Effect of advanced oxidation on *N*-nitrosodimethylamine (NDMA)
formation and microbial ecology during pilot-scale biological activated
carbon filtration

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Abstract

Water treatment combining advanced oxidative processes with subsequent exposure to biological activated carbon (BAC) holds promise for the attenuation of recalcitrant pollutants. Here we contrast oxidation and subsequent biofiltration of treated wastewater effluent employing either ozone or UV/H₂O₂ followed by BAC during pilot-scale implementation. Both treatment trains largely met target water quality goals by facilitating the removal of a suite of trace organics and bulk water parameters. *N*-nitrosodimethylamine (NDMA) formation was observed in ozone fed BAC columns during biofiltration and to a lesser extent in UV/H₂O₂ fed columns and was most pronounced at 20 min of empty bed contact time (EBCT) when compared to shorter EBCTs evaluated. While microbial populations were highly similar in the upper reaches, deeper samples revealed a divergence within and between BAC filtration systems where EBCT was identified to be a significant environmental predictor for shifts in microbial populations. The abundance of *Nitrospira* in the top samples of both columns provides an explanation for the oxidation of nitrite and corresponding increases in nitrate concentrations during BAC transit and support interplay between nitrogen cycling with nitrosamine formation. The results of this study demonstrate that pretreatments using ozone versus UV/H₂O₂ impart modest differences to the overall BAC microbial population structural and functional attributes, and further highlight the need to evaluate NDMA formation prior to full-scale implementation of BAC in potable reuse applications.

Keywords: Biological activated carbon; Advanced oxidation; *N*-nitrosodimethylamine; Biofiltration; Indirect potable reuse

Introduction

As continued population growth, increasing urban density, and varying climate place heavy burdens on our nation's water supplies, water agencies and policy makers are examining innovative ways to enhance reliability and redundancy in their supply portfolio. As such, water agencies throughout the United States and beyond have been turning to potable reuse of municipal wastewater, either directly or indirectly, to help meet growing demands and/or water shortages. Advances in the engineering and application of water reuse technologies combined with more than 50 years of experience in groundwater recharge of recycled water effluents makes planned indirect potable reuse (IPR) a feasible scenario for utilities (USEPA, 2012). Potable reuse schemes currently in operation or in planning phases often employ reverse osmosis (RO) membranes and advanced oxidation processes (AOPs) to ensure maximum contaminant removal and salinity reduction (Trussell et al., 2012). However, the use of RO-based technology can be expensive, energy intensive, and requires the disposal of a concentrated brine stream (Plumlee et al., 2014). In cases where salinity removal is not the driver for RO membrane use, non-salinity reducing options such as combinations of ozone with biological activated carbon (BAC) and other treatment technologies may provide an alternative approach to potable reuse provided they can produce a water that meets public health and regulatory standards (Gerrity and Snyder, 2011; Gerrity et al., 2014).

Treatment of secondary wastewater with ozone is an effective disinfection process and known to attenuate many commonly-occurring trace organic contaminants (Dickenson et al., 2009; Pisarenko et al., 2012; Snyder et al., 2006; Wert et al., 2009a and 2009b). While not universally regulated, these contaminants which include pharmaceuticals, personal care products, household chemicals, endocrine disruptors, pesticides, and disinfection by-products

have been extensively reported and create large concerns due to their ubiquity and potential adverse impacts on human and aquatic health (Benotti et al., 2009; Schwarzenbach et al., 2006). AOPs such as ozone (O₃), ultraviolet radiation (UV) with hydrogen peroxide (H₂O₂) and combinations therein form highly reactive aqueous species such as non-selective hydroxyl radicals that are capable of oxidizing a wide range of these otherwise persistent pollutants (Esplugas et al., 2007; Hübner et al., 2012) and conversion to more bioavailable products for microbial metabolism. While efficient for the removal of several trace organic contaminants, potential problems have been identified related to ozone treatment. Ozone treatment of wastewater can lead to the formation of ozonation byproducts such as ketoacids, aldehydes and carboxylic acids (Wert et al., 2007), the formation of bromate which is regulated in drinking water at a 10 µg/L maximum contaminant level (MCL), and increases in the fraction of assimilable organic carbon (Hammes et al., 2006). Furthermore, observations of direct formation of *N*-nitrosodimethylamine (NDMA) and other nitrosamines during ozone or ozone/peroxide treatment of secondary wastewater have been reported in the literature (Andrzejewski et al., 2008; Pisarenko et al., 2012; Gerrity et al., 2015; Pisarenko et al., 2015). In some cases, ozonation of tertiary treated wastewater in a water reuse pilot-scale system led to the net formation of tens to hundreds of ng/L of NDMA (Stanford et al., 2014; Gerrity et al., 2015), with approximately 50% of this NDMA subsequently passing through RO treatment. NDMA formation from ozone/advanced oxidation (AOP) treatment has also been reported during full scale implementation, with both ozonation and ozone/hydrogen peroxide AOP resulting in the formation of approximately 15 ng/L of NDMA (Zimmermann et al., 2011, Hollender et al., 2009).

Biofiltration, wherein an absence of chemical disinfectant residual encourages biomass

growth on the filter media, is emerging as an effective post-AOP polishing step for the removal of trace organic contaminants and assimilable organics liberated during advanced oxidation to reduce baseline toxicity (Gerrity and Snyder, 2011; Reungoat et al., 2010 and 2012). Specifically, biofiltration using biological activated carbon (BAC) has been shown to provide excellent removal of NDMA in recent studies (Farre et al., 2011; Hollender et al., 2009; Reungoat et al., 2010; Sundaram et al., 2014) and is consistent with microbial oxidation of this recalcitrant nitrosamine (Homme et al., 2013; Sharp et al., 2005, 2007 and 2010). The high surface area of BAC and ability to sequester bioavailable substrates lends itself to colonization by a diverse microbial biofilm that can further contribute to water treatment through the metabolism of nutrients and organics. Biofilm formation, growth control, component composition, activity measurement, and chemical removal have been previously studied in BAC (Magic-Knezev and van der Kooij, 2004; Simpson, 2008), and individual bacterial species with desired degradation capabilities have been isolated or identified from BAC samples (Zhou et al., 2011). A more intimate knowledge of microbial phylogenetic composition and selective biases in these systems could enhance functional understanding of BAC treatment perhaps informing the selection of desirable candidate populations, design and management practices. While studies have begun to explore the effects of upstream water properties on the phylogenetic structure of microbial populations in BAC systems (Mikkelsen et al., 2015), the effects of AOPs remain largely unknown and appear limited to BAC systems following ozonation (Kasuga et al., 2007; Liao et al., 2012, 2013a and 2013b). Ozone and UV/H₂O₂ both utilize hydroxyl radicals to break/alter chemical structures of parent organic compounds including persistent pollutants. Although oxidization mechanisms of these AOPs are very similar, it is not clear whether different biodegradable

intermediates will shift microbial populations in the subsequent BAC process, which could result in significant differences in microbial community functional outcomes with respect to downstream water quality.

Despite these uncertainties, the potential for biofiltration as a post-AOP polishing step is promising, and further research is continuing in order to advance its implementation in IPR scenarios and to determine its role in achieving water quality goals along with consistent, reliable, and manageable operation. To this end, a year-long pilot study was conducted on secondary effluent from a municipally-fed PureOx wastewater treatment plant to evaluate parallel treatment trains of combinations of ion exchange with ozone or UV/H₂O₂ both followed by BAC as an alternative to RO membrane-based treatment for brackish aquifer recharge at a wastewater utility in the Southeastern United States. Water within the aquifer was subsequently utilized as the source for abstraction and further treatment by RO or nanofiltration membranes for drinking water production.

Because of the potential for ozone to form NDMA in recycled water matrices and the known ability of BAC to help manage any NDMA that has formed (Farre et al., 2011; Hollender et al., 2009; Reungoat et al., 2010; Sundaram et al., 2014), it was hypothesized that even with potential ozone-mediated formation of NDMA, the contaminant would be managed to below 10 ng/L through BAC. However, this was not the case for either the ozone-BAC or the UV/H₂O₂-BAC combinations. This manuscript provides evidence of the first known observations of consistent, sustained NDMA formation in bulk water samples (i.e., not chlorinated or chloraminated or using any formation potential tests) during biofiltration and offers insights into possible control strategies. Furthermore, this study reveals the possible influence of AOPs on microbial community structure in BAC biofiltration process while

enhancing our broader understanding of microbial community structure across functionally and geographically distinct biofiltration systems.

Materials and Methods

Experimental set-up

The year-long study was conducted at a wastewater treatment plant with a 24 MGD (91 MLD) pure oxygen biological treatment process resulting in limited nitrification, with chlorine disinfection typically discharged to an outfall for secondary effluent disposal. Influent was derived from a municipal collection system with little-to-no industrial influence. The plant also has the capacity to produce 8 MGD of non-potable recycled water using tertiary sand filtration and chlorine disinfection. The tertiary wastewater effluent characteristics are shown in Table S1.

The pilot plant was situated on site at the wastewater plant and fed with effluent from the secondary clarifiers and was operated for a period of 11 months (schemes shown in Figure 1). A Tetra ® Deep Bed Filter (Severn Trent Water Purification, Inc., Horsham, PA, USA) with a 1.9 m² Filtopack II and 1.8 m of Tetra #5 high-density silica sand media was used to simulate tertiary treatment of the full-scale facility. This was followed in series by an anion exchange resin (Thermax A-72 MP, Thermax, Inc., Novi, MI, USA) targeting TOC removal and a cation exchange resin (Zeosand – Clinoptilolite, Zeo, Inc., McKinney, TX) targeting ammonia removal. The UV and ozone trailers were manufactured and supplied by Xylem, Inc. (Rye Brook, NY) with a low pressure high output UV System from WEDECO (a Xylem Brand) designed for a minimum of 400 mJ/cm² designed UV dose at 70% UVT and a WEDECO ozone generator model GSO30 with a design dose range of 1.5 mg O₃/L to 15 mg

O₃/L. The BAC Filter pilot was built by Burt Process Equipment (Hamden, CT, USA) with 2 filter cells in series per train with 90 inches of Cabot Norit Activated Carbon (Hydrosarco 3000, Cabot Corporation, Alpharetta, GA, USA) over top of 30.5 cm of torpedo sand. Virgin carbon was initially used in this setup, thus it was assumed that the pilot plant operated during three major phases of GAC life including sorption-dominated, combined biological and sorptive processes, and biologically dominated. Table S2 provides a summary of the major operational parameters for each process.

All water quality analyses, including sample collection and all quality assurance/quality control (QA/QC) procedures, including periodic duplicate samples were conducted by Test America (NY, NY, USA) according to their standard methods. Samples were collected from secondary effluent (pilot influent) and then deep bed filtration, ion exchange (both units), UV/H₂O₂, and BAC on 39 different sampling events between January 2013 and November 2013 and included over 200 discrete analytes including bulk water quality parameters, trace organic contaminants, and disinfection byproducts such as NDMA. The bulk water quality data associated with the influents and effluents of the two BAC systems are shown in Table 1. Two additional composite and/or grab samples were specifically collected for supplemental nitrosamine analysis in August 2013 and October 2013 (i.e., after 7 months and 9 months of operation).

Batch studies were conducted using BAC filter media harvested from a filter depth equating to approximately 4 minutes of empty bed contact time (EBCT) and at 18 minutes of EBCT. Media were re-suspended in column influent (ozonated ion exchange effluent) to achieve a final volume of 1 L. External substrates were added to the tests to achieve conditions suitable for mimicking the occurrence of nitrification and denitrification. Aerobic

batch incubations were supplemented with 10 mg/L $\text{NH}_3\text{-N}$ to stimulate nitrification and soluble nutrients, TOC, nitrate, nitrite, and DO were monitored every 15 minutes for 75 mins. Anoxic incubations were supplemented with 100 mg acetic acid as COD/L and 10 mg $\text{NO}_3\text{-N/L}$ to stimulate denitrification and soluble nutrients, and TOC, nitrite, nitrate, and DO were similarly monitored for 120 mins. Controls (biological activity repressed with the addition of 20 mg/L copper chloride) were also included under both aerobic and anoxic conditions. NDMA was measured for each batch experiment using EPA Method 521 at Test America's laboratories at the beginning and end of the experiment and no chlorine or chloramines were added. Nitrogen, TOC and NDMA transformation and/or removal rates were measured using protocols described elsewhere (Khunjar et al., 2014).

The two BAC filtration systems had been operated stably for approximately nine months before BAC samples were taken for microbial analysis. From each BAC column set-up, 5-6 BAC samples were taken from the depths of 0-0.3, 0.6, 3, 3.6, and 6 m (corresponding to EBCT of 0-1, 2, 10, 12, 20 min). Samples were frozen at $-20\text{ }^{\circ}\text{C}$ until DNA extraction.

DNA extraction, sequencing and real-time PCR

Extraction and sequencing were in accordance with prior methods (Li et al., 2013). In brief, triplicate DNA extractions were performed for each BAC sample using the PowerSoil kit (MO BIO laboratories, Carlsbad, CA). For each DNA sample, the 16S rRNA gene was amplified using the 515F forward primer (5'-GTGYCAGCMGCCGCGGTAA-3') and the 806R reverse primer (5'-CCGGACTACHVGGGTWTCTAAT-3') which are universal for nearly all bacterial and archaeal taxa (Caporaso et al., 2011). A 12-bp error-correcting Golay barcode was inserted to the reverse primer to identify unique PCR products. Triplicate

amplification products were pooled before purification with Agencourt AMPure XP magnetic microbeads and quantified on a Life Sciences Qubit 2.0 Fluorometer (Thermo Scientific). Products were normalized, mixed, and sequenced on the Illumina MiSeq platform using NEBNext Ultra DNA Library Prep and MiSeq Reagent v2 2x250 500 cycle kits (Illumina, San Diego, CA) in BioFrontiers Institute, University of Colorado Boulder.

Paired end reads were assembled with fastq-join command (<https://github.com/ExpressionAnalysis/ea-utils>) with a minimum of 100 bp overlap. 16S rRNA gene sequences were demultiplexed and processed using the Quantitative Insights Into Microbial Ecology (QIIME v1.8.0) pipeline with default settings (Caporaso et al., 2010). The minimum sequence length was set to 200 base pairs (bp). Sequences were grouped into operational taxonomic units (OTUs) at 97% sequence similarity. Representative sequences for each OTU were picked and taxonomic data assigned using the RDP classifier (Wang et al., 2007). Possible chimeric sequences were checked with the ChimeraSlayer wrapper in QIIME (Haas et al., 2011). The Shannon diversity index was also calculated in QIIME. In total, 150,979 sequences were obtained for the 11 BAC samples from the two BAC systems and archived in MG-RAST (Meyer et al., 2008) with the public accession number as 4636830.3. Most of these sequences were affiliated with Bacteria, with only a very small fraction belonged to Archaea (0.05%-0.58%).

The quantification of denitrification genes *nirS* and *nirK* by using real-time PCR has been described before (Henry et al., 2004 and 2006). The copy numbers of *nirS* and *nirK* have been normalized to gDNA representing microbial biomass determined by a Life Sciences Qubit 2.0 Fluorometer (Thermo Scientific) (Table S3).

The weighted UniFrac distance matrix generated in QIIME was used for Principal Coordinate Analysis (PCoA) with PRIMER 6 (Clarke and Gorley, 2006). Microbial taxonomic groups significantly associated to particular samples were determined with the LEfSe (the linear discriminant analysis effect size) algorithm that employs the non-parametric factorial Kruskal-Wallis test followed by the post-hoc Linear Discriminant Analysis (Segata et al., 2011). Environmental predictor variables for microbial community compositions in both BAC treatments were identified using BEST and DistLM (Distance based linear models) tests of PRIMER 6. BEST and DistLM tests are designed to find the best match between the multivariate among-sample patterns of an assemblage and that from environmental variables associated with those samples, where the extent to which these two patterns match reflects the degree to which the chosen abiotic factor data explains the biotic pattern. Additional statistical analyses such as Spearman rank correlation coefficient, Mann–Whitney *U* test, Kendall's *W* test, Kruskal-Wallis *H* test, ANOSIM, and PERMANOVA were performed using the SPSS package (version 16.0) or PRIMER 6. Significance was reported for *P* values less than 0.05.

Results and Discussion

Contaminant profiles

As shown in Table 1, comparison of BAC influent and effluent water quality revealed similarities in classical indicators of water quality such as TOC, TSS, and nitrate despite different upstream AOP processes (UV/H₂O₂ vs. ozone). The incorporation of BAC into the treatment train increased the concentration of nitrate while it decreased the concentrations of

COD, TOC, NH_4^+ , nitrite, and TOX, suggesting coupled biological and sorptive processes. Phosphate, sulfate, chloride, conductivity, TDS, total nitrogen, BOD_5 and pH appeared static. BAC filtration had similar advantages for the removal of most trace organic contaminants with outcomes that appeared independent of upstream AOP (Table S4) with the notable exception of NDMA (Figure 2), though much of the observed removal was likely due to the remaining sorptive capacity of the GAC as it aged and converted to fully biological BAC. While not reported in the table, specific UV absorbance (SUVA) values in the BAC effluent were similar across all treatment trains (2.6 – 3.9 L/mg-m). However, ozonated water entering the BAC system had lower SUVA values (2.4–3.2 L/mg-m for ozone:TOC ratios of 1:1 and 2:1, respectively) resulting in a net increase of 21% - 28% during BAC transit. In contrast, UV/AOP SUVA values were higher, ranging from 3.2 to 4.8 L/mg-m across multiple measurements at varying UV doses of 400 mJ/cm^2 to 1000 mJ/cm^2 resulting in a net decrease in SUVA of 17% to 33%.

The use of ozone in the pilot feed water was expected to result in an increase in NDMA concentration across the ozone unit (Andrzejewski et al., 2008; Pisarenko et al., 2012), but a subsequent reduction of NDMA was expected after transit through BAC (Gerrity et al. 2015). In contrast, NDMA formation was not expected with the UV/ H_2O_2 and BAC treatment train. Our findings contradicted these assumptions during the 11 months of pilot testing with increases across these systems. Specifically, after several months of operation, NDMA concentrations increased within both BAC columns (Figure 2). Toward the latter part of pilot testing, NDMA concentrations increased across the BAC filter in the ozone-pretreated column and remained relatively similar between influent and effluent samples in the UV/ H_2O_2 treated column. The observation of NDMA formation in the ozone and UV/ H_2O_2

fed columns indicate that the microbial community and/or water quality conditions in the BAC columns were causing a net formation of NDMA.

To investigate this further, aqueous pore-water samples were taken to profile NDMA concentrations across the ozone-fed BAC columns at multiple EBCTs at 6-months into the piloting period. Pore water was analyzed for NDMA, TOC, ammonia, nitrite, and nitrate data; in situ dissolved oxygen data were not available during the sampling period. As shown in Table 2, NDMA formation decreased to below detection limits during the first 4–14 minutes (EBCT) of transit. However, there was an increase above influent concentrations by 20 minutes of EBCT suggesting NDMA formation during longer transit in the ozone-fed BAC column.

Batch experiments using harvested BAC

During the same sampling period (July which was approximately 5 months into pilot system operation), samples of BAC media were collected for batch studies as described previously (Khunjar et al., 2014) to explore the link between biological activity and NDMA fate. Samples of column media were collected from locations with EBCT of approximately 2 minutes and 18 minutes, which was in the upper region of each of the two columns in series.

Results from batch testing under aerobic and anoxic conditions revealed that nitrifier activity as measured by accumulation of nitrate/nitrite was high throughout both column cells (Figure 3a). Despite presumed DO penetration into the second column cell (>10 min EBCT), assayed denitrification activity was observed to increase relative to the first cell. Interestingly, no increase in the abundance of denitrification genes *nirS* and *nirK* was observed from the harvested BAC in the region of question (Figure S1). Cumulatively, this finding indicates that

denitrifiers were able to survive but not proliferate in regions with high DO penetration. Simultaneous nitrification and denitrification occurred throughout the columns; however, it is important to note that nitrifier activity exceeded denitrifier activity in both columns (Figure 3a). As a result, products from nitrification accumulated in the bulk liquid. This finding is corroborated by the presence of nitrate in the BAC effluent (Table 1).

The role of nitrogen metabolism in the formation of NDMA was also investigated. NDMA was produced during anoxic incubations containing biomass with high denitrifier potential (Figure 3a and b). In contrast, NDMA production and/or removal was limited in oxic microcosms. The results imply that NDMA formation in BAC may be linked to the occurrence of denitrification as has been reported by other researchers (Kaplan et al., 1983; Kunisaki and Hayashi 1979, Mills and Alexander, 1976). While the mechanism of NDMA formation was not elucidated in this study, it could be due to denitrifying bacteria catalyzing the nitrosation reaction analogous to those that occur in the human gut (Leach et al., 1987). Nitrous oxide (NO) produced by denitrifiers can react with oxygen and superoxide (O_2^-) to form peroxynitrite ($ONOO^-$), which can nitrosate amines to form NDMA (Grady et al., 1999; Masuda et al., 2000; Soltermann, et al., 2013; Uppu et al., 2000).

Microbial community profile and spatial trends

The overall microbial phylogenetic distribution of BAC samples revealed trends within and across systems (Figure 4). Despite differences in upstream AOP treatment, microbial composition of BAC samples were similar (Mann–Whitney U test, $P=0.442$) when contrasting the upper portions (EBCT of 0-1 min) of the flow-through columns. This early region was dominated by phyla and super-classes including *Nitrospirae*, *Bacteroidetes*,

Planctomycetes, *Chloroflexi*, and *Firmicutes*, as well as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Acidobacteria* which have been also identified in previous studies as major microbial groups in BAC biofiltration systems with ozonation pretreatment when using groundwater or lake water as source water (Li et al., 2010; Liao et al., 2012, 2013a and 2013b). Microbial composition was however found to diverge with depth (Kendall's W test, both $P < 0.001$) (Figure 5). Principal Coordinate Analysis (PCoA) indicated that microbial community structure within the samples from the upper portions (EBCT of both 1 and 2 min) were more similar between ozone and UV/H₂O₂ BAC columns when contrasted with deeper samples having EBCT over 10 min. The UV/H₂O₂ followed by EBCT of 20 min was not included in PCoA analysis due to the abnormal microbial community structure containing predominant uncultivated OD1 phylum (as shown in Figure 4). OD1 bacteria generally contain small genomes and are widely spread in groundwater and sediments (Luef et al., 2015).

Community diversity assessed by Shannon indices revealed similar microbial diversity in both BAC columns regardless of upstream differences in AOP. Interestingly, the highest population diversities were in the top samples (Fig S2) where a significant decrease with depth was found in both columns (Kruskal-Wallis H Test, both $P < 0.001$). Similar phenomena were observed in a previous pilot-scale up-flow BAC filtration system treating lake water with pre-ozonation, in which a marked decrease of microbial diversity in the BAC filtration system was observed along the flow path (Liao et al., 2012) and a lab scale BAC filtration system (Mikkelsen et al., 2015). In contrast, a converse trend with increasing diversity during biofiltration transit has been observed in sand sediment columns exposed to surface and wastewaters (Li et al., 2012 and 2013). The distinctly different water qualities

between AOP treated water and these other waters as well as geomeia provides possible explanations for this discrepancy.

Through a more detailed analysis of specific microbial clades, microbial genera colonizing the two BAC set-ups were similar with some modest shifts in relative abundance (Figure 6). *Nitrospira*, which are important nitrite-oxidizing microorganisms, were highly abundant in both set-ups with an overwhelming dominance in the top samples of both columns (accounting for 14.3% and 20.2% of total population in the top of the ozone and UV/H₂O₂ BAC columns respectively). This phylogenic profile is consistent with nitrate formation during BAC transit as described in the prior section. Analogously, *Nitrospira* were found in the top layers (0.3 m below the surface) of two BAC columns that received ozone treated water (accounting for 3.4% and 5.3% of total microbial population) in a previous study (Liao et al., 2013b) and were dominant (5.1% of total microbial population) in the top layer of a BAC column receiving tap water (Mikkelsen et al., 2015). *Planctomyces* species belong to the phylum *Planctomycetes*, which hosts guilds capable of anammox metabolism (Kartal et al., 2013), were abundant in the top samples of both columns (3.4% and 1.0%, respectively), suggesting a potential interplay with *Nitrospira*. This association was seen in a prior study of ozone followed by BAC mentioned above with a relative abundance of 1.2% and 2.1% *Planctomycetes* respectively (Liao et al., 2013b). These results in conjunction with nitrogen species profiles point to the importance of nitrogen cycling in BAC systems (Tables 1 and 2).

Most of the other abundant bacterial genera in both BAC set-ups were facultative or obligate anaerobes capable of the fermentation of diverse organic compounds including *Ruminococcus*, *Dysgonomonas*, and *Cloacibacterium*. These microbial genera can be explained by anoxic microenvironments in BAC systems. Methylophilic *Hyphomicrobium*

and sulfate-reducing *Desulfosporosinus* bacterial genera were also abundant in the BAC samples. The abundance of *Hyphomicrobium* has been also observed in prior BAC biofiltration systems (Liao et al., 2013b), suggesting that simple carbon sources such as methanol or methane generated during AOPs processes are prevalent in BAC systems following AOPs.

Environmental predictor variables for microbial community compositions in both BAC treatments were identified using BEST and DistLM. Consistent with changes in depth depicted in Figure 5, EBCT (or depth) of each BAC sample was identified as a significant environmental predictor for microbial community composition in BAC treatments (BEST $P=0.001$; DistLM $P=0.008$). Correlations of dominant genera to EBCT were established by calculating Spearman rank correlation coefficients between the square-root transformed relative abundance of each microbial genus in all BAC samples and corresponding Log(X+1) transformed EBCT (Figure 7). The relative abundance of *Halomonas*, *Shewanella*, *Staphylococcus*, and *Acinetobacter* all increased with EBCT in both BAC columns. In contrast, a gradual decrease in abundance with EBCT was observed for *Nitrospira*, *Planctomyces*, *Dysgonomonas*, *Rhodoplanes*, *Hyphomicrobium*, and *Sphingopyxis*. These trends were more pronounced in ozone BAC, perhaps due to some different character of the effluent dissolved organics entering the two BAC treatment trains as indicated by SUVA values. *Sphingopyxis* have been frequently observed in water supply systems and appear metabolically versatile, with the ability to utilize a wide range of natural organic compounds as well as man-made refractory environmental contaminants (Balkwill et al., 2006).

Conclusions

Both ozone and UV/H₂O₂ fed BAC columns provide a long-term source for NDMA production though this trend was less pronounced with time and for UV/H₂O₂ systems. Microbial population structure significantly changed with respect to biofiltration depth in both BAC filtration processes, with EBCT identified to be a more significant environmental predictor variable for microbial population structural change in BAC set-ups than upstream implementation of ozone or UV/H₂O₂. As the effluents from both BAC columns had similar constituent levels with the exception of NDMA, the functional behaviors of microbial populations in both set-ups were also highly analogous. Certain abundant microbial groups including *Nitrospira*, *Planctomyces* and *Hyphomicrobium* have been observed in different systems also investigating AOP-BAC filtration treatment (Kasuga et al., 2007; Liao et al., 2012, 2013a and 2013b), suggesting a ubiquity of these microbial clades and functionality in similar systems. The association of *Nitrospira* and *Planctomyces* in conjunction with nitrogen species profiles point to the importance of nitrogen cycling in BAC systems and its potential interplay with nitrosamine formation. This cycling may vary with an early spatial dominance of net nitrifying conditions followed by more pronounced and/or localized denitrifying microenvironments with the BAC matrix.

From an engineering and design perspective, unintended consequences such as NDMA formation should be investigated and identified at pilot scale prior to making full-scale design recommendations. The impact of EBCT on NDMA formation should be considered when planning for BAC treatment. While data from this study cannot form an exact recommendation on EBCT, shorter EBCTs of 12–16 minutes did not result in NDMA formation during column pore water testing indicating that 20 minutes EBCT may be too long and lead to conditions that favor NDMA formation. It is also not clear what role the

upstream wastewater treatment plant operation may have on subsequent NDMA formation
and therefore should be the subject of future investigations.

Acronyms and abbreviations

AOPs	advanced oxidation processes
BAC	biological activated carbon
EBCT	empty bed contact time
IPR	indirect potable reuse
NDMA	<i>N</i> -nitrosodimethylamine
OTUs	operational taxonomic units
PCoA	Principal Coordinate Analysis
QA/QC	quality assurance/quality control
QIIME	Quantitative Insights Into Microbial Ecology
RNS	reactive nitrogen species
RO	reverse osmosis
TOC	total organic carbon
UV	ultraviolet radiation

Acknowledgements

This material was supported in part by the U.S. National Science Foundation through
CBET-1055396 and the Engineering Research Center for Re-inventing the Nation's Water

Infrastructure (ReNUWIt: EEC-1028968) at CSM. Researchers from Hazen and Sawyer and SNWA were supported by the Water Environment and Reuse Foundation's financial, technical and administrative assistance in funding and managing the project. The authors thank Tara VanEyck, Enrique Vadiveloo, Philip C. Cooke for extensive work, sampling, and project management at the pilot site location; Zackary Jones for technical assistance in DNA extraction, preparation and analysis, and Kristin Mikkelsen for constructive comments on a prior version of this manuscript. The comments and views detailed herein do not necessarily reflect the views of the funding agencies, their officers, directors, employees, affiliates, or agents.

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Figure 1. Pilot layout using secondary clarifier effluent from PureOx wastewater facility. A small amount of chlorine was added to control midges but with no residual after deep bed filtration (chlorine data not shown). The third process train of ozone – BAC- HEUV was not discussed in this manuscript but provided additional NDMA removal using high energy UV ($\sim 1000 \text{ mJ/cm}^2$).

Figure 2. (top) NDMA concentrations in pilot influent, ozone and ozone-BAC effluents; (bottom) NDMA concentrations in pilot influent, UV/H₂O₂ and UV/H₂O₂-BAC effluents.

Figure 3. Specific nitrogen processing activity in oxic and anoxic batch tests (3a); NDMA removal and formation in oxic and anoxic batch tests (3b). Batch studies were conducted using BAC filter media harvested from a filter depth equating to approximately 4 minutes of EBCT (3B) and at 18 minutes of EBCT (4D), respectively.

Figure 4. The relative abundance (%) of microbial phyla. Oz1-Oz20 represent BAC samples with ozone as the pre-treatment and an EBCT of 1-20 min. UV1-UV20 represent BAC samples with UV/H₂O₂ as the pre-treatment and an EBCT of 1-20 min.

Figure 5. Differences in the phylogenetic fingerprints of microbial community composition within the BAC samples using PCoA analysis. Principal coordinates were established using the weighted UniFrac distance matrix generated in QIIME. Oz1-Oz20 represent BAC samples with ozone as pre-treatment and EBCT of 1-20 min. UV1-UV12 represent BAC samples with UV/H₂O₂ as pre-treatment and EBCT of 1-12 min. The BAC sample UV20 with UV/H₂O₂ as pre-treatment and EBCT of 20 min was not included due to the abnormal microbial community structure compared to others.

Figure 6. The relative abundance of the first 15 most abundant microbial genera in both BAC columns following either ozone (a) or UV/H₂O₂ treatments (b). Relative abundance represents a composite of all sampling depths.

Figure 7. Representative microbial genera that were significantly positively or negatively related to EBCT. The relative abundance of microbial genera has been normalized by the Z-score across all data sets. Oz1-Oz20 represent BAC samples with ozone as pre-treatment and EBCT of 1-20 min. UV1-UV20 represent BAC samples with UV/H₂O₂ as pre-treatment and EBCT of 1-20 min.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Table 1. Average influent and effluent water quality data for the BAC biofiltration columns following ozone and UV/H₂O₂ pretreatment during stable operation for approximately 9 months

Parameters ^a	AOP	BAC (ozone)		BAC (UV/H ₂ O ₂)	
	Influent	Influent	Effluent	Influent	Effluent
COD (mg/L)	32±26	41±28	16±10	49±33	14±9
BOD ₅ (mg/L)	2.7±0.7	2.2±0.9	2.0±0.6	2.2±1.2	2.0±0.4
TOC (mg/L)	3.0±1.9	2.4±1.4	1.1±0.5	2.2±1.1	1.1±0.6
NH ₄ ⁺ (mg-N/L)	4.2±3.5	3.6±3.1	0.3±0.3	4.1±3.5	2.2±1.9
Nitrite (mg-N/L)	1.9±1.5	0.5±0.4	0.1±0.1	2.2±1.7	0.1±0.1
Nitrate (mg-N/L)	2.2±2.0	3.4±2.5	6.0±2.4	2.0±1.6	4.2±2.4
Total Nitrogen (mg/L)	9.3±4.5	8.9±5.1	7.8±2.7	9.0±6.0	7.5±4.2
Phosphate (mg/L)	1.2±1.0	1.1±1.0	1.2±0.4	1.1±1.0	1.2±0.5
Chloride (mg/L)	1500±500	1500±300	1400±200	1500±400	1400±300
Sulfate (mg/L)	210±120	160±130	110±90	140±120	120±90
pH	6.5±0.6	6.6±0.3	6.6±0.5	6.6±0.6	6.5±0.5
Conductivity (µs/cm)	5020±1710	4600±1850	4100±1400	4880±1420	5240±1460
TDS (mg/L)	3540±890	2650±660	3460±770	2600±720	3480±600
TOX (µg/L)	230±170	370±220	83±52	320±130	85±61
TSS (mg/L)	3.9±1.9	3.4±1.0	3.5±1.9	3.5±1.9	3.4±2.2
Temp (°C)	28.6±1.8				

^aTDS as total dissolved solids; TOX as total organic halides; TSS as total suspended solids. In total, 39 different sampling events were campaigned between January 2013 and November 2013.

Table 2. Soluble inorganic nitrogen, TOC and NDMA concentrations throughout
UV/H₂O₂-Fed BAC transit (data are similar for ozone-fed BAC)

	TOC	NH ₃	NO ₂	NO ₃	TIN	NDMA
	mg/L	mg-N/L	mg-N/L	mg-N/L	mg/L	ng/L
Influent	1.50	5.65	0.93	2.64	9.22	18
3A (2min)	1.20	3.65	0.22	3.49	7.36	nm
3B (4 min)	1.10	2.81	0.05	4.15	7.01	<2
3C (6 min)	0.91	2.64	0.03	4.27	6.94	nm
3D (8 min)	0.82	2.65	0.02	4.13	6.80	nm
3E (10 min)	0.85	2.56	0.03	3.92	6.51	nm
4A (12 min)	0.72	2.1	0.07	4.49	6.66	nm
4B (14 min)	0.66	2.07	0.05	4.51	6.63	nm
4C (16 min)	0.60	2.01	0.04	4.40	6.45	<2
4D (18 min)	0.52	2.07	0.03	4.03	6.13	nm
4E (20 min)	0.52	1.96	0.04	4.19	6.19	25

nm – not measured; “3A” through “3E” refer to BAC column 1 sampling locations
corresponding to approximately 2 min EBCT intervals (i.e., 2, 4, 6, 8, and 10 min EBCT
sample taps); “4A” through “4E” refer to BAC column 2 sampling locations that followed
column 1, again at approximately 2 min EBCT intervals to give 12, 14, 16, 18, and 20 min
EBCT sample locations.