Environmental Science
Water Research & Technology

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

Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-12-2018-000926.R1
Article Type:	Paper
Date Submitted by the Author:	13-Feb-2019
Complete List of Authors:	Bacaro, Fernanda; University of Nevada Las Vegas; Trussell Technologies, Inc. Dickenson, Eric; Southern Nevada Water Authority, Trenholm, Rebecca; Southern Nevada Water Authority, Water Quality Research and Development Gerrity, Daniel; University of Nevada, Las Vegas, Civil and Environmental Engineering and Construction

N-nitrosodimethylamine (NDMA) Formation and Mitigation in Potable Reuse Treatment Trains Employing Ozone and Biofiltration

Water Impact Statement

The carcinogenic disinfection byproduct NDMA is one of the few compounds with adverse public health impacts at the ng/L level. Some advanced treatment processes used in potable reuse applications are linked to NDMA formation, thereby requiring downstream mitigation measures. This paper evaluates the potential for optimization of biofiltration to remove NDMA and its ozone- and chloramine-reactive precursors.

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

FERNANDA BACARO[1,2], ERIC DICKENSON[3], REBECCA A. TRENHOLM[3], DANIEL GERRITY[1]*

[1]*Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas, Box 454015, 4505 S. Maryland Parkway, Las Vegas, NV 89154-4015, United States*

[2]*Trussell Technologies, Inc., 232 N. Lake Avenue Suite 300, Pasadena, CA 91101, United States.*

[3]*Applied Research and Development Center, Southern Nevada Water Authority, P.O. Box 99954, Las Vegas, NV 89193, United States*

*Corresponding author. Mailing address: Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas, Box 454015, 4505 S. Maryland Parkway, Las Vegas, NV 89154-4015, United States. Phone: (702) 895-3955. Fax: (702) 895-3936. Email: Daniel.Gerrity@unlv.edu.

Abstract

Ozone and chloramines are critically important for achieving stringent public health criteria and operational water quality objectives in potable reuse treatments trains, but these disinfectants are also linked to the formation of *N*-nitrosodimethylamine (NDMA). In the current study, a pilot-scale ozone-biofiltration system was used to treat membrane bioreactor (MBR) filtrate from a full-scale water reclamation facility. Experiments were designed to assess the roles of preoxidation, empty bed contact time (EBCT), and media type on NDMA formation and removal. In biological activated carbon (BAC) and anthracite columns receiving pre-ozonated MBR filtrate, EBCTs ≥10 min achieved >90% NDMA removal, while an EBCT of 2 min achieved only 30-40% NDMA removal. A control BAC column receiving non-ozonated MBR filtrate achieved a maximum removal of ~45%, even with an EBCT of 20 min. Moreover, this non-ozonated BAC column still exhibited inferior performance during a short-term transition to pre-oxygenated or pre-ozonated MBR filtrate. This suggests that media conditioning with pre-ozonated MBR filtrate selected for a microbial community that was better adapted to NDMA biodegradation. The presence of monooxygenase genes and microbial taxa suspected to be involved in NDMA biodegradation was also confirmed in the biofiltration columns. When subjected to final chloramination, pre-ozonation (but not biofiltration alone) was effective in transforming NDMA precursors and reducing NDMA formation by up to 96%. Ancillary monitoring of trace organic compounds (TOrCs) also highlighted potential concerns related to the persistence of perfluoroalkyl acids (PFAAs) in potable reuse applications.

Keywords: Potable reuse; ozone; biofiltration; *N*-nitrosodimethylamine (NDMA); monooxygenase; perfluoroalkyl acid (PFAA).

1.0 Introduction

To ensure adequate public health protection in potable reuse systems, regulatory frameworks often mandate the use of a multi-barrier treatment train capable of mitigating risks associated with a wide range of chemical and biological constituents.[1] In many potable reuse systems, wastewater effluent is purified at an advanced water treatment facility (AWTF) employing microfiltration (MF) or ultrafiltration (UF), reverse osmosis (RO), and a UV advanced oxidation process (AOP)—a combination described as full advanced treatment (FAT).[2] However, FAT is energy-intensive, costly from a capital and operational perspective, and requires disposal of concentrated brine streams.[3]

As an alternative to FAT, treatment trains employing ozone-biofiltration are gaining interest in the water reuse industry due to their 'equivalency' in the context of public health protection,[4] but more specifically because of their lower costs.[5-6] Ozone-biofiltration has been shown to transform and/or remove significant quantities of trace organic compounds (TOrCs) and bulk organics,[7-10] in part by increasing the level of biodegradable dissolved organic carbon (BDOC). Specifically, oxidation by ozone and hydroxyl radicals transforms effluent organic matter (EfOM)—measured as total organic carbon (TOC) or dissolved organic carbon (DOC)—into smaller, more assimilable compounds.[11] This decreases the aromaticity of the water as the EfOM is transformed into short-chain molecules (e.g., aldehydes and ketones) that are subsequently removed during downstream biofiltration.[12-13] This has the added benefit of reducing disinfection byproduct (DBP) formation during final disinfection.[2,14-16]

However, ozone-biofiltration has a practical limit with respect to overall TOC removal. FAT is capable of removing more than 90% of the TOC present in the wastewater-derived feed,[17] while TOC removal with ozone-biofiltration ranges from only 5% to 50%,[8-9,16,18]

64 depending on operational conditions and biofiltration media type and age. This presents an issue
65 in jurisdictions with stringent TOC guidelines, such as California's limit of 0.5 mg/L of
66 wastewater-derived TOC.[19] In order to maximize TOC removal, minimize DBP formation
67 potential, and reduce concentrations of recalcitrant TOrCs [e.g., perfluoroalkyl acids (PFAAs)],
68 some ozone-biofiltration systems are being supplemented with granular activated carbon (GAC)
69 for final 'polishing'.[20]

70 Another critical public health concern for potable reuse is the formation of the DBP and
71 probable human carcinogen *N*-nitrosodimethylamine (NDMA), which has a notification level of
72 10 ng/L in California[19] and a 10^{-6} risk level of 0.69 ng/L.[21] NDMA has been shown to form
73 during chloramination[15,22-24] and ozonation[15,24-25] of wastewater effluents. Because there are
74 distinct groups of precursors for each oxidant,[26] pre-ozonation can achieve net NDMA
75 reductions in some chloraminated systems,[27] but systems with high concentrations of ozone-
76 reactive precursors may experience net increases in NDMA.[5,24]

77 For some DBPs, specifically trihalomethanes (THMs) and haloacetic acids (HAAs), TOC
78 concentration can be a useful predictor of DBP formation potential.[16,28] However, the wide range
79 of precursors present at trace concentrations and with varying molar yields[26] makes it difficult to
80 predict NDMA formation potential with easily measured surrogates, thereby necessitating
81 multiple treatment barriers for NDMA mitigation. High-dose UV irradiation is often identified as
82 the best available treatment technology for NDMA,[29] but biofiltration has also been shown to
83 achieve NDMA concentrations <10 ng/L.[2,7,15,24]

84 In addition to the knowledge gap related to NDMA formation mechanisms and major
85 precursors, NDMA biodegradation has not yet been fully elucidated. Some pathways for NDMA
86 biodegradation are known,[30-31] with many studies focusing on individual bacterial strains and

their respective enzymes (e.g., monooxygenases).[30-34] Although these studies are useful for a fundamental understanding of the co-metabolic pathway, they may not be broadly applicable to environmental systems (e.g., wastewater biofiltration) harboring diverse microbial communities[35-36] and potentially containing promoting or inhibitory substances.

Therefore, additional studies are needed to understand the role of individual unit processes and operational conditions in potable reuse treatment trains on the formation and attenuation of NDMA. Ultimately, this knowledge can be leveraged to further enhance, stimulate, or select for conditions that minimize NDMA concentrations in potable reuse product waters. Within this context, the aim of this study was to investigate the role of different operational parameters, including empty bed contact time (EBCT), dissolved oxygen (DO), BDOC, and media type, on NDMA formation (with ozone and chloramines) and attenuation in a pilot-scale ozone-biofiltration system. Ancillary TOrC monitoring was also performed to validate the performance of the system with respect to well-characterized indicator compounds and to characterize the occurrence of recalcitrant PFAAs. This study also evaluated microbial community structure and the presence of monooxygenase genes within the biofiltration system.

2.0 Material and methods

2.1 Study site and pilot-scale ozone-biofiltration system

A 1-liter-per-minute pilot-scale ozone-biofiltration system was constructed and operated at a full-scale water reclamation facility in Nevada. The full-scale facility employs a membrane bioreactor (MBR) with full nitrification (solids retention time of 8-10 days) and partial denitrification, and solids separation is achieved with a 0.04-µm membrane. Ozonated MBR filtrate was fed to two biofilters: one containing anthracite with a diameter of 1.2 mm and another containing biological activated carbon (BAC) with a diameter of 0.95 mm. The

anthracite was obtained from the San Jose Creek Water Reclamation Plant in Los Angeles, CA, and the BAC was exhausted GAC (Norit 820, Cabot Corporation, Alpharetta, GA) with over 10 years of use at the F. Wayne Hill Water Resources Center in Gwinnett County, GA. A separate column filled with the same BAC received non-ozonated MBR filtrate and served as the experimental control. All three columns were 2.5 cm in diameter and packed with media to a depth of approximately 70 cm. Filter media sampling ports were located at bed depths of 7.6 cm and 42 cm. A more detailed description of the ozone-biofiltration system is provided in Text S1 in the Supplementary Information (SI).

2.2. Analytical methods

2.2.1 Bulk organic matter characterization and nutrient quantification

Text S2 provides a summary of the methods used for TOC quantification, bulk organic matter characterization with UV absorbance and fluorescence, and nutrient quantification.

2.2.2 NDMA

Reagent grade NDMA was purchased from Sigma-Aldrich (St. Louis, MO) and used to spike the non-ozonated and ozonated MBR filtrate in a subset of the experiments. Samples intended for NDMA analysis were collected in 1-L amber glass bottles containing 1 g/L of sodium azide for preservation and 80 mg/L of sodium thiosulfate for quenching. NDMA was analyzed using gas chromatography tandem mass spectrometry (GC-MS/MS) and isotope dilution based on a previously described modification of U.S. EPA method 521.[24,37-38] Briefly, automated solid phase extraction was performed using a Dionex AutoTrace workstation (Thermo Scientific, Sunnyvale, CA, USA). Analyses were performed using an Agilent 7890B GC with a PAL RSI 85 autosampler and a 7010 triple quadrupole MS (Agilent, Santa Clara, CA, USA). NDMA was monitored by multiple reaction monitoring in electron ionization mode using ion

transitions 74 → 44 m/z for quantification and 74 → 42 m/z for confirmation. Quantification was done using isotope dilution with d_6-NDMA (80 → 50 m/z). Method reporting limits (MRLs) for NDMA ranged from 2.9 to 28 ng/L, depending on the sample matrix and dilution.

2.2.3 Indicator TOrCs and PFAAs

Samples for TOrC or PFAA analysis were collected in 1-L amber glass or high density polyethylene bottles, respectively, containing 1 g/L of sodium azide for preservation and 50 mg/L of ascorbic acid for quenching. The target TOrCs included acetaminophen, atenolol, caffeine, carbamazepine, N,N-diethyl-meta-toluamide (DEET), fluoxetine, gemfibrozil, ibuprofen, meprobamate, naproxen, primidone, sucralose, sulfamethoxazole, tris(2-chloroethyl) phosphate (TCEP), triclocarban, triclosan, and trimethoprim. The target PFAAs included perfluorodecane sulfonic acid (PFDS), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluoropentanoic acid (PFPeA), perfluorobutane sulfonic acid (PFBS), and perfluorobutanoic acid (PFBA). TOrCs were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) with isotope dilution according to Vanderford et al.[39] and Vanderford and Snyder.[40] The PFAAs were analyzed using LC-MS/MS with isotope dilution, surrogate standard, or external calibration according to Appleman et al.[41] and Pisarenko et al.[38] LC-MS/MS was performed with an API 4000 triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA) operated in positive (TOrCs) and negative (TOrCs and PFAAs) ionization modes. MRLs were established at 3-5 times the method detection limits and ranged from 0.25 ng/L to 10,000 ng/L for the TOrCs and 0.5 ng/L to 5 ng/L for the PFAAs, depending on the target compound, sample matrix, and dilution.

2.3. Microbiological analyses

2.3.1 Attached ATP

Attached adenosine triphosphate (ATP) was used as a surrogate for biomass density on the biofiltration media. The Deposit and Surface Analysis ATP test kit (Hach, Loveland, CO) was used to extract ATP from the attached biomass, and a PhotonMaster Luminometer (LuminUltra Technologies Ltd, New Brunswick, Canada) was used to measure the ATP in each sample via luminescence. Duplicate samples of 1 gram of wet BAC and anthracite were dried at 105°C for 24 hours to determine the average moisture content for each media type (57% for BAC and 33% for anthracite). These values were then used to adjust and report the ATP concentrations based on dry media weight.

2.3.2 Monooxygenase genes

BAC and anthracite media were collected from both sampling depths in the biofilter columns (7.6 cm and 42 cm), and DNA was extracted using a DNeasy PowerBiofilm DNA Isolation Kit (QIAGEN, Hilden, Germany) according to manufacturer instructions. Purified DNA was quantified using a Nanodrop 1000 (ThermoFischer Scientific, Waltham, MA) and a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA). Target DNA sequences were then quantified with quantitative polymerase chain reaction (qPCR) using a CFX96 Touch Real-Time PCR Detection System (Bio-RAD, Hercules, CA) with the following temperature profile: 2 min of initial denaturation at 95°C; 40 cycles of 15 s of denaturation at 95°C, 30 s of annealing at 55°C, and 30 s of extension at 72°C; and 5 min of final extension at 72°C. The target 16S rRNA and monooxygenase genes and associated primers are summarized in Table 1, and additional details are included in Text S3.

178 **2.3.3 Microbial community structure**

179 The same DNA extracts were frozen and shipped to RTLGenomics (Lubbock, TX), where
180 the samples were amplified using degenerate primers targeting the V1-2 region of the 16S rRNA
181 gene (28F and 388R; Table 1) and then sequenced using a MiSeq sequencer (Illumina, San
182 Diego, CA). The raw sequencing data provided by RTLGenomics were deposited in NCBI's
183 Sequence Read Archive (SRA) under BioProject ID PRJNA521999. Additional details related to
184 DNA extraction, primer coverage, sequencing, and data analysis were described previously in
185 Gerrity et al.[36] and are also summarized in Text S5.

186 **2.4. Experimental approach**

187 **2.4.1. Phase 1: NDMA biodegradation as a function of EBCT**

188 All experimental phases were performed once consistent TOC removal was observed
189 through each of the biofilter columns. The primary focus of phase 1 testing was evaluating
190 NDMA biodegradation as a function of EBCT, pre-ozonation, and media type. Ozonated
191 (O_3/TOC = 1.0) and non-ozonated MBR filtrate were spiked with NDMA to target a final
192 concentration of ~300 ng/L. This allowed for quantification across a wider range of
193 concentrations. The 300-ng/L target is also representative of systems with extremely high
194 concentrations of ozone-reactive NDMA precursors.[4] EBCTs of 2, 10, and 20 min were tested in
195 the ozonated BAC, ozonated anthracite, and non-ozonated BAC. The biofiltration columns were
196 operated for three times the target EBCT to promote steady state conditions prior to sample
197 collection. Each condition was tested with experimental duplicates, and removal percentages
198 were calculated relative to the actual concentrations in the feed tank, which were evaluated at the
199 beginning and end of each experiment. The feed concentrations were consistent across each
200 testing phase.

201 **2.4.2. Phase 2: NDMA biodegradation as a function of pretreatment**

202 Phase 2 testing aimed to compare the effects of pre-ozonation (high BDOC and high
203 DO), pre-oxygenation (oxygen concentrator on but ozone generator off; low BDOC and high
204 DO), and no pretreatment (low BDOC and low DO) on NDMA biodegradation with BAC only.
205 Pre-ozonation was qualitatively assumed to achieve 'high' BDOC concentrations, which is
206 supported by the greater TOC removal observed during biofiltration in the presence vs. absence
207 of pre-ozonation (shown later in Figure 1). DO concentrations in the biofilter feed ranged from
208 16-21 mg/L for pre-ozonation and pre-oxygenation and 4-5 mg/L for the ambient MBR filtrate.
209 The feed waters were again spiked with NDMA to a final concentration of ~300 ng/L, and the
210 biofiltration system was operated for three times the target EBCT prior to sample collection. The
211 O_3/TOC was 1.2 for the ozonated feed water, and the EBCT was fixed at 10 minutes. Each water
212 was fed to the typically ozonated BAC column and the typically non-ozonated BAC column, and
213 each condition was evaluated using experimental triplicates.

214 **2.4.3. Phase 3: NDMA formation upon final chloramination**

215 Phase 3 testing focused on NDMA formation during chloramination and the role of ozone
216 and/or biofiltration in oxidizing/removing chloramine-reactive NDMA precursors. Chloramine
217 dosing followed the uniform formation conditions (UFC) approach, which targets a total chlorine
218 residual of 1.0±0.4 mg/L as Cl_2 after 3 days in the dark at room temperature.[42-43] UFC dosing
219 was performed in duplicate for each sample. The O_3/TOC for these experiments was 1.6, and the
220 EBCT was fixed at 10 minutes for the ozonated BAC, ozonated anthracite, and non-ozonated
221 BAC columns.

222 **2.4.4. Phase 4: Evaluation of TOrC and PFAA attenuation**

Phase 4 testing focused on attenuation of the target TOrCs and PFAAs. The O_3/TOC was 1.3, and the EBCT was fixed at 10 min for the ozonated BAC and non-ozonated BAC columns. Only single samples were analyzed for each experimental condition.

3.0 Results and discussion

3.1. General water quality parameters

Table 2 summarizes the results from weekly monitoring of general water quality parameters during long-term operation of the pilot-scale ozone-biofiltration system. Ozone alone generally resulted in a minimal reduction in TOC (5% on average) but a clear transformation of bulk organic matter, as demonstrated by the reduction in UV_{254} absorbance (Table 2) and changes in EEM 'fingerprints' (Figure S2). Biofiltration was critical for the removal of TOC, with pre-ozonation coupled with BAC or anthracite achieving average TOC reductions of 22% and 15%, respectively. The superior performance of BAC relative to anthracite has often been attributed to greater biomass density.[44] However, recent studies suspect that differences in performance may actually be attributable to adsorption/desorption dynamics or differences in microbial community structure.[16] This is because biomass density, as measured by attached ATP, is not always greater in ozonated BAC systems and does not always correlate with TOC removal.[16] In the current study, total attached ATP levels were estimated to be $(7.71\pm5.74)\times10^{10}$ pg, $(3.82\pm2.11)\times10^{10}$ pg, and $(4.01\pm2.23)\times10^{11}$ pg in the ozonated BAC, ozonated anthracite, and non-ozonated BAC columns, respectively (Table S4).

In reuse applications, TOC removal has also been shown to be a function of O_3/TOC and EBCT,[16] although the 'optimal' EBCT for TOC removal is generally less than 15 min.[45] In conjunction with phase 1 testing, TOC removal was also evaluated as a function of EBCT but with a constant O_3/TOC of 1.0 (Figure 1). The raw data for Figure 1 are summarized in Table S1.

Consistent with the literature and the data in Table 2, TOC removal followed a trend of O_3-BAC≥O_3-ANT>BAC, and TOC removal plateaued at an EBCT of 10 min. For this particular experiment, maximum TOC removal ranged from ~10% for biofiltration to 35-40% for ozone-biofiltration, thereby resulting in minimum effluent TOC concentrations of 4.5-5.0 mg-C/L. Both ozone-biofiltration effluents actually contained higher TOC concentrations with an EBCT of 20 min, either due to experimental variability (e.g., changes in TOC concentration of the feed water) or potentially the release of soluble microbial products (SMPs) by the microbial community. The latter theory is supported by the increase in fluorescence observed in the ozone-BAC effluent relative to the ozonated MBR filtrate (Figure S2).

Maximum TOC concentrations are not always stipulated in potable reuse regulations, but regulatory limits have been established in some states in the U.S., ranging from 0.5 mg/L in California[19] to 3 mg/L in Florida.[1] Consistent with U.S. EPA recommendations,[1] Arnold et al.[16] identified a target effluent TOC concentration of 2.0-3.3 mg/L to ensure compliance with the 80-µg/L maximum contaminant level (MCL) for total THMs. Therefore, additional treatment (e.g., GAC) or dilution with low-TOC surface or ground water would likely be necessary to mitigate DBP concerns if this ozone-biofiltration effluent was intended for a potable reuse application.

3.2. Phase 1: NDMA biodegradation as a function of EBCT

Based on recent literature, there appears to be a weak relationship between direct NDMA formation and ozone dose. Figure 2 provides a summary of bench-scale data from the literature for a range of secondary effluent water qualities, including both non-nitrified (F) and fully nitrified (A-E) wastewaters.[5,46] The data indicate that direct formation during ozonation ranges from the low ng/L level to more than 100 ng/L and is not necessarily a function of nitrification. Moreover, NDMA formation seems to be independent of ozone dose beyond an O_3/TOC of 0.5;

Pisarenko et al.[38] showed a weak relationship for O_3/TOC<0.5 and minimal impact from H_2O_2 addition. Considering that many potable reuse systems will employ an O_3/TOC of at least 0.5,[5] at which point NDMA formation has reached a maximum, the current study focused only on the effects of EBCT for NDMA attenuation during biofiltration.

During phase 1 testing, the ambient NDMA concentration in the MBR filtrate was 7 ng/L, and direct NDMA formation with an O_3/TOC of 1.0 was 26 ng/L, thereby suggesting a moderate concentration of ozone-reactive NDMA precursors. Figure 3 illustrates NDMA removal as a function of EBCT; again, the feed waters had been spiked with ~300 ng/L of NDMA prior to biofiltration. Interestingly, the ozonated BAC and ozonated anthracite achieved similar NDMA removal (maximum removal > 90%), but there were diminishing returns beyond an EBCT of 10 min. This is consistent with the TOC removal and reductions in DBP formation potential observed in Gifford et al.[45] and Arnold et al.[16] Wu and Xie[47] observed improvements in HAA mitigation with longer EBCTs but only at low temperatures (<10°C). Despite a lower overall removal (maximum removal <50%), longer EBCTs were advantageous for the non-ozonated BAC, as NDMA removal doubled for an EBCT of 20 min vs. 10 min. Assuming first order kinetics (Eq. 1), the rate constants describing biodegradation of NDMA relative to EBCT (k_{NDMA}) were 0.20 min^{-1}, 0.19 min^{-1}, and 0.03 min^{-1} for ozonated BAC, ozonated anthracite, and non-ozonated BAC, respectively.

$$\ln\left(\frac{NDMA_f}{NDMA_0}\right) = - k_{NDMA} \text{ x EBCT} \quad \text{(Eq. 1)}$$

Although there were only small improvements in removal percentage for the 20-min EBCTs in the ozonated biofiltration columns, those improvements might be necessary when targeting stringent potable reuse regulations (e.g., the 10-ng/L notification level) in systems with high concentrations of ozone-reactive NDMA precursors. For ozone-biofiltration, the 10-min

EBCT achieved an average effluent NDMA concentrations of ~30 ng/L, whereas the 20-min EBCT achieved effluent NDMA concentrations <10 ng/L. The non-ozonated BAC column achieved a minimum effluent NDMA concentration of 130 ng/L. The superior performance of the ozonated biofiltration columns suggested a positive correlation between BDOC and/or DO (byproducts of ozonation) and NDMA removal, which was the focus of phase 2 testing.

3.3 Phase 2: NDMA biodegradation as a function of pretreatment

3.3.1 Phase 2a: NDMA biodegradation profile

The defining feature of phase 2 was the fact that MBR filtrate with three different pretreatment conditions (high BDOC/high DO, low BDOC/high DO, low BDOC/low DO) was fed to the typically ozonated BAC column and the typically non-ozonated BAC column. Figure 4 shows that NDMA removal for the typically ozonated BAC column was consistent with the 10-min EBCT data from phase 1 (i.e., ~90% removal for ozone-biofiltration), but interestingly, NDMA removal was independent of pretreatment. As expected, the greatest level of TOC removal was observed for the ozonated feed water (31±1.4%), but the oxygenated (24±1.0%) and untreated (22±1.4%) MBR filtrates also exhibited relatively high TOC removals for that column. As this was only a short-term test, TOC removal (and possibly NDMA removal) would be expected to decrease for the oxygenated and untreated MBR filtrates over a longer operational period, but this was not confirmed in the current study.

The typically non-ozonated column exhibited considerably different removal profiles. With respect to TOC, the ozonated feed water exhibited the greatest removal (30±1.0%), presumably due to its higher BDOC concentration, while the oxygenated (11±3.9%) and untreated (22±0.4%) MBR filtrates exhibited expectedly lower removals. With respect to the relatively poor performance for the oxygenated MBR filtrate, it is possible that the microbial

community was actually inhibited by the 4-fold increase in DO (up to 20 mg/L), particularly when also challenged by the limited BDOC content relative to the ozonated feed water. Under supersaturated conditions, accumulation of reactive oxygen species (e.g., hydrogen peroxide and superoxide) has been shown to be toxic to microorganisms,[48-49] although this was not observed for pre-ozonation. Since NDMA biodegradation is proposed as a cometabolic pathway,[32] NDMA removal was expected to be higher for the ozonated feed water, but the opposite trend was actually observed. NDMA removal was lowest for the ozonated feed water (36±0.0%) and then increased for the oxygenated (45±2.3%) and untreated MBR filtrate (51±4.3%). The 51% removal for the MBR filtrate was also more than double the removal observed for the 10-min EBCT in phase 1.

Collectively, these data suggest that TOC removal, BDOC concentration, and DO concentration are unreliable surrogates/predictors of NDMA removal during biofiltration. Instead, long-term exposure to higher concentrations of NDMA and/or ozone transformation products may be a more significant factor affecting NDMA removal in biofiltration systems. In support of this hypothesis, a recent study demonstrated changes in the microbiota of BAC biofilters over 60 days of exposure to 1 μg/L of NDMA and other nitrosamines, although the study did not evaluate changes in gene expression that could be explicitly linked to NDMA biodegradation.[50] Using soil columns receiving chloraminated/dechlorinated secondary effluent with nitrification and denitrification, Trussell et al.[51] also showed increases in NDMA and TOC removal over time, thereby suggesting microbial community acclimation, selection of relevant microbiota, or upregulation of relevant gene expression.

3.3.2 Phase 2b: Microbial community characterization

Propane monooxygenases have been linked to NDMA cometabolism (Text S3), thereby making them a potentially important target for characterizing NDMA biodegradation potential. As such, media samples were collected from each of the biofilter columns with the intent of comparing relative abundance of monooxygenase genes and overall microbial community structure. However, following extraction and purification, DNA quantification indicated high levels of interference and/or extraction efficiency issues for the ozonated BAC and non-ozonated BAC samples (Table S5). This was observed with repeated extractions using multiple kits, with varying quantities of BAC media, with a supplemental freeze/thaw step, and with bead beating. The UV_{260} absorbance measurements by Nanodrop indicated relatively high concentrations of DNA for all samples (13.7±1.1 ng/μL), which were expected based on the aforementioned ATP data (Table S4). In contrast, the fluorescence-based Qubit measurements indicated high DNA concentrations in the anthracite extracts (12.2±4.2 ng/μL) but very low DNA concentrations for the BAC extracts (0.08±0.05 ng/μL). This was confirmed by quantification of the 16S rRNA gene in each sample, with anthracite yielding $(5.7\pm4.4)\times10^7$ gene copies per gram and BAC yielding only $(3.2\pm0.17)\times10^4$ gene copies per gram (Table S6). Possible reasons for these discrepancies include stronger adsorption/biofilm attachment to the activated carbon and/or the presence of inhibitory substances adsorbed onto the BAC (e.g., organics, heavy metals, etc.) that may have interfered with DNA extraction.[52] Unfortunately, this meant that the molecular analyses were effectively limited to the ozonated anthracite media.

Figure 5 summarizes the results from the qPCR assays for the 16s rRNA gene (as a surrogate for overall biomass) and the *prm*A, *prm*B, and *prm*E monooxygenase genes. All four target sequences exhibited higher counts in the upper portion of the biofiltration column, consistent with the ATP data in Table S4. More importantly, all three monooxygenase genes

appeared to be abundant, ranging from 0.01% to 1% relative abundance. Their detection also provides support for the theory that monooxygenases may be responsible for NDMA biodegradation in environmental systems.

Text S5 summarizes the results of the 16S rRNA gene sequencing analysis. Among genus-level taxa, *Mycobacterium* was differentially abundant in the upper portion of the anthracite biofilter (high = 31%, low = 8%), and Bradyrhizobiaceae (family; unclassified at the genus level) (high = 6%, low = 13%) was differentially abundant in the lower portion of the anthracite biofilter (Table S8). In a previous study of this same system, *Bradyrhizobium* was highly prevalent[36] and was suspected in generating ammonia during biofiltration;[16] this genus is capable of nitrogen fixation.[53] The high relative prevalence of *Mycobacterium* is significant because previous studies have linked several species of *Mycobacterium* to NDMA biodegradation (Table S2). Interestingly, *Mycobacterium* also comprised ~2-5% of the sequences from the ozonated BAC samples but 0% of the sequences from the non-ozonated BAC samples (data not shown). These BAC data are potentially unreliable because of the DNA extraction issue, but they may provide insight into the superior performance of the ozonated vs. non-ozonated BAC column. *Rhodococcus* is also important for NDMA biodegradation (Table S2) but was detected in only the lower ozonated BAC sample (not in the ozonated anthracite, upper ozonated BAC, or non-ozonated BAC samples). *Rhodococcus* is similar to *Mycobacterium* and other members of the order *Corynebacterium*,[54] which were highly prevalent in all ozonated media but not in the non-ozonated BAC.

Therefore, monooxygenase genes and/or taxa that are known to possess these genes were detected in the ozonated anthracite and ozonated BAC biofilter media. However, additional metagenomics testing would be needed to simultaneously detect the observed taxa and confirm

that they possessed those specific genes within their genomes. Alternatively, qPCR assays targeting species/strains linked to NDMA biodegradation (Table S2) could provide a valuable point of comparison for systems exhibiting differing NDMA biodegradation rates. This could also be accomplished with detection and quantification of mRNA to demonstrate differential expression of monooxygenase genes. In the current study, these types of comparisons could not be performed with confidence because of the nucleic acid extraction issues for the BAC. Nevertheless, these findings highlight existing knowledge gaps related to NDMA biodegradation and leave room for further research in this area.

3.4. Phase 3: NDMA formation upon final chloramination

As illustrated in Figure 6, the ambient NDMA concentration in the MBR filtrate was <MRL during phase 3 testing, but upon chloramination, the MBR filtrate contained 960 ng/L of NDMA. This was higher than secondary effluent D presented earlier in Figure 2 (590 ng/L) but lower than secondary effluent F (1,600 ng/L). It must be noted that the data in Figure 2 are based on 'formation potential' conditions[43,55] as compared with the 'uniform formation conditions' in the current study. Using the UFC approach, Zeng et al.[43] reported NDMA formation for a range of wastewater effluents ranging from as low as 14 ng/L to as high as 833 ng/L. These comparisons confirm that the MBR filtrate in the current study contained exceptionally high concentrations of chloramine-reactive precursors.

Biofiltration removed only a small portion of the chloramine-reactive precursors, which reduced NDMA formation from 960 ng/L to 930 ng/L. Consistent with the data from phase 1, ozonation resulted in direct formation of 20 ng/L but dramatically reduced formation during chloramination. Assuming 20 ng/L resulted from ozonation, chloramination generated only an additional 21 ng/L—an overall reduction in NDMA of 96% relative to the chloraminated MBR

406 filtrate. Krasner et al.[56] and Marti et al.[57] also demonstrated that pre-oxidants (e.g., ozone, free
407 chlorine, chlorine dioxide) reduce overall NDMA levels in some treated wastewaters, depending
408 on the composition of the precursors. Ozone is known to rapidly oxidize primary, secondary, and
409 tertiary amines—typical chloramine-reactive precursors—into nitrated byproducts and N-
410 oxides,[58] thereby reducing NDMA formation during chloramination. However, since each pre-
411 oxidant results in a unique DBP profile, the risks posed by other DBPs (e.g., bromate during pre-
412 ozonation)[59] must be evaluated against the potential formation of NDMA to identify the
413 preferred treatment sequence for a given matrix.[15]

414 Finally, the ozone-biofiltration data in Figure 6 demonstrate that biofiltration was able to
415 eliminate the low levels of NDMA formed during ozonation (i.e., ~20 ng/L). However, ozone-
416 biofiltration was unable to eliminate all of the chloramine-reactive NDMA precursors, as there
417 was still 17 ng/L and 23 ng/L of NDMA formation upon chloramination of the ozone-BAC and
418 ozone-anthracite effluents, respectively. This may have been due to the release of SMPs during
419 biofiltration, which are known to be chloramine-reactive precursors.[60] Nevertheless, the
420 combination of ozone-biofiltration-chloramination resulted in a 98% reduction in NDMA
421 formation relative to the MBR filtrate, consistent with other studies in the literature.[61]

422 The final concentrations observed in the current study would still exceed the 10-ng/L
423 notification level (NL) in California potable reuse applications, which might necessitate an
424 alternative residual disinfection strategy (e.g., free chlorine) in a potable reuse application.
425 Again, this would require an evaluation of the overall DBP profile because the TOC
426 concentration in the ozone-BAC effluent was 4.6 mg-C/L, which would likely create THM
427 compliance issues with free chlorine.[16] Alternatively, a potable reuse system could implement an
428 additional polishing step (e.g., GAC and/or UV/H_2O_2). Zeng et al.[2] demonstrated that ambient

NDMA concentrations could be reduced to <MRL with UV/H_2O_2, but they still observed some reformation due to reactions between residual chloramines and recalcitrant chloramine-reactive precursors. These additional polishing steps might also be warranted to address recalcitrant TOrCs that persist through typical ozone-biofiltration systems,[20,62] as noted in the following section.

3.5. Phase 4: Evaluation of TOrC and PFAA attenuation

As expected, compounds susceptible to biological treatment, such as acetaminophen, caffeine, and ibuprofen, were present at low concentrations in the MBR filtrate (<5 ng/L), whereas biologically recalcitrant compounds, such as sucralose and sulfamethoxazole, were found at higher concentrations (i.e., >1-50 µg/L) (Table 3). Some of the biologically recalcitrant compounds typically found in secondary wastewater effluents are effectively oxidized during ozonation, particularly with high ozone doses (O_3/TOC = 1.3 in phase 4). This includes compounds that are susceptible to ozone (e.g., carbamazepine, fluoxetine, naproxen, sulfamethoxazole, triclocarban, triclosan, and trimethoprim) and compounds that are susceptible to hydroxyl radicals (e.g., atenolol and gemfibrozil).[10] All of these compounds were detected in the MBR filtrate but were <MRL after ozonation. However, there are also compounds that are resistant to biodegradation, ozone, and hydroxyl radicals (e.g., DEET, primidone, meprobamate, sucralose, and TCEP),[10] and they are often detected in ozonated secondary effluents, including the ozonated MBR filtrate in the current study.

In wastewater/reuse applications, activated carbon is quickly exhausted by high bulk organic loadings, thereby limiting TOrC adsorption in BAC systems. Moreover, because many biodegradable TOrCs are removed/transformed in the upstream activated sludge process and then further oxidized during pre-ozonation, minimal TOrC attenuation is expected in

452 downstream biofiltration processes. In other words, biofiltration is critically important for the
453 removal of bulk organic matter that is transformed during pre-ozonation, but it is often
454 inconsequential in terms of TOrC removal, except in the case of NDMA that forms during pre-
455 ozonation. In the current study, biofiltration actually resulted in increases in the concentrations of
456 some TOrCs, presumably due to temporal variability and/or desorption from the BAC. This was
457 more apparent with biofiltration alone (i.e., MBR+BAC) than with biofiltration following pre-
458 ozonation (i.e., MBR+O_3+BAC). Desorption can occur when there is a concentration gradient in
459 the water promoting the release of adsorbed compounds from the activated carbon.[63-64] For
460 example, after discontinuing their TOrC feed to a pilot-scale biofiltration system, Greenstein et
461 al.[65] observed an increase in effluent TOrC concentrations as the system established a new
462 equilibrium condition. This can also occur with a longer EBCT, which allows more time for the
463 system to equilibrate with variable feedwater concentrations.[63] These data also suggest that
464 biofiltration systems exhibiting high removals of biologically recalcitrant, oxidant-resistant
465 TOrCs (e.g., TCEP) might still possess adsorption capacity for trace organics even if they appear
466 to be exhausted toward bulk organic matter.[8-9]

467 Ambient PFAA concentrations in the MBR filtrate were relatively low, and there was no
468 apparent removal by ozone and/or biofiltration (Table 3). PFBS and PFHpA both increased in
469 concentration by a factor of ~10 with biofiltration alone (i.e., 3-4 ng/L to 23-45 ng/L), but
470 additional sampling would be needed to confirm whether the increase was due to temporal
471 variability, desorption, or precursor transformation. Oxidation and biological treatment have
472 been shown to increase PFAA concentrations in some matrices due to degradation of higher-
473 chain precursors.[20,37,66] From a public health perspective, the combined concentration of PFOS
474 and PFOA was ≤23 ng/L in all samples, which is less than the U.S. EPA Health Advisory Level

of 70 ng/L.[67] However, California also has notification levels of 13 ng/L and 14 ng/L for PFOS and PFOA, respectively,[68] and all samples exceeded the PFOA notification level with concentrations ≥20 ng/L. Again, this would likely necessitate an additional adsorption-based GAC 'polishing' step, as GAC is often identified as the best available technology for PFAA attenuation.[69-71] Other treatment processes, including nanofiltration, RO, and ion exchange, have also been evaluated for PFAA abatement.[20,70-72]

4.0 Conclusions

Ozone-biofiltration is an established and viable alternative to reverse osmosis in some potable reuse applications, but there are still opportunities to improve the design and performance of ozone-biofiltration with respect to certain treatment objectives, including NDMA attenuation. Longer EBCTs achieved greater NDMA removal, but short-term changes in DO and BDOC had minimal impact on NDMA attenuation. Instead, the current study identified long-term exposure of biofiltration systems to ozonated water as being the critical factor affecting NDMA biodegradation rates, presumably due to shifts in microbial community structure and function. The qPCR assays detected the presence of monooxygenase genes that have been linked to NDMA biodegradation, but additional studies are needed to confirm that these genes are responsible for NDMA attenuation in complex environmental systems. Ozonation was also effective for the oxidation of chloramine-reactive NDMA precursors, which might be important for systems trying to control membrane biofouling or targeting final disinfection with chloramines. However, the benefits for chloramination must be weighed against the potential for ozone-induced NDMA formation and other potential byproducts. In most potable reuse systems, ozone-biofiltration must be coupled with additional treatment barriers to achieve additional

pathogen log removal credits or attenuation of recalcitrant organics, including PFAAs, residual NDMA, and even bulk organic matter.

Conflicts of Interest Statement

There are no conflicts to declare.

Acknowledgements

This publication was made possible by U.S. EPA grant R835823 (Early Career Award – Framework for Quantifying Microbial Risk and Sustainability of Potable Reuse Systems in the United States) and USGS grant G16AP00069. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the U.S. EPA or USGS. We would also like to thank the following personnel from UNLV and SNWA for their assistance: Peter Faught, Katerina Papp, Brett Vanderford, Oscar Quinones, Janie Zeigler-Holady, and Jennifer Fuel.

References

1. EPA, 2017 Potable Reuse Compendium, United States Environmental Protection Agency, 2017, https://www.epa.gov/sites/production/files/2018-01/documents/potablereusecompendium_3.pdf (accessed February 2019).
2. T. Zeng, M. J. Plewa, and W. A. Mitch, *N*-Nitrosamines and halogenated disinfection byproducts in U.S. full advanced treatment trains for potable reuse, *Water Res.*, 2016, **101**, 176-186.

3. R. W. Holloway, L. Miller-Robbie, M. Patel, J. R. Stokes, J. Munakata-Marr, J. Dadakis, and T. Y. Cath, Life-cycle assessment of two potable water reuse technologies: MF/RO/UV-AOP treatment and hybrid osmotic membrane bioreactors. *J. Membrane Sci.*, 2016, **507**, 165-178.
4. R. R. Trussell, A. Salveson, S. Snyder, R. S. Trussell, and D. Gerrity, Equivalency of advanced treatment trains for potable reuse, Final Report for Reuse-11-02, *Water Environment & Reuse Foundation*, 2016, Alexandria, VA.
5. D. Gerrity, E. Owens-Bennett, T. Venezia, B. D. Stanford, M. H. Plumlee, J. Debroux, and R. S. Trussell. Applicability of ozone and biological activated carbon for potable reuse, *Ozone Sci. Eng.*, 2014, **36(2)**, 123-137.
6. M. H. Plumlee, B. D. Stanford, J. Debroux, D. C. Hopkins, and S. A. Snyder, Costs of advanced treatment in water reclamation, *Ozone Sci. Eng.*, 2014, **36(5)**, 485-495.
7. J. Hollender, S. G. Zimmermann, S. Koepke, M. Krauss, C. S. McArdell, C. Ort, H. Singer, U. von Gunten, and H. Seigrist, Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration, *Environ. Sci. Technol.*, 2009, **43**, 7862-7869.
8. D. Gerrity, S. Gamage, J. C. Holady, D. B. Mawhinney, O. Quiñones, R. A. Trenholm, and S. A. Snyder, Pilot-scale evaluation of ozone and biological activated carbon for trace organic contaminant mitigation and disinfection, *Water Res.*, 2011, **45(5)**, 2155-2165.
9. J. Reungoat, B. I. Escher, M. Macova, F. X. Argaud, W. Gernjak, and J. Keller, Ozonation and biological activated carbon filtration of wastewater treatment plant effluents, *Water Res.*, 2012, **46(3)**, 863-872.
10. Y. Lee, D. Gerrity, M. Lee, A. E. Bogeat, E. Salhi, S. Gamage, R. A. Trenholm, E. C. Wert, S. A. Snyder, and U. von Gunten, Prediction of micropollutant elimination during ozonation

of municipal wastewater effluents: Use of kinetic and water specific information, *Environ. Sci. Technol.*, 2013, **47(11)**, 5872-5881.

11. B. S. Sidhu, L. Taylor-Edmonds, M. J. McKie, and R. C. Andrews, Pre-oxidation strategies for biofiltration performance improvement, *J. Water Process Eng.*, 2018, **26**, 116-123.
12. S. D. Richardson, A. D. Thruston, T. V. Caughran, P. H. Chen, T. W. Collette, T. L. Floyd, K. M. Schenck, B. W. Lykins, G. Sun, and G. Majetich, Identification of new ozone disinfection byproducts in drinking water, *Environ. Sci. Technol.*, 1999, **33(19)**, 3368-3377.
13. C. von Sonntag and U. von Gunten, Chemistry of Ozone in Water and Wastewater Treatment, *IWA Publishing*, 2012, London.
14. M. J. Farré, K. Döderer, L. Hearn, Y. Poussade, J. Keller, and W. Gernjak, Understanding the operational parameters affecting NDMA formation at advanced water treatment plants, *J. Hazard. Mater.*, 2011, **185(2-3)**, 1575-1581.
15. Y. Chuang and W.A. Mitch, Effect of ozonation and biological activated carbon treatment of wastewater effluents on formation of *N*-nitrosamines and halogenated disinfection byproducts, *Environ. Sci. Technol.*, 2017, **51**, 2329-2338.
16. M. Arnold, J. Batista, E. Dickenson, and D. Gerrity, Use of ozone-biofiltration for bulk organic removal and disinfection byproduct mitigation in potable reuse applications, *Chemosphere*, 2018, **202**, 228-237.
17. S. L. Kim, J. P. Chen, and Y. P. Ting, Study on feed pretreatment for membrane filtration of secondary effluent, *Sep. Purif. Technol.*, 2002, **29(2)**, 171-179.
18. C. O. Lee, K. J. Howe, and B. M. Thomson, Ozone and biofiltration as an alternative to reverse osmosis for removing PPCPs and micropollutants from treated wastewater, *Water Res.*, 2012, **46(4)**, 1005-1014.

19. CDPH, NDMA and Other Nitrosamines - Drinking Water Issues, California Department of Public Health, 2014, http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/NDMA.shtml (accessed August 2018).
20. C. M. Glover, O. Quinones, and E. R. V. Dickenson, Removal of perfluoroalkyl and polyfluoroalkyl substances in potable reuse systems. *Water Res.*, 2018, **144**, 454-461.
21. EPA, Integrated Risk Information System, United States Environmental Protection Agency, 2018, https://www.epa.gov/iris (accessed August 2018).
22. J. Choi and R. L. Valentine, Formation of *N*-nitrosodimethylamine (NDMA) from reaction of monochloramine: A new disinfection by-product, *Water Res.*, 2002, **36(4)**, 817-824.
23. W. A. Mitch and D. L. Sedlak, Formation of *N*-nitrosodimethylamine from dimethylamine during chlorination. *Environ. Sci. Technol.*, 2002, **36(4)**, 588-595.
24. D. Gerrity, A. N. Pisarenko, E. Marti, R. A. Trenholm, F. Gerringer, J. Reungoat, and E. Dickenson, Nitrosamines in pilot-scale and full-scale wastewater treatment plants with ozonation, *Water Res.*, 2015, **72**, 251-261.
25. C. Lee, C. Schmidt, J. Yoon, and U. von Gunten, Oxidation of N-nitrosodimethylamine (NDMA) precursors with ozone and chlorine dioxide: Kinetics and effect on NDMA formation potential, *Environ. Sci. Technol.*, 2007, **41(6)**, 2056-2063.
26. E. J. Marti, A. N. Pisarenko, J. R. Peller, and E. R. V. Dickenson, *N*-Nitrosodimethylamine (NDMA) formation from the ozonation of model compounds, *Water Res.*, 2015, **72**, 262-270.
27. S. Eden, S. B. Megdal, and J. McLain, Potable reuse of water: A view from Arizona, *Water Resour. Impact*, 2016, **18(4)**, 10-11.

587 28. D. M. Golea, A. Upton, P. Jarvis, G. Moore, S. Sutherland, S. A. Parsons, and S. J. Judd,
588 THM and HAA formation from NOM in raw and treated surface waters, *Water Res.*, 2017,
589 **112**, 226-235.
590 29. C. M. Sharpless and K. G. Linden, Experimental and model comparisons of H_2O_2 assisted
591 UV photodegradation of microcystin-LR in simulated drinking water, *Environ. Sci. Technol.*,
592 2003, **37(9)**, 1933-1940.
593 30. D. Fournier, J. Hawari, S. H. Streger, K. McClay, and P. B. Hatzinger, Biotransformation of
594 *N*-nitrosodimethylamine by *Pseudomonas mendocina* KR1, *Appl. Environ. Microbiol.*, 2006,
595 **72(10)**, 6693-6698.
596 31. D. Fournier, J. Hawari, A. Halasz, S. H. Streger, K. R. McClay, H. Masuda, and P. B.
597 Hatzinger, Aerobic biodegradation of *N*-nitrosodimethylamine by the propanotroph
598 *Rhodococcus ruber* ENV425, *Appl. Environ. Microbiol.*, 2009, **75(15)**, 5088-5093.
599 32. J. O. Sharp, T. K. Wood, and L. Alvarez-Cohen, Aerobic biodegradation of *N*-
600 nitrosodimethylamine (NDMA) by axenic bacterial strains, *Biotechnol. Bioeng.*, 2005, **89(5)**,
601 608-618.
602 33. J. O. Sharp, C. M. Sales, J. C. LeBlanc, J. Liu, T. K. Wood, L. D. Eltis, W. W. Mohn, and L.
603 Alvarez-Cohen, An inducible propane monooxygenase is responsible for N-
604 nitrosodimethylamine degradation by *Rhodococcus sp.* strain RHA1, *Appl. Environ.*
605 *Microbiol.*, 2007, **73(21)**, 6930-6938.
606 34. T. S. Webster, C. Condee, and P. B. Hatzinger, Ex situ treatment of N-nitrosodimethylamine
607 (NDMA) in groundwater using a fluidized bed reactor, *Water Res.*, 2013, **47(2)**, 811-820.

608 35. Q. Li, S. Yu, L. Li, Z. Gu, M. Liu, Z. Liu, Y. Ye, Q. Xia, and L. Ren, Microbial communities
609 shaped by treatment processes in a drinking water treatment plant and their contribution and
610 threat to drinking water safety, *Front. Microbiol.*, 2017, **8**, 2465.
611 36. D. Gerrity, M. Arnold, E. Dickenson, D. Moser, J. D. Sackett, and E. C. Wert, Microbial
612 community characterization of ozone-biofiltration systems in drinking water and potable
613 reuse applications, *Water Res.*, 2018, **135**, 207-219.
614 37. J. C. Holady, R. A. Trenholm, and S. A. Snyder, Use of automated solid-phase extraction and
615 GC-MS/MS to evaluate nitrosamines in water matrices, *Am. Lab.*, 2012, 6-13.
616 38. A. N. Pisarenko, E. J. Marti, E.J., D. Gerrity, J. R. Peller, and E. R. V. Dickenson, Effects of
617 molecular ozone and hydroxyl radical on formation of *N*-nitrosamines and perfluoroalkyl
618 acids during ozonation of treated wastewaters, *Environ. Sci. Water Res. Technol.*, 2015, **1(5)**,
619 668-678.
620 39. B. J. Vanderford, R. A. Pearson, D. J. Rexing, and S. A. Snyder, Analysis of endocrine
621 disruptors, pharmaceuticals and personal care products in water using liquid
622 chromatography/tandem mass spectrometry, *Anal. Chem.*, 2003, **75(22)**, 6265-6274.
623 40. B. J. Vanderford and S. A. Snyder, Analysis of pharmaceuticals in water by isotope dilution
624 liquid chromatography/tandem mass spectrometry, *Environ. Sci. Technol.*, 2006, **40(23)**,
625 7312-20.
626 41. T. D. Appleman, C. P. Higgins, O. Quiñones, B. J. Vanderford, C. Kolstad, J. C. Zeigler-
627 Holady, and E. R. V. Dickenson, Treatment of poly- and perfluoroalkyl substances in U.S.
628 full-scale water treatment systems, *Water Res.*, 2014, **41**, 246-255.
629 42. T. Zeng and W. A. Mitch, Contribution of *N*-nitrosamines and their precursors to domestic
630 sewage by greywaters and blackwaters, *Environ. Sci. Technol.*, 2015, **49(22)**, 13158-13167.

631 43. T. Zeng, C. M. Glover, E. J. Marti, G. C. Woods-Chabane, T. Karanfil, W. A. Mitch, and E.
632 R. V. Dickenson, Relative importance of different water categories as sources of N-
633 nitrosamine precursors, *Environ. Sci. Technol.*, 2016, **50**, 13239-13248.
634 44. O. D. Basu, S. Dhawan, and K. Black, Applications of biofiltration in drinking water
635 treatment–A review, *J. Chem. Technol. Biotechnol.*, 2016, **91(3)**, 585-595.
636 45. M. Gifford, A. Selvy, and D. Gerrity, Optimizing ozone-biofiltration systems for organic
637 carbon removal in potable reuse applications, *Ozone Sci. Eng.*, 2018, **40(6)**, 427-440.
638 46. S. Snyder, U. von Gunten, G. Amy, J. Debroux, and D. Gerrity, Use of ozone in water
639 reclamation for contaminant oxidation, Final report for WRF-08-05, *WateReuse Research*
640 *Foundation*, 2014, Alexandria, VA.
641 47. H. Wu and Y. F. Xie, Effects of EBCT and water temperature on HAA removal using BAC,
642 *J. Am. Water Works Assoc.*, 2005, **97(11)**, 94-101.
643 48. S. Korshunov and J. A. Imlay, Detection and quantification of superoxide formed within the
644 periplasm of *Escherichia coli*, *J. Bacteriol.*, 2006, **188**, 6326-6334.
645 49. J. A. Imlay, The molecular mechanisms and physiological consequences of oxidative stress:
646 Lessons from a model bacterium, *Nature Rev. Microbiol.*, 2013, **11(7)**, 443-454.
647 50. W. Wang, Y. Guo, Q. Yang, Y. Huang, C. Zhu, J. Fan, and F. Pan, Characterization of the
648 microbial community structure and nitrosamine-reducing isolates in drinking water biofilters,
649 *Sci. Tot. Environ.*, 2015, **521-522**, 219-225.
650 51. B. Trussell, S. Trussell, Y. Qu, F. Gerringer, S. Stanczak, T. Venezia, I. Monroy, F. Bacaro,
651 R. Trussell, A four-year simulation of soil aquifer treatment using columns filled with San
652 Gabriel Valley sand, *Water Res.*, 2018, **144**, 26-35.

653 52. J. M. Young, N. J. Rawlence, L. S. Weyrich, and A. Cooper, Limitations and
654 recommendations for successful DNA extraction from forensic soil samples: A review, *Sci.*
655 *Justice*, 2014, **54(3)**, 238-244.
656 53. J. L. Grönemeyer, P. Chimwamurombe, and B. Reinhold-Hurek, *Bradyrhizobium*
657 *subterraneum sp. nov.*, a symbiotic nitrogen-fixing bacterium from root nodules of
658 groundnuts, *Int. J. Syst. Evol. Microbiol.*, 2015, **65(10)**, 3241-3247.
659 54. M. Cappelletti, A. Presentato, G. Milazzo, R. J. Turner, S. Fedi, D. Frascari, and D. Zannoni,
660 Growth of *Rhodococcus sp.* strain BCP1 on gaseous n-alkanes: New metabolic insights and
661 transcriptional analysis of two soluble di-iron monooxygenase genes, *Front. Microbiol.*,
662 2015, **6**, 393.
663 55. W. A. Mitch and D. L. Sedlak, Characterization and fate of *N*-nitrosodimethylamine
664 precursors in municipal wastewater treatment plants, *Environ. Sci. Technol.*, 2004, **38**, 1445-
665 1454.
666 56. S. W. Krasner, W. A. Mitch, D. L. McCurry, D. Hanigan, and P. Westerhoff, Formation,
667 precursors, control, and occurrence of nitrosamines in drinking water: A review, *Water Res.*,
668 2013, **47(13)**, 4433-4450.
669 57. E. J. Marti, E. R. V. Dickenson, R. A. Trenholm, and J. R. Batista, Treatment of specific
670 NDMA precursors by biofiltration, *J. Am. Water Works Assoc.*, 2017, **109(6)**, E273-E286.
671 58. D. L. McCurry, A. N. Quay, and W. A. Mitch, Ozone promotes chloropicrin formation by
672 oxidizing amines to nitro compounds, *Environ. Sci. Technol.*, 2016, **50(3)**, 1209-1217.
673 59. Y. Lee, D. Gerrity, M. Lee, S. Gamage, A. Pisarenko, R. A. Trenholm, S. Canonica, S. A.
674 Snyder, and U. von Gunten, Organic contaminant abatement in reclaimed water by UV/H_2O_2
675 and a combined process consisting of O_3/H_2O_2 followed by UV/H_2O_2: Prediction of

abatement efficiency, energy consumption, and byproduct formation, *Environ. Sci. Technol.*, 2016, **50(7)**, 3809-3819.

60. Z. Bukhari, L. Weinrich, M. Surmeier, and R. M. Vega, Impact of Filtration Media Type/Age on Nitrosamine Precursors, Final Report for Project 4532, *Water Research Foundation*, 2017, Denver, CO.
61. X. Liao, C. Chen, B. Yuan, J. Wang, and X. Zhang, Control of nitrosamines, THMs, and HAAs in heavily impacted water with O_3-BAC, *J. Am. Water Works Assoc.*, 2017, **109(6)**, 215-225.
62. D. Gerrity, Y. Lee, S. Gamage, M. Lee, A. N. Pisarenko, R. A. Trenholm, U. von Gunten, and S. A. Snyder, Prediction of trace organic contaminant abatement with UV/H_2O_2: Development and validation of semi-empirical models for municipal wastewater effluents, *Environ. Sci. Water Res. Technol.* 2016, **2**, 460-473.
63. P. C. To, B. J. Mariñas, V. L. Snoeyink, and J. N. Wun, Effect of pore-blocking background compounds on the kinetics of trace organic contaminant desorption from activated carbon, *Environ. Sci. Technol.*, 2008, **42(13)**, 4825-4830.
64. C. J. Corwin and R. S. Summers, Adsorption and desorption of trace organic contaminants from granular activated carbon adsorbers after intermittent loading and throughout backwash cycles, *Water Res.*, 2011, **45(2)**, 417-426.
65. K. E. Greenstein, J. Lew, E. R. V. Dickenson, and E. C. Wert, Investigation of biotransformation, sorption, and desorption of multiple chemical contaminants in pilot-scale drinking water biofilters, *Chemosphere*, 2018, **200**, 248-256.
66. E. F. Houtz and D. L. Sedlak, Oxidative conversion as a means of detecting precursors of perfluorolkyl acids in urban runoff, *Environ. Sci. Technol.*, 2012, **46(17)**, 9342-9349.

699 67. EPA, Fact Sheet PFOA & PFOS Drinking Water Health Advisories, United States
700 Environmental Protection Agency, 2016, https://www.epa.gov/sites/production/files/2016-
701 06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf (accessed
702 August 2018).
703 68. DDW, State Water Board Releases Guidelines for Testing and Reporting on PFOA and
704 PFOS in Drinking Water, California Division of Drinking Water, 2018,
705 https://www.waterboards.ca.gov/press_room/press_releases/2018/pr071318_pfoa_nl.pdf
706 (accessed August 2018).
707 69. M. Inyang and E. R. V. Dickenson, The use of carbon adsorbents for the removal of
708 perfluoroalkyl acids from potable reuse systems, *Chemosphere*, 2017, **184**, 168-175.
709 70. T. D. Appleman, E. R. V. Dickenson, C. Bellona, and C. P. Higgins, Nanofiltration and
710 granular activated carbon treatment of perfluoroalkyl acids, *J. Hazard. Mater.*, 2013, **260**,
711 740-746.
712 71. P. McCleaf, S. Englund, A. Östlund, K. Lindegren, K. Wiberg, and L. Ahrens, Removal
713 efficiency of multiple poly- and perfluoroalkyl substances (PFASs) in drinking water using
714 granular activated carbon (GAC) and anion exchange (AE) column tests, *Water Res.*, 2017,
715 **120**, 77–87.
716 72. C. Zhao, J. Zhang, G. He, T. Wang, D. Hou, and Z. Luan, Perfluorooctane sulfonate removal
717 by nanofiltration membrane the role of calcium ions, *Chem. Eng. J.*, 2013, **233**, 224-232.

Figure 1. TOC removal as a function of EBCT with a constant O_3/TOC of 1.0. Data represent averages ±1 standard deviation of duplicate experimental samples. The corresponding raw data are summarized in Table S1.

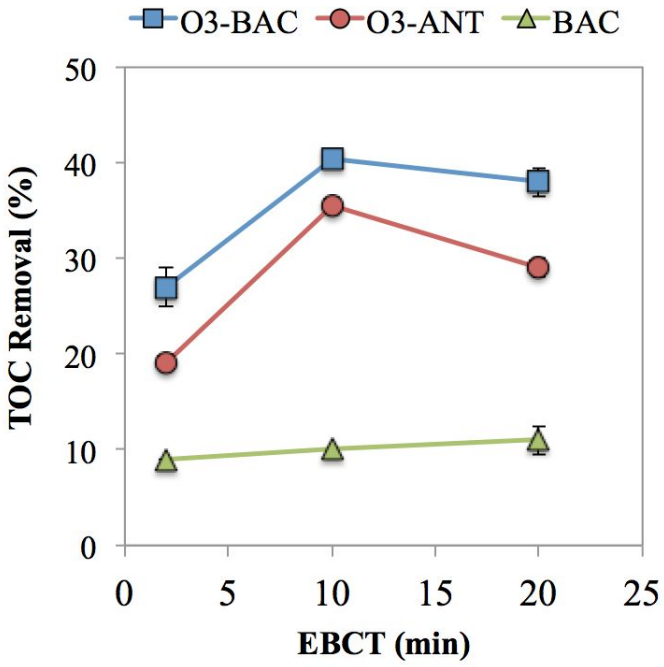

Figure 2. Literature summary of NDMA formation during ozonation and chloramination of six secondary effluents.[5,46] The first column in each group indicates the ambient NDMA concentration of each secondary effluent. The second and third columns represent the NDMA concentrations after ozonation with O_3/TOC of 0.5 and 1.0. The fourth through sixth columns represent those same samples after 10 days of chloramines exposure with an initial dose of 140 mg/L as Cl_2. The average NDMA formation during ozonation (left side of solid lines at top of figure) and the average reduction in NDMA formation following ozonation and chloramination (right side of solid lines at top of figure) are also shown for each secondary effluent.

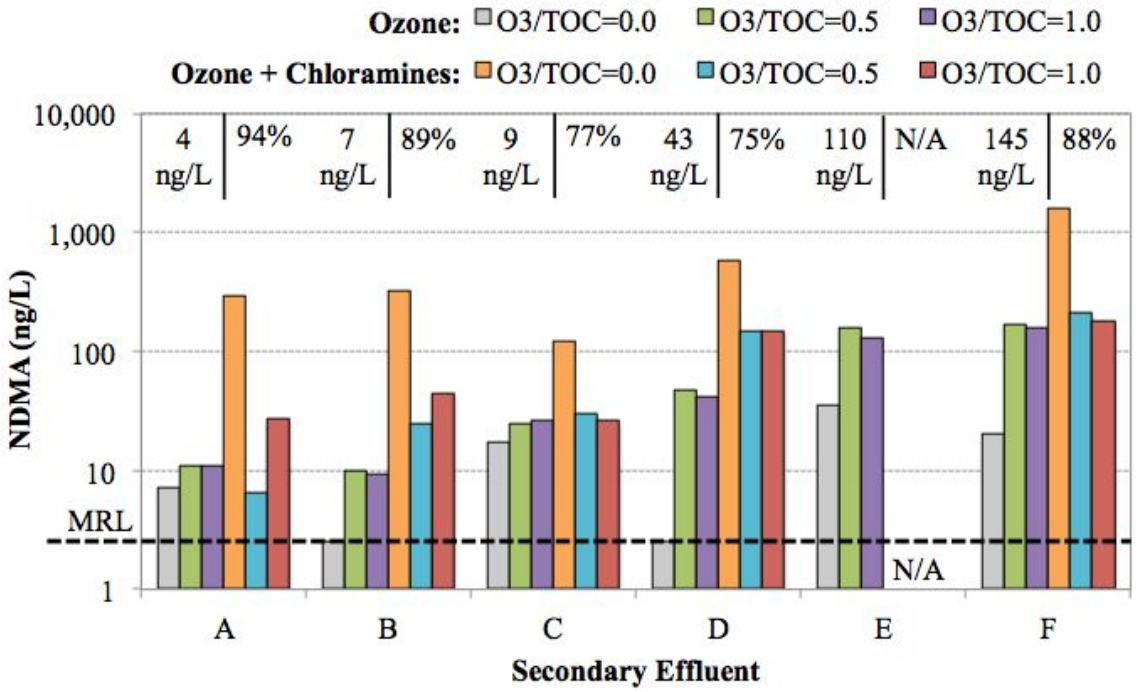

Figure 3. NDMA removal as a function of EBCT with a constant O_3/TOC of 1.0 and NDMA spiked at ~300 ng/L. Data represent averages ±1 standard deviation of duplicate experimental samples.

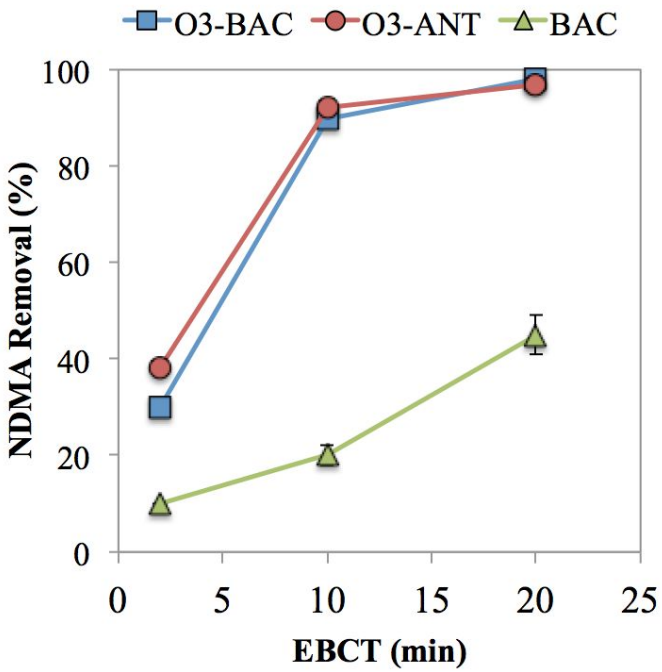

Figure 4. NDMA removal as a function of pretreatment with a constant O_3/TOC of 1.4, EBCT of 10 min, and NDMA spiked at ~300 ng/L. Pre-ozonation = high BDOC/high DO; pre-oxygenation = low BDOC/high DO; MBR filtrate = low BDOC/low DOC. Data represent averages ±1 standard deviation of triplicate experimental samples.

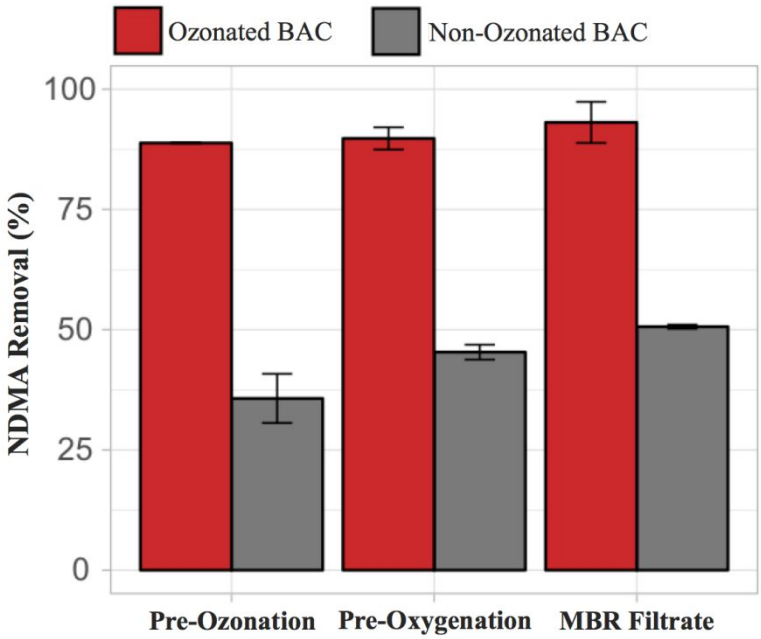

Figure 5. Abundance of the 16S rRNA gene and a subset of the monooxygenase genes that have been linked to NDMA biodegradation. Data represent averages ±1 standard deviation of triplicate qPCR assays for a single DNA extract from each ozonated anthracite media sample. The ozone residual in the feed water had decayed completely prior to reaching the anthracite biofilter.

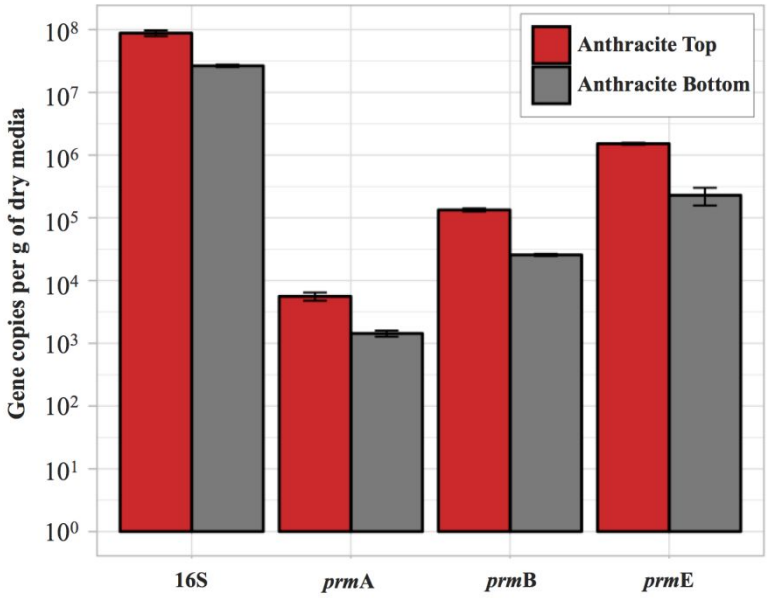

Figure 6. NDMA formation after chloramination with the UFC approach. Data represent averages ±1 standard deviation of duplicate chloraminated samples.

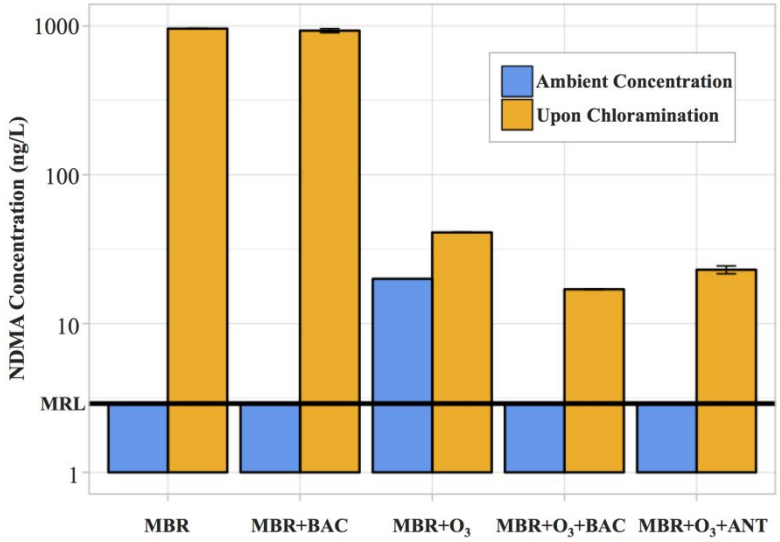

Table 1. 16S rRNA and propane monooxygenase gene targets and associated primers.

Gene	Sequence (5' to 3')	Reference
prmA – f	CGCGGCGAACATCTACCT	33
prmA – r	TGGCTACGAACAGGGTGTTG	33
prmB – f	GGACGAGGATTGACGGATTTC	33
prmB – r	CGGCGGGTCCATCGAT	33
prmE – f	GGAACTACTACGTCGTCGGG	33
prmE – r	GAGCCGACGAGATTTCCGAT	33
16S rRNA – 28F	GAGTTTGATCNTGGCTCAG	36
16S rRNA – 388R	TGCTGCCTCCCGTAGGAGT	36

Table 2. Summary of weekly water quality monitoring. Data represent averages ±1 standard deviation over 17 weeks.

	UVA_{254} cm^{-1}	**PO_4^{3-} mg/L**	**NO_3^- mg-N/L**	**NO_2^- mg-N/L**	**NH_3 mg-N/L**	**TOC mg-C/L**	**TOC removal[1] %**
MBR	0.14±0.01	5.9±1.1	6.7±1.9	0.025±0.009	0.01±0.02	7.5±0.5	N/A
MBR+O_3	0.07±0.02	5.5±1.5	5.6±2.0	<0.005	0.01±0.01	7.1±1.1	5±1
MBR+O_3+BAC	0.07±0.02	5.8±1.2	5.2±1.2	<0.005	0.02±0.02	5.1±0.9	22±5
MBR+O_3+Ant	0.07±0.01	5.3±1.6	5.6±2.0	0.017±0.008	0.02±0.02	6.2±0.5	15±7
MBR+BAC	0.13±0.01	5.1±1.3	5.7±2.0	0.007±0.003	<0.02	6.3±0.3	14±5

[1]Represents an average of all TOC removal percentages during the study period

Table 3. Summary of trace organic compounds concentrations (ng/L). Ozone was applied at an O_3/TOC of 1.3, and the BAC biofilters were operated with an EBCT of 10 min.

Compound	MBR	MBR+BAC	MBR+O_3	MBR+O_3+BAC
TOrCs (listed alphabetically)				
Acetaminophen	<5	<5	<5	<5
Atenolol	53	160	<20	<20
Caffeine	<5	<100	<5	<100
Carbamazepine	150	220	<1	3
DEET	59	58	3	7
Fluoxetine	74	32	<1	<1
Gemfibrozil	3	16	<1	<1
Ibuprofen	3	3	<1	<1
Meprobamate	480	490	71	79
Naproxen	34	120	<1	<1
Primidone	300	390	13	16
Sucralose	51,000	61,000	19,000	21,000
Sulfamethoxazole	1,400	2,900	<5	<5
TCEP	150	280	190	270
Triclocarban	43	<2	<2	<2
Triclosan	35	24	<1	<1
Trimethoprim	60	72	<1	<1
PFAAs (listed in descending order of carbon chain length)				
PFDS	<1	<1	<1	<1
PFDA	4	<1	5	3
PFNA	1	1	2	1
PFOS	1	1	1	1
PFOA	22	21	22	20
PFHpA	3	23	5	5
PFHxS	<1	<1	<1	<1
PFHxA	27	22	31	33
PFPeA	48	39	47	47
PFBS	4	45	10	10
PFBA	<5	5	7	7

N-nitrosodimethylamine (NDMA) Formation and Mitigation in Potable Reuse Treatment Trains Employing Ozone and Biofiltration

Table of Contents Entry

This paper evaluates how changes in operational conditions affect NDMA formation and biodegradation in ozone-biofiltration systems, including aspects of microbial community structure and function.