two replicate experiments with varying DO concentration are reported in Table 13.

Table 13. Concentration of sodium sulfite added to achieve desired DO influent concentrations and TOC removal by BAC column under different DO concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\text{Na}_2\text{SO}_3) added, mg/L</th>
<th>DO influent, mg/L</th>
<th>DO effluent, mg/L</th>
<th>DO consumed, mg/L</th>
<th>TOC effluent, mg/L</th>
<th>TOC Removal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone effluent</td>
<td>N/A</td>
<td>21.1</td>
<td>-</td>
<td>-</td>
<td>7.1</td>
<td>-</td>
</tr>
<tr>
<td>BAC DO 1</td>
<td>0</td>
<td>21.1</td>
<td>9.4</td>
<td>11.7</td>
<td>5.0</td>
<td>29.2%</td>
</tr>
<tr>
<td>BAC DO 2</td>
<td>~40</td>
<td>15.3</td>
<td>8.3</td>
<td>7.0</td>
<td>6.8</td>
<td>4.2%</td>
</tr>
<tr>
<td>BAC DO 3</td>
<td>~96</td>
<td>7.9</td>
<td>3.7</td>
<td>4.2</td>
<td>6.9</td>
<td>2.8%</td>
</tr>
<tr>
<td>BAC DO 4</td>
<td>~154</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>8.1</td>
<td>-14.1%</td>
</tr>
</tbody>
</table>
Based on the results of this experiment, dissolved oxygen appears to be a critical factor for TOC removal. Since the feed water to this biofiltration column was always supersaturated with DO, the sudden decrease in DO may have caused stress on the microbial community, compromising its performance. In fact, under low DO conditions (i.e., 0.5 mg/L; Rittman and McCarty, 2001), the biofilters were unable to degrade any organic matter, and there has been a release of cellular debris, thereby resulting in a net increase in effluent TOC concentration. The lack of electron acceptors (O$_2$) for the microorganisms may have also have caused desorption of organic matter attached to the biomass and carbon media. Desorption due to gradient concentrations can happen after long-term loading of a particular contaminant (Corwin and Summers, 2011), or in this case, loading of organics with a lack of electron acceptors and/or simultaneous loading of inhibitory substances.

In natural environments, such as seawater, changes or stratification in DO cause changes in microbial community, mainly its richness (i.e., the abundance of species found) and sometimes its total biomass (Beman and Carolan, 2013). Some species are more commonly found in environments with relatively high DO concentrations rather than environments exhibiting ‘threshold’ DO concentrations (Spietz et al., 2015). In this case, the sudden drop in DO concentration might have caused stress to species sensitive to changes in DO, thereby causing their passive detachment and expulsion from the system.

Yadav et al. (2014) assessed the microbial community composition of activated sludge under different DO concentrations. Their results showed a decrease in relative abundance of alpha-Proteobacteria in lower DO levels, suggesting this class is sensitive to lower DO levels (Yadav et al., 2014). In fact, alpha-Proteobacteria are usually found extensively in wastewater systems – along with other Proteobacteria, Actinobacteria,
and Acidobacteria (Ju et al., 2014) – and in biofiltration systems (Wang et al., 2015). Therefore, shifting the environment to conditions that are unfavorable for these taxa may have been responsible for the poor biofilter performance observed for low DO levels.

Besides the negative impacts of low oxygen concentrations, excess of dissolved oxygen in the water can cause stress to microorganisms as well. Hyperoxia (i.e., exposure of cells to elevated amounts of oxygen) cause oxidative stress and higher production of reactive oxygen species such as superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) in elevated amounts. They accumulate in the cells via the respiratory chain mechanism, and these oxidants have toxic effects for them. This toxicity will lead to increased DNA damage, genetic changes (mutagenesis) and impaired growth (Baez and Shiloach, 2014). However, the dissolved oxygen concentrations used here are not in the toxic level.

As an alternative theory to rapid changes in DO, the high concentrations of sodium sulfite may have resulted in toxicity/inhibition of the microbial community. Sulfites are used in the food industry as antioxidants and preservatives. These compounds can destroy thiamine, or vitamin B$_1$, an essential cofactor for all organisms (Ramis-Ramos, 2003). Recently, compounds targeting the abatement of thiamine production in microorganisms have been investigated as potential antibiotic agents (Du et al., 2011). In addition, in water and wastewater, the sulfite can undergo several reactions that generate potential electron donors for the microorganisms, thereby competing with the bulk organic matter as the preferred electron donor and decreasing treatment performance.
4.3.2. NDMA Molecular Biology Tests

DNA extracts quantification revealed low concentrations, as shown in Table 14 below. The noticeable difference of methods detection is also shown in the table.

Table 14. DNA extracts concentrations using Nanodrop and Qubit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA Concentration, ng/µL</th>
<th>Nanodrop</th>
<th>Qubit</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC High</td>
<td>12.7</td>
<td></td>
<td>0.036</td>
</tr>
<tr>
<td>BAC Low</td>
<td>12.2</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Anthracite High</td>
<td>14.9</td>
<td></td>
<td>15.2</td>
</tr>
<tr>
<td>Anthracite Low</td>
<td>14.7</td>
<td></td>
<td>9.2</td>
</tr>
<tr>
<td>BAC Control High</td>
<td>14.5</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>BAC Control Low</td>
<td>13.2</td>
<td></td>
<td>0.1</td>
</tr>
</tbody>
</table>

PCR tests were performed to identify the presence or absence of propane monooxygenases (prmA, prmB, and prmE) in the media samples. A picture of the gel demonstrating the findings can be visualized in Figure 30.

Figure 30. Gel electrophoresis product from prmB PCR. Columns a through f are DNA samples: a) BAC top; b) BAC bottom; c) Anthracite top; d) Anthracite bottom; e) BAC Control top; f) BAC Control bottom. g column is empty and h column is the DNA ladder (100 – 1000 nucleotides) for comparison.
Once the presence of monooxygenase genes was confirmed, quantitative PCR (qPCR) tests were performed to quantify these genes in the DNA extracts from the biofilter media. The quantification cycles (C<sub>q</sub>) are presented in Table 15 along with the standard C<sub>q</sub>s (for 10<sup>8</sup> copies/µL).

Table 15. Average (n=3) C<sub>q</sub> values for the biofilters samples and for each of the sets of primers tested.

<table>
<thead>
<tr>
<th></th>
<th>Average C&lt;sub&gt;q&lt;/sub&gt;</th>
<th>prmA</th>
<th>prmB</th>
<th>prmE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>10&lt;sup&gt;8&lt;/sup&gt; copies/µL</td>
<td>22</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>BAC</td>
<td>High</td>
<td>36</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>36</td>
<td>34</td>
<td>44</td>
</tr>
<tr>
<td>Anthracite</td>
<td>High</td>
<td>27</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>29</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>BAC Control</td>
<td>High</td>
<td>36</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>36</td>
<td>37</td>
<td>41</td>
</tr>
</tbody>
</table>

Since the DNA extracts concentrations were relatively low and the C<sub>q</sub> values were relatively high, the same DNA extracts were shipped to a genomics laboratory (RTL Genomics, Lubbock, Texas) for microbial quantification using qPCR targeting 16S rRNA gene (forward primer: CCATGAAGTCGGAATCGCTAG, reverse primer: GCTTGACGGGGCGGTGT, probe: TACAAGGCCCCGGGAACGTATTCCCG). For both ozonated and non-ozonated BAC samples, the C<sub>q</sub> values were below the method’s level of detection (above 30 cycles out of 35 cycles used for the qPCR assay), but the C<sub>q</sub> value was approximately 18 for the anthracite samples, which implies in 8 x 10<sup>8</sup> and 6.28 x 10<sup>8</sup> copies per gram of dry media for high and low sampling locations, respectively.

The low (below detection limit) number of copies from BAC columns might be explained by a few possibilities for the observed DNA extraction limitation: (i) GAC has a higher adsorption capacity than anthracite, (ii) the GAC biofilm may have been
well established and stable, or (iii) the presence of certain adsorbed compounds on the GAC (e.g., organics, heavy metals, etc.) may have compromised DNA extraction (Young et al., 2014). According to Young et al. (2014), in the presence of multivalent cations (e.g., Ca$^{2+}$, Mg$^{2+}$), DNA adsorption onto clay particles is enhanced. In Las Vegas wastewater, TDS concentrations are high, usually close to 1,000 mg/L, and multivalent cations such as Ca$^{2+}$ and Mg$^{2+}$ comprise a large percentage of the TDS. Ethylenediaminetetraacetic acid (EDTA), a chelating agent, is usually present in DNA extraction kits to avoid this issue (Young et al., 2014), but the excessive TDS present in this particular water might not have been entirely buffered by the conventional kit. Therefore, normal biofilm DNA extraction kits might not be efficient for these media types. However, this requires a more extensive evaluation of DNA extraction efficiency, which was beyond the scope of the current study.

Additionally, the presence of certain compounds in the matrix can decrease PCR and qPCR efficiency such as humic and fluvic acids – organic compounds naturally found in water (Gentry-Shields et al., 2013). Humic acids can inhibit these tests by: disturbing the DNA polymerase; binding to the DNA template; and/or interfering with the fluorescence signal of dyes used (e.g., SYBR Green) during qPCR by quenching them, resulting in longer C$q$s to reach the target threshold. In the presence of certain ions such as iron and calcium, humic acids can form colloids that can interfere with the PCR elements such as by bonding with magnesium ions that are essential cofactors for PCR (Sidstedt et al., 2015). Besides humic acids, other PCR inhibitory substances are phenol, ethanol, polysaccharides, some proteins and proteinases (Schrader et al., 2012).

The copies of monooxygenases per gram of dry media were calculated and are presented in Figure 31. The values can be seen in Appendix 3.
These results are somewhat surprising since the ozonated BAC column had overall better performance for NDMA removal (Chapter 3). However, this may be due to DNA extraction efficiency limitations, as previously mentioned, and noticed from the Qubit method in Table 14. Nevertheless, the anthracite data still provide some degree of confidence in the fact that monooxygenase genes are highly abundant in this ozone-biofiltration system and may explain the NDMA removal observed during the aforementioned experiments.

Correlations between NDMA and TOC removal were determined for the ozonated BAC and anthracite columns. The results, described in units of ng of NDMA removed per mg of TOC removed, are shown in Table 16.

<table>
<thead>
<tr>
<th>Column</th>
<th>EBCT = 2 min</th>
<th>EBCT = 10 min</th>
<th>EBCT = 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC</td>
<td>39</td>
<td>76</td>
<td>89</td>
</tr>
<tr>
<td>Anthracite</td>
<td>66</td>
<td>88</td>
<td>111</td>
</tr>
</tbody>
</table>

Table 16. ng of NDMA removed for each mg of TOC removed.
On a mass basis, the anthracite column removed a greater amount of NDMA relative to the amount of TOC removed. Despite the DNA extraction limitations, this stoichiometric relationship supports the theory that monooxygenase genes were more abundant in the anthracite column. However, as mentioned in the previous chapter, the first order rate constant for NDMA biodegradation was higher for BAC than for anthracite. Therefore, NDMA biodegradation may have been more rapid in the BAC column, but higher concentrations of monooxygenase genes in the anthracite column may have compensated for the slower kinetics and achieved greater NDMA removal relative to the corresponding TOC removal. Since NDMA is co-metabolized, TOC (or BDOC) is assumed to be the primary substrate driving the biodegradation process.

Some studies found that media type can play an interesting factor in microbial community development. Using the same pilot-scale ozone-biofiltration system as in this study, Gerrity et al. (2018) performed 16S rRNA gene sequencing tests (primer set: 28F-388R) in order to characterize the microbial community of these biofilters. Principal coordinate analyses, which illustrate relative similarity/dissimilarity between samples, indicated that the microbial communities for the ozonated BAC and non-ozonated BAC control columns were more similar to each other than the anthracite column (Gerrity et al., 2018; Figure 32). A closer look into the microbial community on those biofilters showed a higher occurrence (relative abundance) of alpha-Proteobacteria, which is a common class in wastewaters and sensitive to low DO levels, as previously mentioned. Gerrity et al. (2018) also looked into other biofilters, but at DWTPs, and also noticed similarities in microbial community structure among filters using the same media type.
The difference in microbial community structure within filters containing different media types might be explained by the properties of the media. Anthracite is a natural type of coal and the media grains are large (1.2 mm in diameter), while GAC is manmade (i.e., burned carbon material such as coconut shell) with smaller grain size (0.95 mm in diameter). GAC also contains small pores that increase the surface area, potentially harboring more biomass (Appendix 2).

Nevertheless, the relatively low presence of *prm* genes in the ozonated BAC column and its ability to degrade NDMA (Chapter 3) suggests that other monooxygenase genes or even other enzymes might be present in this column.

Alternatively, environmental samples are known to have limitations. Even though the bacterial distribution (and, therefore genetic material distribution) is believed and assumed to be homogeneous in its microscale, only a small amount of media (0.3-0.4 g) was used for DNA extraction, which might not be completely representative of the real

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1 Bray-Curtis analysis evaluates the dissimilarities among samples, i.e., how similar or how different they are from one another. Weighted means that the number of times a same operational taxonomic unit showed in that community.
microbial community. These results show the complexity of NDMA biodegradation under actual treatment conditions rather than controlled laboratory experiments.

Among the different genes tested, higher quantities were observed for *prmE*, which was unexpected because the genes should theoretically been present in similar relative quantities. This suggests that the PrMO operon and its genes might not be present or that the microbial community might consist of bacterial strains capable of NDMA biodegradation but without the full complement of monoxygenase genes.

Except for the non-ozonated BAC control column, the overall starting quantities are higher for the top of the column and lower for the bottom. This is consistent with previous studies highlighting the amount of biomass and relative abundance of microbial communities with depth. As the water travels in a column, the organic matter starts being consumed by the microbiota, and less BDOC is left towards the bottom of the column. This gradient of nutrients causes changes in microbial community structure within depth (Liao et al., 2013). Even though the higher quantity at the top was more noticeable for the anthracite column, in the ozonated BAC column, there was no significant difference between top and bottom quantities of the *prmA* and *prmB* genes, but there were noticeable differences for *prmE*. In the non-ozonated BAC control column, the differences between top and bottom were not statistically significant (p=0.18).

Alternative extraction methods should be investigated in the future in an attempt to obtain higher DNA yield from BAC media particles such as using bead-beaters.
4.4 Conclusions

Biofilter resilience towards changes in the environment, such as pH, presence of inhibitory substances, and, to a lesser extent, DO concentration, makes them attractive options for potable reuse treatment, particularly when seeking greater reliability in achieving water quality targets and public health protection.

In ozone-biofiltration systems, DO levels in the biofiltration feed water will likely be supersaturated, and the microbial community in that system will likely be acclimated to that condition. However, even when fed with low BDOC levels (pre-oxygenation) or low BDOC and low DO levels (ambient MBR filtrate), the typically ozonated column was able to achieve significant TOC and NDMA removal. On the other hand, typically non-ozonated biofiltration systems may be more sensitive to spikes in DO level, although these are not expected to occur under normal operating conditions. Furthermore, sodium sulfite addition appeared to have a significant adverse impact on biofilter performance, perhaps due to its role as a biological preservative/inhibitory agent. Again, such high concentrations of inhibitory compounds are not expected to occur, although there have been notable spikes even at full-scale facilities (e.g., acetone spike at Orange County’s AWTF).

The higher quantities of monooxygenase genes in the anthracite column were surprising since the ozonated BAC achieved greater NDMA removal. These findings disagree with the rate constants found, in which the rates for BAC were higher than for anthracite. Moreover, the ozonated BAC and non-ozonated BAC control columns showed similar monooxygenase levels. This might be explained by the limitations of the DNA extraction method, which yielded less purified DNA for those samples than for the corresponding anthracite samples from 16S rRNA qPCR tests. Alternatively, other monooxygenases not investigated in this study, or even other enzymes, might be
contributing to NDMA biodegradation in the complex wastewater matrix. These findings highlight the need for further study in this area to achieve a greater understanding of NDMA biodegradation in biofiltration systems from a molecular microbiology perspective.
Chapter 5 – Conclusions

Depletion of conventional water supplies has stimulated potable reuse throughout the world since last century. But it is in this century that research and technology have advanced and made potable reuse a safe and reliable strategy to overcome water issues. Although very effective and consistent across a range of feed water qualities, some treatment trains (e.g., full advanced treatment) are costly and energy-intensive, hindering their use in many places, especially small-sized facilities and in inland locations. In this context, ozone-biofiltration has been proposed as an alternative form of advanced treatment for potable reuse applications, but several knowledge gaps still require further investigation.

NDMA, specifically its formation and subsequent attenuation, constitutes one of those knowledge gaps. This potential carcinogenic disinfection byproduct results from chloramination or ozonation of wastewater and is a public health concern even at trace levels, thereby warranting a notification level of 10 ng/L in California. Its biodegradation processes in ozone-biofiltration systems has not been completely elucidated.

Here, the removal of this DBP was investigated in ozonated BAC and anthracite columns and in a non-ozonated BAC (control) column. In a spiking test (~300 ng/L), increasing EBCT enhanced NDMA removal in the ozonated columns (~30% for 2-minute EBCT vs. ~95% for 20-minute EBCT), and the correlations exhibited a pseudo first order decay profile, which is supported by existing literature. Pre-ozonation appeared to play a significant role in NDMA attenuation considering the ozonated BAC and anthracite columns both achieved >90% NDMA removal, while the control BAC column achieved <50% NDMA removal even at the longest EBCT.
Because the ozonated columns achieved greater NDMA abatement than the non-ozonated column, the different features of ozonation were investigated in an attempt to isolate the critical feature(s) of pre-ozonation: ozone itself; high dissolved oxygen levels; or the greater amount of biodegradable dissolved organic carbon (BDOC) generated by pre-ozonation. This experiment was performed in the ozonated and non-ozonated BAC columns using three different water types: ozonated MBR filtrate, oxygenated MBR filtrate, and ambient MBR filtrate. The results from this novel experiment showed no differences in NDMA removal by those different operational conditions in the typically ozonated BAC column (~90%), whereas the typically non-ozonated BAC control column still achieved <50% NDMA removal regardless of the feed water. These findings suggest that the microbial community is the major feature controlling NDMA removal. Therefore, long-term exposure to ozonated MBR filtrate (maybe consistent exposure to ozone-induced NDMA) or the high DO concentrations characteristic of the ozonated MBR filtrate appears to select for microbial taxa that are better adapted to NDMA biodegradation.

Since NDMA can also be formed from chloramination and that a final residual disinfectant such as chloramine needs to be added to control bacterial regrowth in distribution systems (e.g., in direct potable reuse applications), NDMA formation potential tests were performed under uniform formation conditions (UFC). Biofiltration alone (i.e., non-ozonated BAC column) had minimal impact on NDMA precursor concentrations, with a final NDMA concentration after chloramination approaching 1 μg/L (100 times higher than the California notification level). Ozonation oxidized the chloramine-reactive NDMA precursors (primary, secondary, and tertiary amines) and resulted in a total of 41 ng/L of NDMA after pre-ozonation and chloramination. Post-ozone biofiltration eliminated the NDMA formed during pre-ozonation and also
eliminated some of the chloramine-reactive precursors, resulting in ~20 ng/L of NDMA after chloramination. Nevertheless, final polishing would likely be needed for ozone-biofiltration effluents in potable reuse applications to comply with existing U.S. EPA regulations on THMs and HAAs (i.e., when free chlorine is used) or with state notification levels for NDMA (i.e., when chloramines are used). Because of the efficacy of ozone-biofiltration, the operational requirements for downstream treatment processes (e.g., UV irradiation) would likely be reduced, thereby reducing capital and O&M costs.

Biofilm development stage and biofilter resilience were tested with abrupt changes in pH and DO levels in the feed water, and by the introduction of inhibitory substances. Results showed that the biofilms colonizing the biofilters are in the latest stage, in which load shocks do not disturb filters performance significantly. DO decreases in the feed water adversely impacted the BAC column, presumably because that column had been acclimated to high DO levels for months prior to the challenge testing. Also, the reagent tested for reduction in DO (sodium sulfite) is an inhibitory substance that can degrade Vitamin B₁, an essential coenzyme for all organisms. Sodium sulfite can also undergo chemical reactions in water and disturb the microbial community’s equilibrium with the usual electron donors.

DNA extracts from the biofilter media were tested for the presence of several monooxygenase genes linked to NDMA co-metabolism via quantitative PCR. Results showed higher quantities of these genes in the anthracite column than in the ozonated and non-ozonated BAC columns. The near absence of the tested monooxygenase genes in the BAC columns, despite the high level of NDMA removed, indicates that there might be other monooxygenases—or other enzymes entirely—responsible for NDMA biodegradation in those systems. Alternatively, this might be attributable to the low DNA extraction yields observed for BAC vs. anthracite. These results prove the
complexity of understanding NDMA biodegradation in complex matrices such as wastewater and leave room for further research in this area.

In conclusion, these data suggest that ozone-biofiltration would be effective for NDMA mitigation in some potable reuse systems, particularly when chloramines are expected to be used as a final disinfectant. However, UV photolysis might still be necessary as a final polishing step to ensure compliance with relevant guidelines and regulations (e.g., 10-ng/L notification level in California). Also, additional studies are needed to better characterize microbial community structure and function in potable reuse systems.
Appendix 1

Wet media was used for ATP measurements since the drying process could affect bacterial growth and ATP concentrations. The ATP concentrations for the wet media were then adjusted based on moisture content to determine the corresponding dry weight ATP concentrations. Moisture content was determined by drying each media type at 105 °C for 24 hours (Stoddart et al., 2016). The ATP concentrations for wet media were then converted and reported based on dry weight (i.e., pg ATP/g dry media). To report the ATP concentrations based on biofilter volume (i.e., pg ATP/cm³ of bulk media), the ATP concentrations were multiplied by the bulk density of the media (0.5 g/cm³ for BAC and 0.83 g/cm³ for anthracite).

<table>
<thead>
<tr>
<th>Media Type</th>
<th>Wet weight, g</th>
<th>Dry weight, g</th>
<th>Correlation, g dry/g wet</th>
<th>Moisture Content, %</th>
<th>Average Moisture Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC</td>
<td>1.316</td>
<td>0.603</td>
<td>0.458</td>
<td>54.2</td>
<td>57%</td>
</tr>
<tr>
<td></td>
<td>1.033</td>
<td>0.442</td>
<td>0.428</td>
<td>57.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.184</td>
<td>0.515</td>
<td>0.435</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.856</td>
<td>0.348</td>
<td>0.407</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>Anthracite</td>
<td>1.075</td>
<td>0.768</td>
<td>0.714</td>
<td>28.6</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>1.065</td>
<td>0.667</td>
<td>0.627</td>
<td>37.3</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 2

The total surface area of the column bed for the media types was calculated to allow comparison (Arnold et al., 2018). Media type parameters are presented below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>BAC</th>
<th>Anthracite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle diameter</td>
<td>mm</td>
<td>0.95</td>
<td>1.2</td>
</tr>
<tr>
<td>Bulk density</td>
<td>g/cm³</td>
<td>0.50</td>
<td>0.83</td>
</tr>
<tr>
<td>Volume (particle)</td>
<td>mm³</td>
<td>0.45</td>
<td>0.90</td>
</tr>
<tr>
<td>Surface area (particle)</td>
<td>mm²</td>
<td>2.83</td>
<td>4.52</td>
</tr>
</tbody>
</table>

The volume of the filter bed can be calculated as the height multiplied by the area of the circumference:

\[ V = h \pi r^2 = 70 \text{ cm} \times \pi \times (2.54 \text{ cm}/2)^2 = 354.7 \text{ cm}^3 = 354,700 \text{ mm}^3 \]

Assuming a 64% maximum packing arrangement (i.e., a maximum volume fraction of 64% is occupied by media and the remaining is occupied by water), the total bed volume occupied by media grains is 0.64 x 354,700 mm³ = 227,000 mm³

The total number of particles in the columns can be estimated by dividing this volume by the volume of each media type particle (0.45 mm³ for BAC and 0.90 mm³ for anthracite). Therefore, the BAC filters contain around 504,444 particles whereas the anthracite column contains around 252,222 particles.

The total media grain surface area for each filter bed is calculated by multiplying the number of particles by the surface area of individual grains, as shown below. Based on this analysis, filters with BAC as the media type have 25% more surface area available for biomass growth than anthracite filters.

BAC: 504,444 particles x 2.83 mm²/particle = 14,276 mm²

Anthracite: 252,222 particles x 4.52 mm²/particle = 1,140 mm²

% Difference: \((14,276 \text{ mm}^2 - 11,400 \text{ mm}^2) / 11,400 \text{ mm}^2 = 0.252 = 25.2\%\)
Appendix 3

The average copies of monooxygenases per µL for each sample is represented below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>prmA</th>
<th>prmB</th>
<th>prmE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC High</td>
<td>3.0 E+04</td>
<td>5.4 E+02</td>
<td>1.7 E+04</td>
</tr>
<tr>
<td>BAC Low</td>
<td>2.7 E+04</td>
<td>4.5 E+02</td>
<td>5.9 E+02</td>
</tr>
<tr>
<td>Anthracite High</td>
<td>7.4 E+06</td>
<td>8.7 E+03</td>
<td>1.3 E+09</td>
</tr>
<tr>
<td>Anthracite Low</td>
<td>1.9 E+06</td>
<td>3.1 E+03</td>
<td>2.8 E+08</td>
</tr>
<tr>
<td>BAC Control High</td>
<td>2.2 E+04</td>
<td>1.5 E+02</td>
<td>2.5 E+03</td>
</tr>
<tr>
<td>BAC Control Low</td>
<td>2.6 E+04</td>
<td>1.4 E+02</td>
<td>4.5 E+03</td>
</tr>
<tr>
<td>Blank</td>
<td>5.3 E+01</td>
<td>1.6 E+01</td>
<td>1.5 E+02</td>
</tr>
</tbody>
</table>
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Curriculum Vitae

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N-nitrosodimethylamine (NDMA) formation and mitigation in potable reuse treatment trains employing ozone and biological activated carbon (BAC)
Expected to consist of 2 years of graduate research experience focused on the formation and subsequent removal of carcinogenic NDMA in potable reuse applications. Will serve as M.S. thesis.

Natural Vulnerability and Risk to Contamination of Aquifers
12 months of undergraduate research experience on aquifers and their geological characteristics and vulnerability to contamination by various point and area sources, including gas stations, cemeteries, and landfills. Research involved use of ArcGIS software. Served as undergraduate dissertation.

Environment & Public Health Research Unit (EPHRU)
3 months of undergraduate research experience in an environmental and public health laboratory at the University of Brighton, England, UK. The research focused on analysis of water, wastewater, shellfish, and sediments in addition to water and wastewater treatment. Advisor: Dr. Huw Taylor.

Hooke Laboratory of Mass Spectrometry
13 months of undergraduate research experience in a microbiology and mass spectrometry laboratory at Sao Paulo State University, Brazil. The research efforts focused on the analysis of cyanotoxins in water with mass spectrometry.
WORK EXPERIENCE

Temporary Engineer Assistant  
Summer 2017  
3 months of experience at Trussell Technologies, Inc., in Pasadena (CA). Worked in several projects, including soil aquifer treatment for reuse purposes, secondary treatment characterization, and evaluation of drinking water treatment processes alternatives.

Natural Vulnerability and Risk to Contamination of Aquifers  
Fall 2015  
2 months of internship experience at the Rio Claro City Hall and the Department of Environment, Sao Paulo, Brazil. Performed inspections and surveys on spring and river areas to verify compliance with existing laws.

AWARDS & SCHOLARSHIPS

Nevada Water Resources Association  
Best Poster of the Conference. March, Las Vegas.  

WateReuse Association Graduate Scholarship  
Award: $1,000. In recognition of superior academic achievement and future promise in the water reuse industry.

Superior Academic Progress. Graduate College, UNLV  
Award: $2,000. In recognition of superior academic progress during the M.S. course.

WateReuse Association Graduate Scholarship  
Award: $1,000. In recognition of superior academic achievement and future promise in the water reuse industry.

Scientific Foundation Research (Undergraduate)  
Vulnerability and Risk to Contamination of the Free Aquifer in the Urban Area of Rio Claro City – SP, Brazil. Funding agency: São Paulo Research Foundation (FAPESP). Award: $2,300 as annual stipend.

Scientific Foundation Research (Undergraduate)  
Exchange Program Science without Borders – University of Brighton (UK). Funding agency: Higher Education Personnel Improvement Coordination (CAPES), Brazil. Award: U$ 12,200 as annual stipend; U$ 19,000 as tuition and fees.

XXIV CIC (Scientific Foundation Congress) of Sao Paulo State University, Brazil 2012  
Award among the best presentations (poster) of the Conference. Rio Claro, Sao Paulo, Brazil.

BrMass (Brazilian Conference of Mass Spectrometry)  
Best Poster of the Conference. Campinas, Sao Paulo, Brazil.  

Scientific Foundation Research (Undergraduate)  
Identification and Characterization of microcystins during cyanobacteria flourishing by MALDI-TOF-MS. Funding Agency: São Paulo Research Foundation (FAPESP). Award: $2,100 as annual stipend.
PUBLICATIONS AND PRESENTATIONS

- Bacaro, F., Gerrity, D. N-nitrosodimethylamine (NDMA) formation and mitigation in potable reuse treatment trains employing ozone and biological activated carbon (BAC). In prep.

EXTRA CURRICULAR ACTIVITIES

- American Water Works Association AWWA – UNLV Student Chapter
- Tau Beta Pi – UNLV Chapter
- Phi Kappa Phi Honors Society– Chapter 100 (UNLV)
- Volunteer with Outside Las Vegas, Friends of Nevada Wilderness, and Green Our Planet Las Vegas