Biotransformation and Sorption of Trace Organic Compounds in Biological Nutrient Removal Treatment Systems

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Highlights

- Biotransformation rate and sorption distribution coefficient of TOrCs in different redox conditions
- Biotransformation rate coefficients affected by redox condition and SRT
- High correlation of sorption distribution coefficients in anaerobic and anoxic sludge
- Fate parameters useful in predictive models
Abstract

This study determined biotransformation rates ($k_{bio}$) and sorption-distribution coefficients ($K_d$) for a select group of trace organic compounds (TOrCs) in anaerobic, anoxic, and aerobic activated sludge collected from two different biological nutrient removal (BNR) treatment systems located in Nevada (NV) and Ohio (OH) in the United States (US). The NV and OH facilities operated at solids retention times (SRTs) of 8 and 23 days, respectively. Using microwave-assisted extraction, the biotransformation rates of the chosen TOrCs were measured in the total mixed liquor. Sulfamethoxazole, trimethoprim, and atenolol biotransformed in all three redox regimes irrespective of the activated sludge source. The biotransformation of $N$, $N$-diethyl-3-methylbenzamide (DEET), triclosan, and benzotriazole was observed in aerobic activated sludge from both treatment plants; however, anoxic biotransformation of these three compounds was seen only in anoxic activated sludge from NV. Carbamazepine was recalcitrant in all three redox regimes and both sources of activated sludge. Atenolol and DEET had greater biotransformation rates in activated sludge with a higher SRT (23 days), while trimethoprim had a higher biotransformation rate in activated sludge with a lower SRT (8 days). The remaining compounds did not show any dependence on SRT. Lyophilized, heat inactivated sludge solids were used to determine the sorption-distribution coefficients. Triclosan was the most sorptive compound followed by carbamazepine, sulfamethoxazole, DEET, and benzotriazole. The sorption-distribution coefficients were similar across redox conditions and sludge sources. The biotransformation rates and sorption-distribution coefficients determined in this study can be used to improve fate prediction of the target TOrCs in BNR treatment systems.

Keywords: Trace organic compounds, pharmaceuticals and personal care products, biotransformation, sorption, biological nutrient removal treatment
1. Introduction

Consumer product ingredients have been identified as a contributor to anthropogenic pollution. With the advancement of analytical techniques, a wide range of trace organic compounds (TOrCs) are now being detected in different environmental matrices. While these compounds are introduced into the environment through a number of pathways, wastewater treatment plant (WWTP) effluents are a major source of these chemicals in receiving waters (Drewes and Shore, 2001). Since wastewater treatment plants were originally designed to remove conventional pollutants (solids, bulk organics, and nutrients), the removal of TOrCs occurs to various degrees by virtue of the treatment process and the physico-chemical properties of the compound (Rogers, 1996). The major mechanisms of removal are biotic transformation or abiotic processes such as sorption, volatilization, isomerization, and hydrolysis (Pomies et al., 2013).

Mathematical models for assessing the fate of TOrCs in wastewater treatment are effective tools for process design, optimization of existing processes, or augmentation using tertiary treatment processes for improved effluent quality (Clouzot et al., 2013). Several modeling frameworks such as ASTREAT (McAvoy et al., 1999), TOXCHEM+ (Melcer et al., 1999), WATER9 (U.S. EPA, 2001), and SimpleTreat (Struijs et al., 2016) have been developed to predict the removal of organic compounds in conventional activated sludge (CAS) systems. The ASM-X model has also been developed to include an anoxic process for denitrifying systems (Plosz et al., 2010).

Biotransformation and sorption are typically the two important fate mechanisms for assessing the removal of organic compounds in WWTPs. These fate processes have predominantly been studied in aerobic activated sludge from CAS treatment systems. State-of-the-art reviews by Clouzot et al. (2013) and Pomies et al. (2013) have discussed the absence of fate parameters in
different redox conditions for micropollutant modeling. Biological nutrient removal (BNR) treatment processes, like the A\textsuperscript{2}O design, employ anaerobic and anoxic zones in addition to an aerobic zone to promote the growth of phosphorus accumulating organisms (PAO) and denitrifiers, which are responsible for the removal nitrogen and phosphorus from wastewater. It has been hypothesized that BNR treatment systems may create favorable conditions for TOrC removal because of the different redox regimes that harbor a diverse consortium of microorganisms and enzyme pools to promote biotransformation (Xue et al., 2013). Nutrient removal processes may also be more effective in reducing the toxicity of treated effluents on aquatic life forms when compared to CAS treatment systems (Parker et al., 2014). In particular, the removal of estrogenicity has been reported in anaerobic, anoxic, and aerobic redox conditions in a pilot-scale University of Cape Town BNR (UCT-BNR) design (Ogunlaja and Parker, 2015). Hence, it is worthwhile to study fate mechanisms of TOrCs in BNR treatment systems and model these systems to advance our current understanding of their removal.

In this study seven TOrCs were chosen for investigation: atenolol (ATN), benzotriazole (BTA), carbamazepine (CBZ), \textit{N}, \textit{N}-diethyl-3-methylbenzamide (DEET), sulfamethoxazole (SMX), trimethoprim (TMP) and triclosan (TCS). These TOrCs were selected because they: (1) are commonly detected in wastewater effluents throughout the world including Europe (Loos et al., 2013), Germany (Ternes et al., 1998), South Korea (Behera et al., 2011), Spain (Rosal et al., 2010; Santos et al., 2007), Switzerland (Eggen et al., 2014), and the United States (Salveson et al. 2012); (2) have been identified as priority contaminants of concern in several priority lists (Higgins et al., 2010; Diamond et al. 2010), and (3) exposure to these compounds has been known to elicit toxic effects in aquatic life forms. DEET, a biocide used in insect repellants, has been shown to cause reproductive disorders after prolonged environmental exposures.
(Manikkam et al., 2012). Isidori et al. (2005) reported sulfamethoxazole, an antibiotic, to cause chronic toxicity to aquatic organisms at part-per-billion levels. Tatarazako et al. (2004) has shown triclosan, an antibacterial agent, to be highly toxic to aquatic biota. The corrosion inhibitor benzotriazole has been shown to present an estrogenic response in bioassays (Harris et al., 2007) and exert toxicity to plants (Cancilla et al., 1997), while the accumulation of carbamazepine and triclosan have been observed in fish liver tissue (Diamond et al. 2010).

The primary objective of this study was to experimentally derive biotransformation kinetic rates and sorption-distribution coefficients of a select group of TOrCs in activated sludge collected from three redox conditions at two different BNR treatment plants with an A2O configuration. In activated sludge systems, the mechanism of biodegradation is often a topic of contention (Pomies et al., 2013). Most studies measure biotransformation only in the aqueous phase, while the biodegradation of an organic compound may occur in the aqueous phase or on the solids while being sorbed. Compounds may also desorb from the solids and become bioavailable for degradation in the aqueous phase. Identifying the biodegradation compartment is challenging because the compound, depending on its physical properties, may be present in both the solid and aqueous phases at a given time. Hence in this study, biotransformation rates were determined as the total compound loss in the mixed liquor by using microwave-assisted extraction. The sorption-distribution coefficients were determined using lyophilized, heat inactivated sludge solids to reduce the occurrence of biotransformation during sample equilibrium. The effect of redox conditions and solids retention time (SRT) were also assessed with respect to biotransformation and sorptive fate of the target TOrCs.
2. Methodology

2.1 Materials

All of these compounds were purchased from Sigma Aldrich (Purity > 97%). The structure of these target compounds, their physico-chemical properties, and location of the isotopic labels in the internal standards are provided in the Supplementary Material (Table S-1 and S-2). Activated sludge for the biotransformation and sorption experiments were obtained from two BNR treatment plants located in Ohio (OH) and Nevada (NV). Both treatment plants were configured in the A²O process design (Figure 1). NV is a 30 MGD BNR treatment facility operated at an SRT of 8 days, while OH is a 9 MGD BNR treatment facility operated at an SRT 23 days. The influent wastewater to both of the wastewater treatment plants is primarily residential. The activated sludge samples were collected from the effluent of each unit process (anaerobic, anoxic, and aerobic) when the treatment plants were operating under normal, steady state conditions. The operational parameters reported by the treatment plants during the time of sampling are provided in the Supplementary Material (Table S-3 and S-4). Activated sludge from both NV and OH were transported to the laboratory on ice and processed within 24 hours of collection.
2.2 Biotransformation Experiment

2.2.1 Experimental Procedure

After receiving samples in the laboratory, 10 L of activated sludge was placed in four separate stainless steel reactors. Two of the four reactors were used as duplicate biotic reactors, one was used as an abiotic reactor (biocide solution containing 10 mM each of NaN$_3$, BaCl$_2$ and NiCl$_2$), and the other as an experimental control (deionized water). All of the reactors were buffered with NaHCO$_3$ to a final equivalence of 3 mM. A nutrient solution was spiked in the aerobic reactors (5 mg NH$_3$-N/L and 10 mg/L PO$_4$-P/L) to assess nitrification and phosphorus uptake, and in the anoxic reactors (30 mg NO$_3$-N/L every 12 hours) to ensure denitrification. Relevant redox conditions (DO > 4 mg/L for aerobic and DO < 0.1 mg/L for anaerobic and anoxic) were maintained by bubbling compressed air or nitrogen gas through the reactors. The reactors were equilibrated for an hour prior to spiking the target TOrCs. The TOrC stock solution was prepared in 100% methanol and stored at 2 °C in amber glass bottles until use. The stock solution was brought to room temperature prior to preparation of the spiking solution, which was
prepared in ultrapure water to achieve an equivalent of 10 μg/L of each TOrC in the reactors at time zero. The methanol addition from the TOrC spiking solution contributed about 7.5 mg-C/L in the system. At specific time points, samples (500 mL) were collected for TOrC analysis in amber bottles containing a final equivalence of 1 g/L NaN₃ and 0.5 g/L ascorbic acid as a preservative. Aerobic and anoxic experiments were run for 1-3 days, while the anaerobic experiments were run for 7 days because of expected slower kinetics. The buffer, nutrient spike, laboratory water, and activated sludge were also analyzed for the target TOrCs to determine background concentrations. The TOrC concentrations (C) measured at different time points were then divided by the measured initial TOrC concentration in the reactor and plotted against time. The results were fit to the following kinetic model:

\[
\frac{dC}{dt} = -k_{bio}[C][X]
\]  

where, \(k_{bio}\) is the pseudo first-order biotransformation rate constant (L/g-day) with assumed constant biomass concentration in the system (X as TSS or VSS).

2.2.2 Analytical Methods

*Conventional Parameters:* The conventional parameters pH, dissolved oxygen (DO), total and volatile suspended solids (TSS/VSS), dissolved organic carbon (DOC), soluble and particulate chemical oxygen demand (sCOD/pCOD), ammonia (NH₃-N), nitrite (NO₂-N), nitrate (NO₃-N) and phosphate (PO₄-P) were analyzed at various time points in the biotic reactors using Standard Methods (provided in the Supplementary Material, Table S-7). Presence of the biocide in the abiotic control reactor interfered with the COD, NO₂-N, NO₃-N, and PO₄-P analysis. Hence, only pH, DO, TSS/VSS, and DOC were analyzed in the abiotic control. The conventional parameter samples were preserved at pH 2 with concentrated sulfuric acid and stored at 2 °C until being
analyzed. The samples for soluble conventional parameters analyses were filtered using a 1.2 µm glass fiber filter (Whatman GF/C).

**TORC Analysis:** TORC extraction and analysis in the total mixed liquor sample consisted of three steps. Firstly, the internal standard solution was added to 10 mL of sample and mixed with 10 mL of methanol. The target compounds were then extracted from the activated sludge/methanol mixture using microwave-assisted extraction (MAE) at 120 °C for 20 min using an EthosUp high performance microwave digestion system from Milestone (Shelton, CT). The MAE liquor was then diluted to 1-L in reagent-grade water and the analytes were isolated via automated solid-phase extraction (ASPE) using HLB (6-mL and 200-mg) cartridges from Waters Corporation (Milford, MA) and an AutoTrace™ ASPE (Dionex Corporation, Sunnyvale, CA). The SPE cartridges were sequentially preconditioned with 5 mL of methyl tert-butyl ether (MTBE), 5 mL of methanol, and 5 mL of reagent-grade water. Each sample was loaded onto a cartridge at 15 mL/min and then rinsed with 5 mL of reagent-grade water and subsequently dried under a nitrogen stream for 30 min. Each cartridge was eluted with 5 mL of methanol followed by 5 mL of 10/90 (v/v) methanol/MTBE and the extract was evaporated under a N₂ stream to a 500 µL final volume. The final step was instrumental analysis of the ASPE extracts using isotope dilution liquid chromatography-tandem mass spectrometry (Vanderford and Snyder, 2006). All analytes were separated by a 100 × 4.6 mm Onyx Monolithic C18 column (Phenomenex, Torrance, CA) at 40 °C. Chromatographic separation was accomplished with a binary gradient of 5 mM ammonium acetate (v/v) in reagent-grade water (A) and 100% methanol (B) at a flow rate of 0.8 mL/min. The target analytes and isotopic surrogates were quantified by tandem mass spectrometry using an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Depending on the compound, either an ESI negative mode (TCS) or ESI
positive mode (ATN, BTA, CBZ, DEET, SMX, TMP) was used for quantitation. The analytical parameters for the target compounds are provided in the Supplementary Material (Table S-5).

2.3 Sorption Experiment

2.3.1 Sludge Handling Procedure

The inactivation of activated sludge was carried out following the procedure described in Kerr et al. (2000). Upon receipt of the activated sludge samples in the laboratory, the solids were allowed to settle for an hour in their sample containers and the supernatant was discarded. The remaining thickened solids solution was centrifuged at 2800 g for 5 min in 50 mL polypropylene centrifuge tubes. The supernatant was discarded and 30 mL of deionized (DI) water was added. The centrifuge tubes were then shaken by hand and centrifuged again at 2800 g for 5 min. This DI water washing step was done three times. After the final washing and decantation, the solids were placed in a freeze drying dish and pre-frozen at –20 °C. Pre-frozen solids were then lyophilized in a benchtop LABCONCO freeze dryer at –60 °C for at least 24 hour. After lyophilization the freeze dried solids were ground lightly using a mortar and pestle to break down the bulk solids. For inactivation, the solids were placed in an oven at 103 °C overnight prior to being stored in clean amber glass bottles. Prior to being used in the sorption experiments, a portion of the freeze dried solids were again heated at 103 °C in an oven overnight. A 90 mg sample of the dried solids were carefully weighed into 50-mL polypropylene centrifuge tubes and 30 mL of synthetic wastewater was introduced to achieve a final solids concentration of approximately 3 g/L. The synthetic wastewater was composed of: 6.5 mg/L KH$_2$PO$_4$; 22 mg/L K$_2$HPO$_4$; 33 mg/L Na$_2$HPO$_4$; 2 mg/L LN$_4$HCl; 22 mg/L MgSO$_4$; 36 mg/L CaCl$_2$; 0.3 mg/L FeCl$_3$; 300 mg/L NaHCO$_3$. The samples were centrifuged at 2800 g for 5 min and the supernatant removed using a pipet. This procedure was repeated two more times to remove any dissolved
organic carbon that was released from the solids. The inactivated sludge was then re-suspended in synthetic wastewater and used immediately in the sorption experiments to minimize the potential for biodegradation.

2.3.2 Sorption Experimental Procedure

After the third washing step, synthetic wastewater was added to maintain a solids-to-liquid ratio of 3 g/L. A spike solution was prepared similar to the biotransformation experiment except that only five of the seven compounds (BTA, CBZ, DEET, SMX, TCS) were added in the sorption experiment. The reactors were spiked at five different concentrations (0.5, 1, 2.5, 5 and 10 µg/L) to develop sorption isotherms. After spiking the test compounds, the centrifuge tubes were thoroughly mixed by end-over-end hand rotation for approximately 30 s. The centrifuge tubes were then equilibrated in a rotational tumbler at room temperature (~23 °C) for 4 h (equilibrium time determined by Steven-Garmon et al. (2011) and Kerr et al. (2000)). Following equilibrium, the test vessels were centrifuged (2800 g for 5 min) and 50 mL of centrate (aqueous phase) was transferred via a pipet into clean 60 mL sampling vials. The TOrC concentrations were measured only in the aqueous phase, and the solid phase TOrC concentrations were assumed to be the difference between the spiking concentration and the aqueous phase concentration. The solid phase concentrations were plotted against the measured aqueous phase concentrations and a linear regression analysis was performed to determine the sorption distribution coefficient (slope of the line fit through the origin) for each compound.

\[
K_d = \frac{C_{sor}/(MLSS \times 10^{-3})}{C_{sol}}
\]  

(2)

where, \(K_d\) is the sorption-distribution coefficient (L/kg), MLSS – concentration of the mixed liquor suspended solids (g/L), \(C_{sor}\) – concentration of TOrCs in the sorbed phase (µg/L) \(C_{sol}\) – Concentration of TOrCs in the aqueous phase (µg/L).
2.3.3 TOrCs Extraction and Analysis

Analytes from the aqueous phase were extracted using 150-mg hydrophilic-lipophilic balance (HLB) cartridges from Waters Corporation (Milford, MA). The SPE cartridges were first preconditioned with 10 mL of reagent-grade water followed by 50 mL of sample loading at 15 mL/min. The analytes were then eluted using 10 mL of acetonitrile in two 5 mL elution steps and collected in 10 mL calibrated centrifuge tube. The extracts were then concentrated by a gentle stream of nitrogen to a volume less than 1 mL and then brought up to exactly 1-mL with acetonitrile. An Agilent G6410A Triple Quadrupole mass spectrometer was used for quantitation of the analytes. All of the compounds, except triclosan, were measured in ESI positive mode. Triclosan was measured in ESI negative mode. The analytes were separated using an Agilent ZORBAX C18 column (2.1x100 mm) at 25 °C. Chromatographic separation was accomplished using a binary gradient of 0.1% formate in reagent-grade water (A) and 0.1% formate in acetonitrile (B) for the positive mode method and 5 mM ammonium acetate in reagent-grade water and 5 mM ammonium acetate in methanol for the negative mode method. An injection volume of 5 μL at 500 μL/mL flow rate was used for all analyses. The analytical parameters for the target compounds are provided in the Supplementary Material (Table S-6).

3. Results and Discussion

3.1 Biotransformation Kinetics

3.1.1. Analysis of Conventional Parameters

The conventional parameters in six biotransformation experiments are discussed in the Supplementary Material (Section 4, Table S-8 and Figures S-1, S-2 and S-3). The conventional parameter monitoring revealed that the microbial process for each of the redox conditions (nitrification in aerobic, denitrification in anoxic, fermentation or phosphorus release in
anaerobic) were operating properly. Monitoring these parameters also ensured that the biomass was active throughout the experiment.

### 3.1.2 TOrCs Kinetics

The pseudo first-order biotransformation rates ($k_{\text{bio}}$) were normalized with the average TSS and VSS concentration over the duration of the experiment (Table 1). While the concentration of VSS better represents the biomass concentration, TSS was also included so that the rates could be compared with literature values. The biotransformation profiles for all the experiments can be seen in Figure S-4 (a-n) in the Supplementary Material. Minimal residual values (< 10% most of the times) and $R^2$ values > 0.8 show very good data fit for the kinetic model.

Results from the abiotic reactor, experimental control, and activated sludge before spiking are also provided in the Supplementary Material (Figures S-5 and S-6). Except for atenolol, all of the compounds remained stable in the abiotic kill control (10 mM Na$_3$N + NiCl$_2$ + BaCl$_2$ biocide) with activated sludge. Atenolol degradation of approximately 40% occurred in the abiotic control during the course of the experiments (Supplementary Material, Figure S-1 c, d), suggesting either that the biocide used was ineffective for this compound or another fate mechanisms were involved. Hydrolysis was ruled out because atenolol was relatively stable in the experimental control reactor with buffered DI water. Sorption to activated sludge was also ruled out because the TOrCs analysis determined total mixed liquor concentration (i.e., inclusive of aqueous and solid phases). It was hypothesized that the enzymes responsible for degradation of atenolol was not inactivated with the biocide. To evaluate this hypothesis, methanol (8% final concentration) was added to the abiotic control reactor and the kinetics were studied. Results from this experiment (Supplementary Material, Figure S-7) showed the atenolol concentration to decrease by only 15% over the course of 7 days, which is much less than the 40% loss observed
without the methanol addition. These results confirm that the biocide used in this study only arrested the microbial respiration and the addition of methanol was needed to denature the enzymes responsible for degrading atenolol.
Table 1. Biotransformation rates ($k_{bio}$ ± 95% CI in L/gTSS-day and L/gVSS-day) of the target TOrCs in activated sludge from three redox conditions in two BNR treatment plants, and a comparison with literature values.

<table>
<thead>
<tr>
<th>TOrC</th>
<th>Kinetic Parameter</th>
<th>OH (SRT = 23 days)</th>
<th>NV (SRT = 8 days)</th>
<th>Literature $k_{bio}$ in L/gTSS-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>Anoxic</td>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
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<td>----------</td>
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<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Group 1</strong> (Biotransformation under all conditions)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATN</td>
<td>L_{G_{TSS}}-day</td>
<td>0.14 ± 0.19</td>
<td>2.56 ± 0.01</td>
<td>2.92 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>L_{G_{VSS}}-day</td>
<td>0.42 ± 0.18</td>
<td>3.36 ± 0.004</td>
<td>3.58 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>R^2 (RMSE)</td>
<td>0.83 (0.14)</td>
<td>0.99 (0.08)</td>
<td>0.85 (0.11)</td>
</tr>
<tr>
<td><strong>Group 2</strong> (No biotransformation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBZ</td>
<td>L_{G_{TSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>NB</td>
</tr>
<tr>
<td></td>
<td>L_{G_{VSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>0.16 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>R^2 (RMSE)</td>
<td>0.54 (0.11)</td>
<td>0.96 (0.04)</td>
<td>0.81 (0.06)</td>
</tr>
<tr>
<td><strong>Group 3</strong> (Biotransformation dependent on redox condition and SRT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTA</td>
<td>L_{G_{TSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>0.19 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>L_{G_{VSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>4.43 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>R^2 (RMSE)</td>
<td>5.43 ± 0.12</td>
<td>0.54 (0.11)</td>
<td>0.96 (0.04)</td>
</tr>
<tr>
<td>DEET</td>
<td>L_{G_{TSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>L_{G_{VSS}}-day</td>
<td>NB</td>
<td>NB</td>
<td>0.60 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>R^2 (RMSE)</td>
<td>0.97 (0.06)</td>
<td>0.97 (0.06)</td>
<td>0.97 (0.06)</td>
</tr>
</tbody>
</table>

NB - No biotransformation, NA – Not Available, RMSE – Root Mean Squared Error. References: a) Helbling et al. (2010); b) Horsing et al. (2011); c) Inyang et al. (2016); d) Wick et al. (2009); e) Mazioti et al. (2015); f) Abegglen et al. (2009); g) Fernandez-Fonataina et al. (2013); h) Suarez et al. (2010); i) Salveson et al. (2012); j) Polz et al. (2010)
The compounds were classified into three groups based on their biotransformation fate in different redox conditions and activated sludge source. It should be noted that this classification is based only on the biotransformation fate and the degradation rates vary in each group.

**Group 1: Compounds that biotransformed under all three redox conditions and both sludge sources.** Atenolol readily biotransformed under all three redox conditions in both treatment plants. The biotransformation rates of atenolol were highest under aerobic conditions in both the OH (3.58 L/g_vss-day) and NV (2.26 L/g_vss-day) activated sludge. The anoxic biotransformation rate of atenolol in OH (3.36 L/g_vss-day) was similar to the aerobic rate in OH. Inyang et al. (2016) also observed the biotransformation of atenolol in activated sludge under three redox conditions in BNR treatment (aerobic = 5.28 L/g_TSS-day, anaerobic = 0.43 L/g_TSS-day and anoxic = 1.7 L/g_TSS-day), but their rates were higher than the \( k_{bio} \) (in L/g_TSS-day) values seen here in both the treatment plants (Table1).

Trimethoprim biotransformed under all three redox condition in activated sludge collected from both treatment plants, but the biotransformation rates were slower than atenolol (TMP OH: aerobic = 0.14 L/g_vss-day, anaerobic = 0.24 L/g_vss-day, anoxic = 0.19 L/g_vss-day and TMP NV: aerobic = 0.75 L/g_vss-day, anaerobic = 0.33 L/g_vss-day, anoxic = 0.81L/g_vss-day). The biotransformation rates of trimethoprim in NV were greater than OH for all three redox conditions. Conflicting observations has been seen in the literature with studies reporting trimethoprim being recalcitrant or being biotransformed (Fernandez-Fontaina et al., 2016; Deng et al., 2016). Inyang et al. (2016) observed the biotransformation of trimethoprim only in aerobic and anoxic activated sludge with no biotransformation in anaerobic activated sludge. Saurez et al. (2010) studied the biotransformation of trimethoprim under nitrifying and denitrifying conditions and reported a biotransformation rate of 0.15 L/g_TSS-day under aerobic
nitrifying conditions, which is similar to the aerobic biotransformation rate for OH (0.11 L/g_{TSS-day}), but they did not observe any biotransformation of trimethoprim in anoxic activated sludge.

Sulfamethoxazole biotransformed at rates similar to trimethoprim. The highest biotransformation rate for sulfamethoxazole was in OH aerobic activated sludge (0.17 L/g_{vss-d}) and the lowest was in the OH anaerobic activated sludge (0.025 L/g_{vss-d}). Plosz et al. (2010) reported both aerobic and anoxic biotransformation rates of 0.41 L/g_{TSS-day} for sulfamethoxazole, which is greater than the rates normalized with TSS in this study.

**Group 2: No biotransformation under any condition.** Carbamazepine was recalcitrant to biotransformation in activated sludge from both sources under all three redox conditions. Others have also reported the recalcitrance of carbamazepine in aerobic activated sludge. (Jelic et al., 2011; Majewsky et al., 2011; Radjenovic et al., 2009; Salveson et al., 2012). However, Hai et al. (2011) observed an exceptionally high removal of carbamazepine (68% removal) under near anoxic (DO = 0.5 mg/L) operational condition in a laboratory scale membrane bioreactor (MBR). The removal observed under aerobic condition (20 %) was also higher than those reported in the literature (Hai et al. 2011). They hypothesized that their MBR may harbor microbes that might degrade carbamazepine, but further microbial community analysis is needed to substantiate their hypothesis (Hai et al. 2011).
**Group 3: Compounds whose biotransformation was dependent on the redox condition and the sludge source.** In this group, there were three compounds, namely benzotriazole, DEET, and triclosan, that biotransformed under aerobic and anoxic conditions in NV, but only under aerobic conditions for OH. The aerobic biotransformation rate of benzotriazole was comparable (0.19 L/g<sub>vss</sub>-d in OH and 0.15 L/g<sub>vss</sub>-d in NV) in activated sludge from both treatment plants. Mazioti et al. (2015) reported biotransformation rates of benzotriazole under aerobic and anoxic conditions to be 0.38 L/g<sub>TSS</sub>-d and 0.24 L/g<sub>TSS</sub>-d, respectively, at rates slightly higher than this study.

Triclosan had aerobic biotransformation rates of 0.49 L/g<sub>TSS</sub>-d in OH and 0.31 L/g<sub>TSS</sub>-d in NV activated sludge. Triclosan is well known to biodegrade under aerobic conditions (Inyang et al., 2016; McAvoy et al., 2002; Salveson et al. 2012). Inyang et al. (2016) reported significant removal of triclosan in batch degradation studies using anaerobic and anoxic activated sludge, but the mechanism was predominantly sorption. Similarly, Phan et al. (2016) attributed the anoxic removal of triclosan in a laboratory scale anoxic-aerobic membrane bioreactor to be mostly due to sorption on the biosolids. Chen et al. (2011) observed biodegradation of triclosan to methyl-triclosan in anoxic activated sludge, but they did not study the kinetics of the reaction. To our knowledge this is the first study to report an anoxic biotransformation rate for triclosan.

DEET biotransformed rapidly in OH aerobic activated sludge (5.43 L/g<sub>vss</sub>-d), but no biotransformation was observed under anaerobic or anoxic conditions in OH activated sludge. In NV activated sludge, DEET biotransformed under aerobic and anoxic conditions, although the rate was much slower in anoxic activated sludge (0.04 L/g<sub>vss</sub>-d). Inyang et al. (2016) observed the biotransformation of DEET only in aerobic activated sludge.
The target compounds were also classified into rapid biotransformation rates ($k_{bio} > 1 \text{ L/g}_{\text{vss}-d}$), moderate biotransformation rates ($1 \text{ L/g}_{\text{vss}-d} > k_{bio} > 0.1 \text{ L/g}_{\text{vss}-d}$) and slow biotransformation rates ($0.1 \text{ L/g}_{\text{vss}-d} > k_{bio} > 0.01 \text{ L/g}_{\text{vss}-d}$). DEET and atenolol were the only two compounds to biotransform rapidly. All of the other compounds biotransformed either moderately or slowly. All the anoxic and anaerobic biotransformation rates were either moderate or slow, except for atenolol that biotransformed rapidly in anoxic activated sludge from NV.

### 3.1.3 Impact of Redox Condition on Biotransformation

All of the compounds evaluated except carbamazepine biotransformed in aerobic activated sludge collected from both treatment plants. Compounds such as benzotriazole, DEET, and triclosan biotransformed only in aerobic activated sludge and not in anoxic or anaerobic activated sludge from OH. Thus, aerobic redox conditions may be more favorable for metabolism of the target TOxCs due to maximum free energy available for substrate utilization (Inyang et al., 2016). Moreover, it has been shown that improvement in removal of TOxCs in wastewater treatment can be achieved under high DO conditions (Xue et al., 2010), nitrifying conditions where NH$_3$ oxidation is catalyzed by ammonia monooxygenase (AMO) enzymes (Anderson et al., 2003; Joss et al., 2004; Li et al., 2011; Suarez et al., 2010; Phan et al., 2014) and the presence of archaea (Helbling et al., 2012). The AMO enzyme is known to catalyze the biotransformation of compounds like bisphenol A (Sun et al., 2012), synthetic and natural estrogens (Khunjar et al., 2011a), iopromide (Batt et al., 2006), and ibuprofen and naproxen (Fernandez-Fontaina et al., 2016). While the AMO enzyme has very low substrate specificity (Rsche et al., 1991), its catalytic effect is highly dependent on the structure of the compound. For example, catalyzation by AMO is hampered by the presence of specific functionalities in the chemical structure like heterocyclic rings, aromatic rings, amide groups,
and amine groups that occur in sulfamethoxazole, trimethoprim, and carbamazepine (Boethling et al. 1994, Keener and Arp 1994, Fernandez-Fontaina et al. 2016). Hence, action of the AMO enzyme on any observed biotransformation of these compounds is highly unlikely. Other ammonia oxidizing enzymes (Helbling et al., 2010; Men et al., 2017) or heterotrophic bacteria (Fernandez Fontaina et al., 2016; Khunjar et al., 2011a) may be responsible for the biotransformation of TOrCs in aerobic activated sludge. For example, atenolol with a primary amide is known to undergo enzymatic hydrolysis to atenololic acid under aerobic conditions by amide hydrolyzing enzymes (e.g., amidase) that are abundant in activated sludge microbial communities (Radjenovic et al., 2008; Helbling et al., 2010). Also, hydrolysis does not require oxygen in the biochemical reactions (Stadler and Love, 2016) and thus enzymatic hydrolysis may explain the anoxic and anaerobic biotransformation of atenolol in this study. This finding is also supported by the methanol inhibition test of atenolol whereby the enzymes responsible for hydrolysis transformation were denatured (Supplementary Material, Figure S-7).

There are instances where the biotransformation rate is comparable or even greater in anoxic activated sludge than aerobic activated sludge (Table 1: ATN and TMP in OH, BTA and TMP in NV). Thus, the absence of oxygen does not necessarily limit biotransformation of TOrCs in activated sludge where other electron acceptors such as nitrate are present. In BNR treatment plants with A²O design, the return activated sludge and the internal recycle activated sludge circulates biomass from aerobic conditions to anaerobic and anoxic conditions, respectively (Figure 1). This recirculation creates a unique environment where facultative heterotrophs and their enzyme pools are shared between redox conditions enabling biotransformation even under low DO conditions (Gomez-Silvan et al., 2014; Phan et al., 2016). This transfer of enzymes may explain the biotransformation of atenolol, sulfamethoxazole, and trimethoprim under all three
redox conditions from both the treatment plants. Several heterotrophic strains of bacteria like 
*Pseudomonas* sp., *Rhodococcus* sp., *Microbacterium* sp., *Rhodococcus* sp., *Achromobacter* sp., 
*Ralstonia* sp., *Tsukumurella* sp., *Brevundimonas* sp., and *Variovorax* sp. have been identified 
from aerobic activated sludge that can biotransform or even mineralize sulfamethoxazole
studied the removal of trimethoprim in UCT-BNR design and concluded that ammonia oxidizing 
bacteria, heterotrophs and PAOs work collaboratively in the overall removal of trimethoprim.
But their individual contribution is at different proportions in the order of heterotrophs > 
ammonia oxidizers > PAOs in aerobic activated sludge and heterotrophs > PAOs in anoxic and 
anaerobic activated sludge (Ogunlaja and Parker, 2018). Phan et al. (2016) studied the bacterial 
communities grown in an anoxic-aerobic MBR and observed a high similarity in structural and 
phylogenetic relationships because of the recycling of aerobic activated sludge. A better 
understanding of the microbial communities may help explain the anoxic biotransformation of 
Group 3 compounds (benzotriazole, DEET and triclosan) in NV activated sludge. However, 
anoxic activated sludge from OH was not able to biotransform these compounds. This lack of 
transformation could be because the facultative microbes responsible for the biotransformation 
of these compounds in NV activated sludge may not be present in the OH activated sludge.
Metagenomic studies on the microbial community for each redox condition at both of the 
treatment plants would be required to confirm the absence or presence of biotransforming 
bacterial communities.

**3.1.4 Impact of Solid Retention Time on Biotransformation**

The average retention time of the microbes held in the system dictates the microbial 
community structure of activated sludge. Changes in SRT have been shown to shift the
community structure by increasing or reducing the abundance of a taxa (Vuono et al., 2015; Phan et al., 2016). If the SRT is not sufficiently long, slow growing microbes that can potentially degrade a particular compound could be washed out of the system. A positive correlation between longer SRT and better removal of TOCs has been reported for: (a) bisphenol-A, ibuprofen, benzafibrate, and natural estrogens (Clara et al. 2005); (b) fluoxetine, naproxen, citalopram, natural and synthetic estrogens (Suarez et al. 2010), (c) trimethoprim (Gobel et al. 2007); and (d) atenolol, DEET and sulfamethoxazole (Gerrity et al., 2013). In this study, atenolol and DEET showed better biotransformation performance under the higher SRT (OH SRT = 23 day). The biotransformation rate of DEET in OH aerobic activated sludge (5.43 L/g vss-day) was more than five times greater than the rate in NV aerobic activated sludge (0.87 L/g vss-day). Similarly, the biotransformation rates of atenolol under all three redox conditions were greater in OH when compared to NV (Table 1).

Better degradation with higher SRT was not consistent for all of the TOCs studied. For example, a higher biotransformation rate occurred with a lower SRT (NV SRT = 8 days) for trimethoprim (Table 1). Since the activated sludge was procured from a BNR treatment plant with biological phosphorus removal, one could expect an abundance in phosphorus accumulating organisms (PAO) in the system that would be favorable under lower SRT conditions. PAOs belonging to bacteria taxa Rhodocyclales and Pseudomonadales have been shown to have greater abundance following a decrease in SRT (Vuono et al., 2015; Phan et al., 2016). Thus, the biotransformation of trimethoprim at NV may be related to PAOs that thrive under lower SRT conditions. However, this relationship would need to be verified with detailed microbial community structure analysis. The SRT did not affect the biotransformation rates of benzotriazole, sulfamethoxazole, and triclosan with rates for a given redox condition comparable
between the two BNR treatment plants. This non-effect of a higher SRT on the biotransformation of benzotriazole, sulfamethoxazole, and triclosan may be due to: (a) slower growing microorganisms are not responsible for their biotransformation, (b) shorter SRT is sufficient to support the microbes responsible for biotransforming these compounds; or (c) the difference in SRT between the treatment plants (NV 8 days and OH 23 days) is not significant enough to observe any difference.

3.2 Sorption distribution coefficient

Five compounds (TCS, SMX, CBZ, DEET and BTA) were chosen for determining their sorption-distribution coefficient (Table 2). These compounds were classified on their average log $K_d$ values as highly sorptive ($\log K_d > 3$), moderately sorptive ($2 < \log K_d < 3$), and lowly sorptive ($\log K_d < 2$).

**Highly sorptive ($\log K_d > 3$):** Triclosan was the only highly sorptive compound in this study with $K_d$ values ranging from 6,516 to 10,541 L/kg-MLSS ($\log K_d = 3.81-4.02$). Hyland et al. (2012) reported log $K_d$ values for triclosan to range between 3.28 and 3.98 for aerobic activated sludge, which are slightly lower than the values found in this study.
Table 2. Sorption-distribution coefficients (at pH = 7.5) in aerobic, anoxic, and anaerobic activated sludge for two BNR treatment plants (OH and NV). $K_d \pm 95\%$ CI. Literature measured $K_d$ values are also provided for comparison.

<table>
<thead>
<tr>
<th>Sorption Capacity</th>
<th>TOC</th>
<th>OH (SRT = 23 days)</th>
<th>NV (SRT = 8 days)</th>
<th>Literature $K_d$ in L/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anaerobic L/kg</td>
<td>Anoxic L/kg</td>
<td>Aerobic L/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>log $K_d$</td>
<td>log $K_d$</td>
<td>log $K_d$</td>
</tr>
<tr>
<td>High</td>
<td>TCS</td>
<td>8058 ± 2107</td>
<td>8828 ± 1895</td>
<td>6515 ± 2115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.91 ± 3.95</td>
<td>3.95 ± 3.81</td>
<td>3.81 ± 3.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9351 ± 1524</td>
<td>10541 ± 1309</td>
<td>9053 ± 844</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.97 ± 3.97</td>
<td>4.02 ± 3.96</td>
<td>3.96 ± 3.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>1905-9550*</td>
</tr>
<tr>
<td>Moderate</td>
<td>SMX</td>
<td>241 ± 24</td>
<td>189 ± 14</td>
<td>26* ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.38 ± 2.28</td>
<td>2.28 ± 1.42*</td>
<td>1.42* ± 2.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>104* ± 8</td>
<td>162 ± 2.21</td>
<td>2.21 ± 2.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.27 ± 2.27</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>CBZ</td>
<td>302 ± 22</td>
<td>208 ± 10</td>
<td>68* ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.48 ± 2.32</td>
<td>2.32 ± 1.83*</td>
<td>1.83* ± 1.34*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>134 ± 8</td>
<td>2.13 ± 2.40</td>
<td>2.40 ± 2.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.40 ± 2.40</td>
<td>3870*</td>
<td>6550*</td>
</tr>
<tr>
<td></td>
<td>DEET</td>
<td>224 ± 55</td>
<td>149 ± 14</td>
<td>62 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.35 ± 2.17</td>
<td>2.17 ± 1.79</td>
<td>1.79 ± 1.46</td>
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<td></td>
<td></td>
<td>146 ± 11</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.17 NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.15 ± 2.15</td>
<td>1550*</td>
<td>1950*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81*, &lt; 31*</td>
<td>850*</td>
<td>81*, &lt; 31* - &lt; 100*</td>
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<td></td>
<td>1&lt; 100*</td>
<td>28-66*</td>
<td>940* 52-76*</td>
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<td></td>
<td></td>
<td>220*</td>
<td>133*</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>BTA</td>
<td>59 ± 5.2</td>
<td>86 ± 11</td>
<td>14* ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.77 ± 1.94</td>
<td>1.94 ± 1.17*</td>
<td>1.17* ± 1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.23 ± 0.29</td>
<td>2.09 ± 2.09</td>
<td>1.86 ± 1.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.09 ± 2.09</td>
<td>71 ± 1.86</td>
<td>1.76 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.86 ± 1.86</td>
<td>58 ± 2.58</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.76 ± 1.76</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.81*</td>
<td>58*</td>
<td></td>
</tr>
</tbody>
</table>

*indicates the $K_d$ values were determined from single point calculation. NA - Not Available. References: a) Hyland et al. (2012); b) Fernandez-Fontaina et al. (2013); c) Abegglen et al. (2009); d) Stevens-Garmon et al. (2011); e) Radjenovic et al. (2009); f) Ternes et al. (2004); g) Wick et al. (2009); h) Urase and Kikuta (2005); i) Xue et al. (2010); j) Mazioti et al. (2015); k) Stasinakis et al. (2013); l) Yan et al. (2014)
**Moderately sorptive** ($2 < \log K_d < 3$): Sulfamethoxazole, carbamazepine, and DEET were classified as moderately sorptive. The $K_d$ values for sulfamethoxazole ranged from 188 to 241 L/kg-MLSS ($\log K_d = 2.27-2.38$), which were similar to the value 269 L/kg-MLSS reported by Hyland et al. (2012) and the value 256±169 L/kg-MLSS reported by Gobel et al. (2005). The $K_d$ values for carbamazepine ranged from 134 to 302 L/kg-MLSS ($\log K_d = 2.13-2.48$) with an average $\log K_d$ value for all redox conditions and treatment plants of $2.34 \pm 0.13$ (n=5). Other studies reported much lower sorption distribution coefficients (< 86 L/kg-MLSS) for carbamazepine (Fernandez-Fonataina et al. 2013; Abegglen et al. 2005; Ternes et al., 2004; Urase and Kikuta, 2005; Stevens-Garmon et al., 2011; Hyland et al., 2012; and Wick et al. 2009). Radjenovic et al. (2009) reported a $K_d$ value of 135 L/kg-MLSS for carbamazepine, which is similar to the values reported herein. The $K_d$ values for DEET ranged from 62 to 224 L/kg-MLSS ($\log K_d = 1.79-2.35$). The average $\log K_d$ value for all redox conditions and treatment plants was $2.12 \pm 0.24$ (n=4). Xue et al. (2010) reported sorption distribution coefficients ($\log K_d$) for DEET of 2.93 (aerobic), 3.29 (anoxic), and 3.18 (anaerobic), which are slightly higher than the values found in the current study.

**Lowly sorptive** ($\log K_d < 2$): The $K_d$ values for benzotriazole ranged from 86 to 123 L/kg-MLSS ($\log K_d = 1.94-2.09$) with an average $\log K_d$ value for all redox conditions and treatment plants of $1.88 \pm 0.14$ (n=5). Mazioti et al. (2015) and Stasinakis et al. (2013) reported higher sorption distribution coefficients for benzotriazole of 220 L/kg-MLSS and 133 L/kg-MLSS, respectively.

### 3.2.1 Impact of Redox Condition and SRT

It has been established that there are significant differences in sorption affinity among primary, activated sludge and digested sludge because of the variation in their composition
(Berthod et al., 2016; Horsing et al., 2011). However, it is not clear if any differences occur in the sorption behavior of TOrCs due to different redox conditions of activated sludge. Due to internal recycle rates and different redox conditions within a BNR treatment system, there are changes in the microbial composition of the sludge (Vuono et al., 2015) that could affect the sorption capacity.

Regression plots of log $K_d$ values for the different redox conditions and two treatment plants are shown in Figure 2. Variability in log $K_d$ values of low to moderately sorptive compounds (BTA, CBZ, DEET and SMX) were more than the highly sorptive compound triclosan (Figure 2). All four plots showed high correlation ($R^2 > 0.8$) between redox conditions. The highest correlation was between anoxic and anaerobic activated sludge ($R^2 = 0.95$) with a slope coefficient close to 1. Horsing et al. (2011) found similar log $K_d$ values for sulfamethoxazole in activated sludge with SRTs of 3 days and 10 days. Hyland et al. (2012) did not observe a significant effect of SRT on the sorption capacity for both ionic and neutral TOrCs. They also characterized the aerobic sludge solids used in their $K_d$ estimations and found no significant change in the cation exchange capacity or the organic carbon fraction among six different activated sludge sources. Other sludge characteristics like particle size distribution, zeta potential, and availability of extracellular polymeric substances (EPS) have been shown to influence the sorption of TOrCs like trimethoprim (Khunjar and Love, 2011b). Further studies are required to explore how sludge morphology varies among redox conditions, SRT, and treatment plant configurations and how these factors impact the sorption process.
3.2.2 Hydrophobicity as a predictor for sorption-distribution coefficient

Several studies have proposed that sorption of TOrCs to sludge solids is a function of their hydrophobicity and empirical models based on the octanol-water partition coefficient ($K_{ow}$) have been developed to predict the sorption distribution coefficient. These estimations take the general form of $\log K_d = a \times \log K_{ow} + b$, where $a$ and $b$ are empirical constants determined from

Figure 2 – Plots of $\log K_d$ values between Aerobic vs Anoxic ($\text{Anoxic} \log K_d = 0.78 \times$ Aerobic $\log K_d + 0.79, R^2 = 0.89, n=9$), Aerobic vs Anaerobic ($\text{Anaerobic} \log K_d = 0.73 \times$ Aerobic $\log K_d + 0.89, R^2 = 0.82, n=9$), Anoxic vs Anaerobic ($\text{Anaerobic} \log K_d = 0.96 \times$ Anoxic $\log K_d + 0.09, R^2 = 0.95, n=9$) and OH vs NV (NV $\log K_d = 0.83 \times$ OH $\log K_d + 0.54, R^2 = 0.85, n=13$). Solid line indicates 1:1 ($y=x$) plot.
an experimental dataset. Several models derived from experiment data using activated sludge solids and their predictions are provided in Table 3. Mattermuller et al. (1980) consistently underpredicted the log $K_d$ value. Stevens-Garmon et al. (2011) and Hyland et al. (2012) proposed models to predict the organic-carbon distribution coefficient ($K_{oc}$) for neutral compounds such as triclosan, DEET, and carbamazepine. While the model by Stevens-Garmon et al. (2011) underpredicts the sorption of these compounds, the model by Hyland et al. (2012) shows good prediction (RMSE = 0.16) for triclosan and carbamazepine. The model by Jacobson et al. (1993) ($a = 0.58, b = 1.14$) showed very good predictions for most compounds with RMSE = 0.25. It is interesting to note that empirical constants and predictions from Jacobsen et al. (1993), where the compounds studied were chlorinated phenols and chlorinated benzene, were similar to the constants using data from this study (measured log $K_d$ vs log $K_{ow}$, $a = 0.54, b=1.18$). Given the differences in the suite of chemicals studied by Jacobsen et al. (1993) that had log $K_{ow} > 3.5$ and the compounds evaluated in this study with log $K_{ow}$ values ranging from 0.89 to 4.76, the similarity between the two equations could be entirely coincidental. While these single parameter models provide a plausible estimate of sorption distribution coefficient, it has been proposed that multi-parameter models with descriptors of the chemical and the solid phase might provide more reliable predictions (Horsing et al., 2011; Sathyamoorthy et al., 2013).
Table 3. Comparison of measured sorption-distribution coefficients (mean log $K_{d}$ ± SD) with predicted values using empirical models based on hydrophobicity.

<table>
<thead>
<tr>
<th>TOC</th>
<th>log $K_{ow}$</th>
<th>Measured log $K_{d}$</th>
<th>Predicted log $K_{d}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCS</td>
<td>4.76</td>
<td>3.94 ± 0.1</td>
<td>3.75</td>
</tr>
<tr>
<td>CBZ</td>
<td>2.45</td>
<td>2.26 ± 0.2</td>
<td>2.50</td>
</tr>
<tr>
<td>DEET</td>
<td>2.18</td>
<td>2.12 ± 0.2</td>
<td>2.36</td>
</tr>
<tr>
<td>BTA</td>
<td>1.23</td>
<td>1.77 ± 0.3</td>
<td>1.84</td>
</tr>
<tr>
<td>SMX</td>
<td>0.89</td>
<td>2.04 ± 0.4</td>
<td>1.66</td>
</tr>
<tr>
<td>Root Mean Squared Error</td>
<td></td>
<td>0.24</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Correlation from this study: $\log K_{d} = 0.53 \times \log K_{ow} + 1.18$

Jacobsen et al. (1993): $\log K_{d} = 0.58 \times \log K_{ow} + 1.14$

Stevens-Garmon et al. (2011): For $\log K_{ow} < 2$, $\log K_{oc} = 0.6 \times \log K_{ow} + 0.69$

Hyland et al. (2012): For $\log K_{ow} < 2$, $\log K_{oc} = 0.79 \times \log K_{ow} + 0.47$

$K_{d} = K_{oc} \times f_{oc}$

$K_{oc}$ - Organic-carbon partition coefficient

$f_{oc}$ – Fraction of organic carbon in activated sludge solids = 0.44 from Hyland et al. (2012)

### 3.3 Uncertainties in Parameter Estimation

There are several factors that may contribute to uncertainty in the measurement of biotransformation rates. For example, the biotransformation of Group 3 compounds in this study occurred only under anoxic conditions. The difference in design and operational conditions renders each treatment plant with different biotransformation potential. The concentration of the target compound being spiked may also be different from one study to another, which can result in variability of the results. Some experimental procedures involve synthetic wastewater (Joss et al., 2006; Pomies et al., 2014) that could effectively change the mode of metabolism and even the biotransformation pathway (Muller et al., 2013).
One source of uncertainty with the sorption-distribution coefficient estimations could be from differences in the sludge handling procedure. In particular, techniques of inactivation may vary among studies such as the addition of a chemical biocide (Hyland et al., 2012; Wick et al., 2009), freeze drying and heat inactivation (Sevens-Garmon et al., 2011; Mazioti et al., 2015), and use of argon gas (Ternes et al., 2004). Other experiments avoided procedures to limit biotransformation during the sorption experiment and used a two phase models to predict the sorption-distribution coefficient through curve fitting (Xue et al., 2010), which may not be accurate for slowly sorbing compounds since instantaneous sorption equilibrium is assumed. On comparison of the $K_d$ values for carbamazepine and DEET between Xue et al. (2010) and several others (Table 2), their model overestimated the measured $K_d$ values in this study. Thus, these factors of uncertainties should be carefully considered before adopting the fate parameters from model predictions.

4. Conclusion

This study determined biotransformation rates and sorption-distribution coefficients for select TOrCs in activated sludge from three different redox conditions in two BNR treatment plants configured in A2O process design. The biotransformation rates ($k_{bio}$) were influenced by redox condition and solid retention time of the treatment plants as each treatment plant is unique in terms of its biological composition. A key finding was that a longer SRT may not necessarily mean better biotransformation if relevant microorganisms are not present (triclosan, DEET and benzotriazole) or if the structure of the compound is highly stable (carbamazepine). For compounds amenable to biotransformation by facultative heterotrophs, BNR treatment may be advantageous for their biotransformation (atenolol, trimethoprim and sulfamethoxazole) because the microbes responsible for degradation are circulated among the three redox zones (anaerobic,
anoxic and aerobic). This study has also identified areas of future study using metagenomics techniques to better understand the biotransformation behavior of TOrCs in activated sludge from different redox conditions and SRTs.

There were high correlations between the sorption-distribution coefficients under different redox conditions, with the highest correlation seen between anaerobic and anoxic activated sludge. The impact of redox condition and SRT on the sorption distribution coefficient of the target TOrCs with activated sludge were compound specific. While the parameters measured in this study can be used as inputs to predictive BNR treatment models for estimating effluent concentrations, the biotransformation rates should be used with caution because of uncertainties associated with different activated sludge sources. The sorption-distribution coefficients do not vary much between activated sludge sources or among redox conditions, so their use in predictive models would have less uncertainty (particularly for the highly sorptive compounds).
Acknowledgement

This research was funded by Water Environment and Reuse Foundation (Project # U2R13). The authors would like to acknowledge support from the Southern Nevada Water Authority: Janie Zeigler-Holady and Brittney Stipanov for their assistance with the analysis of TOrCs in the biotransformation experiments, Mandu Inyang, Mac Gifford, Erica Marti, Marco Velarde and Assumption Grimaldi for their assistance in collecting the activated sludge samples; and David Rexing and Jenifer Fuel for administrative support. The authors also thank Zhiqiang Wang at the University of Cincinnati for his assistance with TOrCs analysis in the sorption study. The authors would also like to thank the wastewater treatment plant personnel at both OH and NV for their sampling efforts and for providing operational data at the time of sampling.
References


Pollution Research and Development, Pergamon, 299-314.

and biodegradation of selected benzotriazoles and hydroxybenzothiazole in activated sludge
and estimation of their fate during wastewater treatment. Chemosphere 131, 117-123.

Version 1.0. The Procter & Gamble Company.

triclosan in wastewater treatment systems. Environmental Toxicology and Chemistry 21 (7),
1323-1329.

Modeling Volatile Organic Contaminants' Fate in Wastewater Treatment Plants. Journal of
Environmental Engineering 120 (3), 588-609.

of ammonia oxidizing bacteria and other members of nitrifying activated sludge communities
to micropollutant biotransformation. Water research 109, 217-226.


Radjenović, J., Petrović, M., Barceló, D. 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water research 43 (3), 831-841.


Supplementary Material

Biotransformation and Sorption of Trace Organic Compounds in Biological Nutrient Removal Treatment Systems

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1. Structure and Properties of Selected TOrCs

Table S-1. Structure and physico-chemical properties of selected TOrCs ([http://toxnet.nlm.nih.gov](http://toxnet.nlm.nih.gov)).

<table>
<thead>
<tr>
<th>TOrC</th>
<th>Category</th>
<th>Chemical Structure</th>
<th>Molecular Formula</th>
<th>Molecular Mass</th>
<th>pKₐ</th>
<th>Log Kₐw</th>
<th>H (atm·m³/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>Beta-Blocker</td>
<td><img src="image1" alt="Atenolol Structure" /></td>
<td>C₁₄H₂₂N₂O₃</td>
<td>266.34</td>
<td>9.6</td>
<td>0.16</td>
<td>1.3 × 10⁻¹⁸</td>
</tr>
<tr>
<td>Benzotriazole</td>
<td>Corrosion Inhibitor</td>
<td><img src="image2" alt="Benzotriazole Structure" /></td>
<td>C₆H₅N₃</td>
<td>119.12</td>
<td>0.42/8.2</td>
<td>1.23</td>
<td>NA</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anticonvulsant</td>
<td><img src="image3" alt="Carbamazepine Structure" /></td>
<td>C₁₅H₁₂N₂O</td>
<td>236.27</td>
<td>13.9</td>
<td>2.45</td>
<td>1.1 × 10⁻¹⁰</td>
</tr>
<tr>
<td>DEET</td>
<td>Insect repellent</td>
<td><img src="image4" alt="DEET Structure" /></td>
<td>C₁₂H₁₀NO</td>
<td>191.27</td>
<td>NA</td>
<td>2.18</td>
<td>2.1 × 10⁻⁸</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>Antibiotic</td>
<td><img src="image5" alt="Sulfamethoxazole Structure" /></td>
<td>C₁₀H₁₁N₃O₃S</td>
<td>253.28</td>
<td>1.7/5.6</td>
<td>0.89</td>
<td>6.42 × 10⁻¹³</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Antibacterial</td>
<td><img src="image6" alt="Triclosan Structure" /></td>
<td>C₁₂H₇Cl₂O₂</td>
<td>289.54</td>
<td>8.1</td>
<td>4.76</td>
<td>4.99 × 10⁻⁹</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Antibiotic</td>
<td><img src="image7" alt="Trimethoprim Structure" /></td>
<td>C₁₄H₁₃N₃O₃</td>
<td>290.32</td>
<td>7.12</td>
<td>0.91</td>
<td>2.4 × 10⁻¹⁴</td>
</tr>
</tbody>
</table>
Table S-2. Source of Internal Standard and position of the deuterated label.

<table>
<thead>
<tr>
<th>TorC</th>
<th>Source of ISTD</th>
<th>Structure of Deuterated Internal Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>CDN Isotopes</td>
<td><img src="atenolol.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>CDN Isotopes</td>
<td><img src="carbamazepine.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>DEET</td>
<td>CDN Isotopes</td>
<td><img src="DEET.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>Triclosan</td>
<td>CDN Isotopes</td>
<td><img src="triclosan.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>Benzotriazol</td>
<td>CDN Isotopes</td>
<td><img src="benzotriazol.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>Sulfamethoxazol</td>
<td>Toronto Research Chemicals, Canada</td>
<td><img src="sulfamethoxazol.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>CDN Isotopes</td>
<td><img src="trimethoprim.png" alt="Structure of Deuterated Internal Standard" /></td>
</tr>
</tbody>
</table>
2. WWTP Operational Data

Table S-3. Treatment plant operating conditions during the sampling days for NV WWTP.

<table>
<thead>
<tr>
<th>Date</th>
<th>Flow (MGD)</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Ortho-P (mg-P/L)</th>
<th>Total P (mg-P/L)</th>
<th>NO₃+NO₂ (mg-N/L)</th>
<th>NH₃ (mg-N/L)</th>
<th>TKN (mg-N/L)</th>
<th>cBOD₅ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-Oct-15</td>
<td>39.4</td>
<td>6.85</td>
<td>26.8</td>
<td>0.02</td>
<td>0.06</td>
<td>N/A</td>
<td>&lt;0.10</td>
<td>N/A</td>
<td>&lt;2</td>
</tr>
<tr>
<td>10/20/2015*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-Nov-15</td>
<td>39</td>
<td>6.78</td>
<td>23.3</td>
<td>0.04</td>
<td>0.08</td>
<td>N/A</td>
<td>0.27</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td>11/17/2015*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Jan-16</td>
<td>46.6</td>
<td>6.70</td>
<td>20.5</td>
<td>0.07</td>
<td>0.13</td>
<td>N/A</td>
<td>0.82</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td>1/12/2016*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-May-16</td>
<td>40.9</td>
<td>6.77</td>
<td>25.9</td>
<td>0.16</td>
<td>0.21</td>
<td>N/A</td>
<td>&lt;0.10</td>
<td>N/A</td>
<td>&lt;2</td>
</tr>
<tr>
<td>5/3/2016*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Oct-16</td>
<td>36.7</td>
<td>6.86</td>
<td>26.1</td>
<td>0.27</td>
<td>0.32</td>
<td>N/A</td>
<td>&lt;0.10</td>
<td>N/A</td>
<td>&lt;2</td>
</tr>
<tr>
<td>10/18/2016*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NO₃+NO₂ and TKN were not analyzed on sampling day, results are for the Tuesday of same week as sampled

Table S-4. Treatment plant operating conditions during the sampling days for OH WWTP.

<table>
<thead>
<tr>
<th>Date</th>
<th>Flow (MGD)</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Turbidity (NTU)</th>
<th>Total P (mg-P/L)</th>
<th>cBOD₅ (mg/L)</th>
<th>NH₃ (mg-N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-June-15</td>
<td>5.21</td>
<td>7.4</td>
<td>22</td>
<td>2.3</td>
<td>0.11</td>
<td>3</td>
<td>0.05</td>
</tr>
<tr>
<td>19-July-15</td>
<td>11.23</td>
<td>7.7</td>
<td>22</td>
<td>2.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>19-Sep-15</td>
<td>4.36</td>
<td>7.9</td>
<td>22</td>
<td>1.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA – Not Analyzed
3. LC MS/MS Analysis Instrumental Parameters

Table S-5. MRM transitions and MS operating parameters used for the analysis of TOCs in positive mode (ESI+) and negative mode (ESI-) for biotransformation experiment.

<table>
<thead>
<tr>
<th>Target Analyte</th>
<th>Q1 Mass</th>
<th>Q3 Mass</th>
<th>Retention Time (min)</th>
<th>DP (V)</th>
<th>EP (V)</th>
<th>CE (V)</th>
<th>CXP (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td>254</td>
<td>156</td>
<td>3.7</td>
<td>66</td>
<td>10</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Sulfamethoxazole confirmation</td>
<td>254</td>
<td>92</td>
<td>3.7</td>
<td>66</td>
<td>10</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>Sulfamethoxazole-d₄</td>
<td>258</td>
<td>160</td>
<td>3.7</td>
<td>56</td>
<td>10</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>DEET</td>
<td>192</td>
<td>119</td>
<td>7.9</td>
<td>76</td>
<td>10</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>DEET confirmation</td>
<td>192</td>
<td>91</td>
<td>7.9</td>
<td>76</td>
<td>10</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>DEET-d₇</td>
<td>199</td>
<td>126</td>
<td>7.9</td>
<td>76</td>
<td>10</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Benzotriazole</td>
<td>120</td>
<td>92</td>
<td>4.6</td>
<td>45</td>
<td>12</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>Benzotriazole confirmation</td>
<td>120</td>
<td>65</td>
<td>4.6</td>
<td>45</td>
<td>12</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>Benzotriazole-d₄</td>
<td>124</td>
<td>69</td>
<td>4.6</td>
<td>45</td>
<td>12</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>Atenolol</td>
<td>267</td>
<td>145</td>
<td>3.3</td>
<td>65</td>
<td>10</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Atenolol confirmation</td>
<td>267</td>
<td>116</td>
<td>3.3</td>
<td>65</td>
<td>10</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Atenolol-d₇</td>
<td>274</td>
<td>145</td>
<td>3.3</td>
<td>61</td>
<td>10</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>291</td>
<td>261</td>
<td>5.0</td>
<td>81</td>
<td>10</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>Trimethoprim confirmation</td>
<td>291</td>
<td>123</td>
<td>5.0</td>
<td>81</td>
<td>10</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Trimethoprim-d₉</td>
<td>300</td>
<td>234</td>
<td>5.0</td>
<td>71</td>
<td>10</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>237</td>
<td>165</td>
<td>7.7</td>
<td>90</td>
<td>10</td>
<td>57</td>
<td>10</td>
</tr>
<tr>
<td>Carbamazepine confirmation</td>
<td>237</td>
<td>194</td>
<td>7.7</td>
<td>90</td>
<td>10</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>Carbamazepine-d₁₀</td>
<td>247</td>
<td>204</td>
<td>7.7</td>
<td>61</td>
<td>10</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Triclosan</td>
<td>287</td>
<td>35</td>
<td>5.2</td>
<td>-45</td>
<td>-10</td>
<td>-30</td>
<td>-4</td>
</tr>
<tr>
<td>Triclosan confirmation</td>
<td>289</td>
<td>37</td>
<td>5.2</td>
<td>-45</td>
<td>-10</td>
<td>-30</td>
<td>-4</td>
</tr>
<tr>
<td>Triclosan-d₁</td>
<td>294</td>
<td>37</td>
<td>5.2</td>
<td>-45</td>
<td>-10</td>
<td>-30</td>
<td>-4</td>
</tr>
</tbody>
</table>
Table S-6. MRM transitions and MS operating parameters used for the analysis of TOrcs in positive mode (ESI+) and negative mode (ESI-) for the sorption experiment.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fragmentor Voltage (V)</th>
<th>MRM Transitions (m/z)</th>
<th>Collision Energy</th>
<th>Dwell Time (msec)</th>
<th>Group</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzotriazole</td>
<td>200</td>
<td>120 =&gt; 65</td>
<td>29</td>
<td>100</td>
<td>ESI+</td>
<td>3.0</td>
</tr>
<tr>
<td>Benzotriazole-d$_4$</td>
<td>200</td>
<td>124 =&gt; 65</td>
<td>33</td>
<td>100</td>
<td>ESI+</td>
<td>3.0</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>110</td>
<td>254 =&gt; 156</td>
<td>15</td>
<td>100</td>
<td>ESI+</td>
<td>4.2</td>
</tr>
<tr>
<td>Sulfamethoxazole-d$_4$</td>
<td>110</td>
<td>258 =&gt; 160</td>
<td>25</td>
<td>100</td>
<td>ESI+</td>
<td>4.2</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>110</td>
<td>237 =&gt; 194</td>
<td>15</td>
<td>100</td>
<td>ESI+</td>
<td>5.3</td>
</tr>
<tr>
<td>Carbamazepine-d$_{10}$</td>
<td>110</td>
<td>247 =&gt; 204</td>
<td>15</td>
<td>100</td>
<td>ESI+</td>
<td>5.3</td>
</tr>
<tr>
<td>DEET</td>
<td>110</td>
<td>192 =&gt; 119</td>
<td>15</td>
<td>100</td>
<td>ESI+</td>
<td>5.9</td>
</tr>
<tr>
<td>DEET-d$_7$</td>
<td>110</td>
<td>199 =&gt; 126</td>
<td>15</td>
<td>100</td>
<td>ESI+</td>
<td>5.9</td>
</tr>
<tr>
<td>Triclosan</td>
<td>75</td>
<td>287 =&gt; 35</td>
<td>5</td>
<td>100</td>
<td>ESI-</td>
<td>7.2</td>
</tr>
<tr>
<td>Triclosan-d$_4$</td>
<td>75</td>
<td>294 =&gt; 37</td>
<td>5</td>
<td>100</td>
<td>ESI-</td>
<td>7.2</td>
</tr>
</tbody>
</table>
4. Analysis of Conventional Parameters

Analytical methods for the conventional parameters are provided in Table S-7. Results of subsamples from the field-sample carboys prior to dosing are provided in Table S-8. These samples were taken approximately 90 min prior to dosing the TOrCs.

Table S-7. Analytical Methods for Conventional Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analysis Method</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>Standard Method 4500-H B</td>
</tr>
<tr>
<td>TSS/VSS</td>
<td>Standard Methods 2540 D</td>
</tr>
<tr>
<td>NO₂/NO₃/PO₄</td>
<td>Standard Method 4110B Ion Chromatography</td>
</tr>
<tr>
<td>NH₃</td>
<td>Standard Method 4500-NH₃ D</td>
</tr>
<tr>
<td>TKN</td>
<td>Method 8075 Total Kjeldahl, Hach Company</td>
</tr>
<tr>
<td>COD</td>
<td>Method 8000 COD, Hach Company</td>
</tr>
<tr>
<td>DOC</td>
<td>Standard Method 5310 B</td>
</tr>
<tr>
<td>DO</td>
<td>Standard Method 4500-0 G</td>
</tr>
</tbody>
</table>

Table S-8. Conventional parameter results for activated sludge collected from two BNR treatment plants (OH and NV). Results in Mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Conventional Parameter (mg/L)</th>
<th>OH</th>
<th></th>
<th>NV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.3 ± 0.1</td>
<td>7.0 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>TSS</td>
<td>1713 ± 90</td>
<td>3497 ± 67</td>
<td>1720 ± 87</td>
<td>3860 ± 296</td>
</tr>
<tr>
<td>VSS</td>
<td>1427 ± 101</td>
<td>2613 ± 51</td>
<td>1328 ± 50</td>
<td>2713 ± 205</td>
</tr>
<tr>
<td>Total COD</td>
<td>1897 ± 40</td>
<td>4837 ± 209</td>
<td>1867 ± 91</td>
<td>3317 ± 413</td>
</tr>
<tr>
<td>sCOD</td>
<td>20 ± 5</td>
<td>12.7 ± 2.1</td>
<td>60 ± 6</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>pCOD</td>
<td>1876 ± 0</td>
<td>4824 ± 210</td>
<td>1807 ± 0</td>
<td>3284 ± 0</td>
</tr>
<tr>
<td>DOC</td>
<td>7.4 ± 0</td>
<td>10.7 ± 0</td>
<td>6.2 ± 0.1</td>
<td>NA</td>
</tr>
<tr>
<td>sNH₃-N</td>
<td>4.6 ± 1</td>
<td>0.22 ± 0</td>
<td>24.7 ± 0.7</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>sNO₂-N</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>sNO₃-N</td>
<td>&lt; 0.1</td>
<td>0.11 ± 0</td>
<td>&lt; 0.1</td>
<td>2.4 ± 0</td>
</tr>
<tr>
<td>sPO₄-P</td>
<td>3.5 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>&lt; 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
</tbody>
</table>

NA – Not Analyzed
The conventional parameters results from the six biotransformation experiments are provided in Figures S-1 to S-3. A general observation for the conventional parameters in the biotic and abiotic reactors was that they behaved similarly over the time course of the studies.

**pH:** Initial pH of the original activated sludge from OH was higher than NV for all three redox conditions (Table S-8). For the OH experiments, the buffer addition held the pH close to 8.0 in the two biotic reactors under all three redox conditions and between 7.1 - 7.5 in the abiotic controls for all three redox conditions (Figures S-1 to S-3). Results were similar for the NV activated sludge experiments, except the pH in the anoxic biotic reactors decreased from 8 to 6.9 over the 72 h study period (Figure S-3 b, d). The abiotic controls in all of the NV activated sludge experiments decreased due to the presence of the biocide (aerobic: 7.4 – 6.9; anaerobic: 7.6 – 6.9; anoxic: 7.3 – 6.7). This decrease in pH was due to the removal of alkalinity from solution via precipitation reactions with the biocide (as seen visually).

**Dissolved Oxygen:** A DO > 4 mg/L was maintained in the aerobic experiment reactors and a DO < 0.1 mg/L was maintained in the anoxic and anaerobic experiments reactors.

**Total/Volatile Suspended Solids:** Total and volatile suspended solids (TSS/VSS) were similar in the initial activated sludge from both treatment plants with the exception of VSS in the NV aerobic activated sludge (1910 ± 212 mg/L), which was less than the VSS in the OH activated sludge (2613 ± 51 mg/L). Generally, the TSS and VSS values decreased with time in all experiments indicating a decay of biomass. Also, the solids concentration values were higher in the abiotic control reactor when compared to the biotic reactors, which may be due to the formation of precipitates by the biocide (as seen visually). In the anaerobic experiment using OH activated sludge, the mixer in one of the biotic reactors failed on day 1 and it was not fixed until day 3. So the day 1 solids sample was not representative. Since the reactor remained anaerobic during this time period, the lack of mixing was not expected to affect degradation of the target TOrCs. Thus, a day 5 sample was collected from biotic reactor B for conventional parameters and TOrC analyses.

**Chemical Oxygen Demand:** An increase in total and soluble COD concentrations was observed for the time zero samples. This increase in COD was due, in part, to the addition of methanol in the TOrC spiking solution (30 mg of COD/L), but the methanol does not explain the entire increase in total COD, which may be due to hydrolysis of the solids during the 90 min equilibration period prior to the methanol addition. In general, the pCOD decreased over time which corresponded with a decrease in VSS over time. This decrease in pCOD and VSS suggests that bacterial cell lysis may be occurring during the experiments. The sCOD decreased in both the aerobic experiments (Figure S-1) indicating substrate utilization by heterotrophic microorganisms. The sCOD increased in the anaerobic experiments (Figure S-2) was possibly due to fermentation. In the anoxic experiment sCOD deceased overtime for NV activated sludge (Figures S-3 b, d), whereas in the OH anoxic experiment it either increased (Figure S-3a) or remained the same (Figure S-3c).
**Dissolved Organic Carbon:** Similar to sCOD, DOC in the reactors increased from the original aerobic activated sludge to the time zero sampling and was due, in part, to the methanol addition from the TOrC spiking solution (7.5 mg-C/L). However, this amount of DOC from methanol does not explain the observed increase of 15-20 mg-C/L over the 90 min equilibration period. During the experiments, DOC decreased over time in the aerobic and anoxic biotic reactors due to carbon utilization and by the end of the experiments the DOC concentration had dropped to background levels (Figures S-1 and S-3). DOC in all of the abiotic controls increased over time, which was probably due to cell lysis by the biocide. Again, similar to sCOD, DOC increased in the biotic reactors of the OH anaerobic experiment (Figures S-2 a, c), which could be due to fermentation.

**Nutrients (NH$_3$-N, NO$_2$-N, NO$_3$-N and PO$_4$-P):** Concentrations of soluble NH$_3$-N (< 0.1 mg/L), NO$_2$-N (< 1.0 mg/L), NO$_3$-N (< 1.0 mg/L), and PO$_4$-P (< 1.0 mg/L) in the original aerobic activated sludge from OH were quite low indicating that the BNR treatment process was operating properly for removing N and P from the wastewater. In the OH aerobic experiment, the phosphate spike (10 mg PO$_4$-P/L) was readily taken up during the 90 min equilibration period prior to time zero indicating the phosphate accumulating bacteria were active with phosphate values near the original activated sludge concentration of 1.5 mg/L. Phosphorus uptake was also observed in the NV aerobic experiment. A significant increase in NO$_3$-N and decrease in NH$_3$-N was seen in both the aerobic experiments (Figures S-1 a-d) indicating nitrification by nitrifying bacteria. Nitrate uptake during the anoxic experiments are shown in Figures S-3 a-d. The nitrate concentration in the reactors increased overtime due to the addition of 30 mg NO$_3$-N/L every 12 hours to maintain anoxic conditions. Overall, the nutrient trends demonstrated not only that relevant redox conditions were maintained, but also relevant microbial population were active through the experiment.
Figure S-1. Conventional parameter results in aerobic biotransformation experiments.
Figure S-2. Conventional parameter results in anaerobic biotransformation experiments.
Figure S-3. Conventional parameter results in anoxic biotransformation experiments.
5. TOrCs Analysis

Figures S-4 (a,b,c,d). Biotransformation profiles for atenolol and trimethoprim in biotic and abiotic reactors containing activated sludge from aerobic, anaerobic and anoxic redox conditions in OH and NV BNR treatment plants.
Figures S-4 (e,f,g,h). Biotransformation profiles for carbamazepine and sulfamethoxazole in biotic and abiotic reactors containing activated sludge from aerobic, anaerobic and anoxic redox conditions in OH and NV BNR treatment plants.
Figures S-4 (i, j, k, l). Biotransformation profiles of DEET, benzotriazole in biotic and abiotic reactors containing activated sludge from aerobic, anaerobic and anoxic redox conditions in OH and NV BNR treatment plants.
Figures S-4 (m,n). Biotransformation profiles for triclosan in biotic and abiotic reactors containing activated sludge from aerobic, anaerobic and anoxic redox conditions in OH and NV BNR treatment plants.
6. Analysis of Blanks and Controls

The background concentration of the target compounds in the original activated sludge before spiking are provided in Figure S-5. Benzotriazole, a corrosion inhibitor, was found to be occurring at very high concentrations in the activated sludge samples from both treatment plants. The background concentration of triclosan was higher in OH when compared to NV, whereas the background concentration of sulfamethoxazole was slightly greater in NV when compared to OH. The background concentrations of atenolol, carbamazepine, DEET and trimethoprim were always close to or below the reporting limit. Little to no loss of the TOrCs was observed in the experimental control (DI water) reactors (Figure S-6), though some loss was observed for triclosan presumably due to sorption onto the reactor walls.

![Figure S-5. TOrCs background concentrations in aerobic, anaerobic and anoxic activated sludge from OH and NV BNR treatment plants. Reporting limit 250 ng/L for all experiments except aerobic NV where it was 2500 ng/L.](image)
Figure S-6. Relative percent difference in TOC concentrations between the initial and final time point in Experimental Control Reactor containing buffered DI water. Error Bars indicate the standard deviation in measurements from six biotransformation experiments.

Figure S-7. Atenolol biotransformation inhibition in abiotic control containing 10 mM biocide solution and 8% methanol. Sludge source – Anaerobic NV.