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# Impact of Biological Wastewater Treatment on the Reactivity of N-Nitrosodimethylamine (NDMA) Precursors

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# IMPACT OF BIOLOGICAL WASTEWATER TREATMENT ON THE REACTIVITY OF *N*-NITROSAMINE PRECURSORS

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## ABSTRACT

*N*-Nitrosamines are a group of probable human carcinogens associated with $10^{-6}$ lifetime cancer risks at low ng/L levels in drinking water. *N*-nitrosamines can form via the reactions between chloramines (i.e., monochloramine, dichloramine) and organic precursors (e.g., secondary, tertiary and quaternary amines). Municipal wastewater effluents are considered one of the major sources of *N*-nitrosamine precursors that can impact downstream drinking water qualities. Although many studies have investigated the formation of *N*-nitrosamines in the influents and effluents of wastewater treatment plants (WWTPs), the major sources of *N*-nitrosamine precursors are still largely unknown. The first objective of this research was to evaluate the occurrences of *N*-nitrosamine precursors in different sewage components (i.e., blackwaters and greywaters). Results showed that urine blackwater (i.e., raw human urine diluted in tap water 250 times) showed exceptionally high NDMA formation potential (FP, >10000 ng/L). The FP of the other *N*-nitrosamines tested were more than one magnitude lower than NDMA FP. Urine blackwater was the predominant contributor to NDMA (i.e., >90%) and *N*-nitrosopyrrolidine (NPYR) FP (i.e., 65%) in domestic sewage, while laundry greywater was the major source of most other *N*-nitrosamine FP (i.e., 55%-100%). In contrast, *N*-nitrosamine formation under the uniform formation condition (UFC) from all sewage components was generally <100 ng/L, far lower than *N*-nitrosamine FP.

Because of the huge discrepancies between *N*-nitrosamine UFC and FP, the potential effects of different factors (i.e., pH, dissolved organic carbon (DOC), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), $Br^-$) on NDMA UFC and FP were examined.

Under different pH conditions (i.e., pH 6.0, 6.8, 7.8 and 8.8), all model NDMA precursors tested achieved peak NDMA UFC at pH 6.8-7.8 in DDW, regardless of compounds' pKa values (i.e., 3.8-13.6). The peak NDMA FP tended to be achieved at a relatively higher pH than NDMA UFC. In surface waters with higher DOC or $SUVA_{254}$, NDMA UFC tended to be lower, while NDMA FP tended to increase with increasing DOC. The effects of $Br^-$ (i.e., 1000 μg/L) on NDMA UFC depended on pH. Linear regression analysis indicated that NDMA UFC poorly correlated ($R^2$ = 0.04-0.06, n = 17) with NDMA FP in different surface waters.

The removal of NDMA FP and removal of NDMA UFC from model NDMA precursors were then evaluated during batch activated sludge (AS) treatment tests. Among the four model compounds tested, trimethylamine (TMA) and minocycline (MNCL) were readily removed (i.e., 77%-100% removals of NDMA FP) during 24-h AS treatment, ranitidine (RNTD) was moderately removed (i.e., 34%-87% removals of NDMA FP), and sumatriptan (SMTR) was the least removable (i.e., 29%-46% removals of NDMA FP). Increasing incubation time (or hydraulic retention time (HRT)) and solids retention time (SRT) favored the removal of NDMA FP from RNTD. Biosorption was found to be the major deactivation pathway of the amine-based pharmaceuticals (i.e., RNTD, MNCL and SMTR) tested, while biodegradation was the major deactivation pathway of TMA. Adding different biostimulants (e.g., glucose, acetate, benzoate and ammonia) insignificantly affected the removal of NDMA FP from RNTD. Non-specific oxygenase (i.e., phenol 2-monooxygenase) may play an insignificant role affecting the removal of NDMA FP from RNTD, especially at extended incubation time (i.e., 5-20 d). Removal of NDMA UFC from

the tested compounds was generally comparable to the removal of their NDMA FP, except for MNCL which yielded negligible NDMA UFC before and after AS treatment.

Finally, removal of *N*-nitrosamine precursors from sewage components (i.e., blackwaters and greywaters) and WWTP influents during the AS treatment was investigated under both FP and UFC tests. Removal of *N*-nitrosamine FP from sewage components depended on precursor sources (i.e., blackwaters and greywaters) and *N*-nitrosamine species. Increasing incubation time from 6 to 24 h enhanced the removal of *N*-nitrosamine FP. Removal of *N*-nitrosamine FP from WWTP influents mainly depended on AS sources during the batch treatment tests, rather than the types of wastewater influents. Among the three AS (i.e., domestic rural, domestic urban and textile AS) tested, the rural domestic AS showed relatively higher removal of *N*-nitrosamine FP from biologically originated precursor sources (e.g., urine blackwater, shower greywater not containing any personal care products, and kitchen greywater containing food leachates only). On the other hand, the textile AS exhibited higher removal of *N*-nitrosamine FP from sewage components containing detergents or personal care products (e.g., shower greywater containing shampoo, kitchen greywater containing dishwashing detergent, and laundry greywater containing laundry detergent). Different from *N*-nitrosamine FP, *N*-nitrosamine UFC from most sewage components increased after 6 or 24-h AS treatment.

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| | |
|---|---|
| 4-EACTC | 4-Epianhydrochlortetracycline |
| 4-EATC | 4-Epianhydrotetracycline |
| 4-ECTC | 4-Epichlortetracycline |
| 4-EOTC | 4-Epioxytetracycline |
| 4-ETC | 4-Epitetracycline |
| ACS | American Chemical Society |
| ACTC | Anhydrochlortetracycline |
| AT | Amitriptyline |
| ATC | Anhydrotetracycline |
| ATCC | American Type Culture Collection |
| AZM | Azithromycin |
| A/O | Anaerobic/Oxic |
| A/A/O | Anaerobic/Anoxic/Oxic |
| AS | Activated Sludge |
| BF | Biological Filter |
| BOD | Biological Oxygen Demand |
| Cal/EPA | California Environmental Protection Agency |
| CAS | Conventional Activated Sludge |
| CCL4 | Contaminant Candidate List 4 |
| CDPH | California Department of Public Health |
| CI | Chemical Ionization |
| CLA | Clarithromycin |
| CLI | Clomipramine |
| COD | Chemical Oxygen Demand |
| CPZ | Chlorpromazine |
| CTC | Chlortetracycline |
| DBP | Disinfection By-product |
| DCM | Dichloromethane |
| DDW | Distilled and Deionized Water |
| DEA | Diethylamine |
| DEET | Diethyltoluamide |
| DEM | Demeclocycline |
| DL | Detection Limit |
| DMA | Dimethylamine |
| DMAN | *N*,*N*-dimethylaniline |
| DMBA | *N*,*N*-dimethylbutylamine |
| DMBzA | *N*,*N*-dimethylbenzylamine |
| DMEA | *N*,*N*-dimethylethylamine |
| DMEDA | *N*,*N*-dimethylethylenediamine |
| DMiPA | *N*,*N*-dimethylisopropylamine |

| | |
|---|---|
| DMNZD | Daminozide |
| DMPhA | *N*,*N*-dimethylphenethylamine |
| DN | Dissolved Nitrogen |
| DO | Dissolved Oxygen |
| DOX | Doxycycline |
| DPD | *N*,*N*-diethyl-p-phenylenediamine |
| DPH | Diphenhydramine |
| DPR | Direct potable reuse |
| DRN | Diuron |
| DTZ | Diltiazem |
| DVS | Desvenlafaxine |
| DXP | Doxepin |
| EDTA | Ethylenediaminetetraacetic Acid |
| EED | Exogenous Electron Donor |
| ERY | Erythromycin·$H_2O$ |
| FAS | Ferrous Ammonium Sulfate |
| FBR | Fluidized Bed Reactor |
| FID | Flame Ionization Detector |
| FP | Formation Potential |
| GC | Gas Chromatogram |
| HAA | Haloacetic Acid |
| HRT | Hydraulic Retention Time |
| HSDB | Hazardous Substances Data Bank |
| IC | Ion Chromatography |
| ICTC | Iso chlortetracycline |
| MB | Methylene Blue |
| MEM | Meropenem |
| MET | Metformin |
| MLSS | Mixed Liquor Suspended Solids |
| MNCL | Minocycline |
| MRL | Minimum Reporting Level |
| MS | Mass Spectrum |
| NB | Nutrient Broth |
| NDBA | *N*-Nitrosodi-n-butylamine |
| NDEA | *N*-Nitrosodiethylamine |
| NDMA | *N*-Nitrosodimethylamine |
| NDPA | *N*-Nitrosodi-n-propylamine |
| NDPhA | *N*-Nitrosodiphenylamine |
| NMEA | *N*-Nitrosodimethylamine |
| NMOR | *N*-Nitrosomorpholine |
| NOM | Natural Organic Matter |
| NPIP | *N*-Nitrosopiperidine |
| NPYR | *N*-Nitrosopyrrolidine |
| OD | Oxidation Ditch |

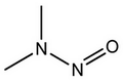
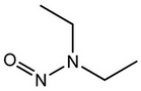
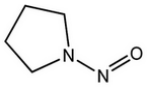
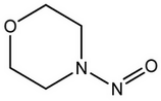
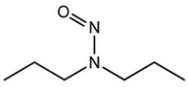
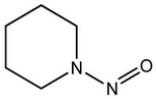
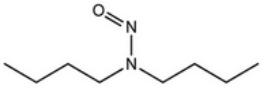
| | |
|---|---|
| OD-PABA | Octyl dimethyl-p-aminobenzoic acid |
| OPPTS | Office of Prevention, Pesticides and Toxic Substances |
| OTC | Oxytetracycline |
| PDS | Primary Dilution Solution |
| PHP | Potassium Hydrogen Phthalate |
| PolyDADMAC | Poly (diallyldimethylammonium chloride) |
| PPCP | Pharmaceutical and Personal Care Products |
| QSBR | Quantitative Structure-Biodegradability Relationship |
| RNTD | Ranitidine |
| ROX | Roxithromycin |
| RSD | Relative Standard Deviation |
| SBR | Sequencing Batch Reactor |
| SM | Standard Method |
| SMTR | Sumatriptan |
| SPE | Solid Phase Extraction |
| SPI | Spiramycin |
| SR | Suwannee River |
| SRT | Solid Retention Time |
| SVI | Sludge Volume Index |
| TCA | Tricarboxylic Acid |
| TCD | Thermal Conductivity Detector |
| TCE | Trichloroethylene |
| TCN | Tetracycline |
| THM | Trihalomethane |
| TKN | Total Kjeldahl Nitrogen |
| TMA | Trimethylamine |
| TRA | Tramadol |
| TSS | Total Suspended Solids |
| TYL | Tylosin |
| UCMR | Unregulated Contaminant Monitoring Rule |
| UDMH | 1,1-dimethylhydrazine |
| UFC | Uniform Formation Condition |
| US EPA | United States Environmental Protection Agency |
| VLFX | Venlafaxine |
| WHO | World Health Organization |
| WWTP | Wastewater Treatment Plant |

# CHAPTER I

# INTRODUCTION

Chloramination has been increasingly used as an alternative disinfection strategy to chlorination in the United States (US), to reduce the formation of regulated disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs). However, chloramination can cause the formation of *N*-nitrosamines (**Table 1.1**). *N*-Nitrosamines can form via the reactions between chloramines (i.e., monochloramine ($NH_2Cl$), dichloramine ($NHCl_2$)) and organic precursors (such as secondary, tertiary and quaternary amines) in drinking waters and wastewaters (Mitch et al., 2003; Oya et al., 2008). Among the seven most reported *N*-nitrosamines, *N*-nitrosodimethylamine (NDMA) was the most frequently detected *N*-nitrosamine in US drinking waters (Krasner et al., 2008; Boyd et al., 2011; Wang et al., 2011; Russell et al., 2012; Uzun et al., 2015; Zeng et al., 2016a). Once formed, NDMA is difficult to remove, because of its high solubility and continuous formation in water distribution systems (Shen and Andrews, 2011a). Controlling NDMA precursors, rather than NDMA itself, can be thus a more effective strategy to reduce NDMA formation in drinking water. One major source of NDMA precursors is municipal wastewater discharge, which can impact downstream source water qualities (Krasner et al., 2013; Sgroi et al., 2018).

**Table 1.1.** Key information of the *N*-nitrosamines that can be analyzed by US EPA Method 521.

| *N*-nitrosamines | Chemical structure | Molecular weight (g/mol) | log $K_{ow}$[a] | $10^{-6}$ cancer risk level[b] (ng/L) |
|---|---|---|---|---|
| *N*-Nitrosodimethylamine (NDMA) |  | 74.0 | -0.57 | 0.7 |
| *N*-Nitrosodiethylamine (NDEA) |  | 102.1 | 0.48 | 0.2 |
| *N*-Nitrosopyrrolidine (NPYR) |  | 100.1 | -0.19 | 15 |
| *N*-Nitrosomorpholine (NMOR) |  | 116.1 | -0.44 | 5 |
| *N*-Nitrosodi-n-propylamine (NDPA) |  | 130.1 | 1.36 | 5 |
| *N*-Nitrosopiperidine (NPIP) |  | 114.1 | 0.36 | 3.5 |
| *N*-Nitrosodi-n-butylamine (NDBA) |  | 158.1 | 2.63 | 3 |

[a]: HSDB (2019). [b]: US EPA (2001).

The presence of *N*-nitrosamine precursors in secondary effluents can pose challenges to direct potable reuse of wastewater. In direct potable reuse, secondary wastewater effluents are further purified with advanced treatment trains typically containing microfiltration and reverse osmosis followed by an advanced oxidation process (e.g., UV/$H_2O_2$). The treated effluents are then directly blended into drinking water treatment plant influents or distribution systems (Gerrity et al., 2013). In directly reused

wastewaters, NDMA has frequently equaled or exceeded its California Notification Level of 10 ng/L (Zeng et al., 2016b; Takeuchi et al., 2018), while other *N*-nitrosamines (e.g., *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR)) have had concentrations exceeding those associated with $10^{-6}$ lifetime cancer risks (Zeng et al., 2016b; Takeuchi et al., 2018; Sgroi et al., 2018). Since the application of wastewater reuse is increasing globally to provide alternative water sources, and advanced wastewater treatment cannot completely remove all *N*-nitrosamine precursors, it is thus important to control *N*-nitrosamine precursors from secondary wastewater effluents (Casey, 2015; Zeng et al., 2016b; Sgroi et al., 2018). So far, however, the sources, fates and removal of different *N*-nitrosamine precursors during biological wastewater treatment have not been comprehensively studied.

The sources of NDMA precursors present in wastewater influents have not been elucidated. Although dimethylamine (DMA) and trimethylamine (TMA) are common ingredients of human urine and feces, their contributions to NDMA FP in wastewater influents have been estimated to be only 11%-21% and 4%-8%, respectively (Mitch and Sedlak, 2004; Wang et al., 2014). Many pharmaceuticals and personal care products (PPCPs) such as ranitidine (RNTD), azithromycin (AZM), clarithromycin (CLA), chlortetracycline (CTC), erythromycin (ERY), roxithromycin (ROX) and tetracycline (TCN) have been shown to be NDMA precursors (Mamo et al., 2016; Shen and Andrews, 2011b). The mean concentrations of RNTD in wastewater influents were generally found to be <0.61 μg/L (Gros et al., 2012). RNTD is a potent NDMA precursor, with its NDMA yields under the formation potential (FP, 100 mg $Cl_2$/L monochloramine dosage, 5-d

contact time) test being approximately 80%-90% on a molar basis (Selbes et al., 2013). Based on its NDMA FP yield and mean concentrations in wastewater influents (Gros et al., 2012), RNTD contributes only <130 ng/L of NDMA FP in wastewater influents on average, which is quite low compared to the thousands of ng/L of NDMA FPs measured in WWTP influents (Sedlak et al., 2005). Other PPCPs such as AZM, CLA, CTC, ERY, ROX and TCN are also at low concentrations (i.e., <5 μg/L) in wastewater influents (Gros et al., 2006; Gobel et al., 2005; Tran et al., 2016; Gobel et al., 2007; Blair et al., 2018; Archer et al., 2017; Ben et al., 2018; Yan et al., 2014; Guerra et al., 2014; Senta et al, 2013; Santos et al., 2013; Gros et al., 2012; Yasojima et al., 2006; Verlicchi et al., 2014). Based on their chemical structures (i.e., none or two carbon atoms between a ring structure and DMA group), the NDMA yields of most of these PPCPs are likely to be low (i.e., <1 % on a molar basis) (Selbes et al., 2013), and their contributions to NDMA FP are thus unlikely to be important. Moreover, some quaternary amines such as tetramethylamine, cetyltrimethylamine, choline, and cocamidopropyl betaine that can be frequently used in manufacturing pharmaceuticals or personal care products, have been found to exhibit low *N*-nitrosamine FP yields (i.e., <0.2% on a molar basis; Kemper et al., 2010).

A recent study indicated that rather than industrial wastewater, domestic and commercial sewage are likely to be the major sources of NDMA precursors (Chuang et al., 2019). In domestic sewage, the occurrences of *N*-nitrosamine precursors from different components (i.e., blackwaters and greywaters) have been investigated. Under a practical chloramination condition (i.e., uniform formation condition (UFC) test; 2-5 mg $Cl_2$/L monochloramine dosage, 1-3 d contact time), laundry greywater was found to be the major

source of *N*-nitrosamine precursors, followed by shower greywater and urine blackwater (Mitch and Sedlak, 2015). However, the UFC test cannot convert all precursors to *N*-nitrosamines and thus cannot measure the amounts of NDMA precursors (Krasner et al., 2013). To evaluate the concentrations of *N*-nitrosamine precursors and compare the formations of *N*-nitrosamines under two different chloramination conditions (i.e., UFC and FP), the FP test with the same samples was required. During the FP test, an excessive monochloramine dosage (i.e., 100-140 mg $Cl_2$/L) and a sufficiently long contact time (i.e., 5-10 d) is applied to convert all precursors into *N*-nitrosamines (Mitch et al., 2003; Mitch and Sedlak, 2004; Selbes et al., 2013, 2014; Uzun et al., 2015; Zeng et al., 2016).

Results from NDMA UFC have been poorly correlated with results from NDMA FP in different water and wastewater samples (Zeng et al., 2016). Although many factors (i.e., pH, bromide, natural organic matters (NOM)) are known to affect NDMA formation, their respective effects on NDMA UFC and NDMA FP are still unclear (Selbes et al., 2013; Shen and Andrews, 2011a, 2013a; Uzun et al., 2015; Zhang et al., 2015). Further, most studies of the factors impacting NDMA formation have focused on single-factor effects in a pure water matrix (i.e., distilled and deionized water (DDW)). In natural surface waters, however, multiple factors co-exist and can simultaneously affect NDMA formation, because there has been no single factor fully attributable to NDMA formation in surface waters (Uzun et al., 2015). To better understand the relationships between NDMA UFC and NDMA FP, it is necessary to investigate the multi-factor effects on NDMA formation in different water matrices (e.g., DDW, NOM solutions, and surface waters).

During biological wastewater treatment (e.g., the activated sludge (AS) process), NDMA precursors can be deactivated to various degrees. Precursors such as DMA and TMA have been shown to be readily biodegradable with >75% and >71% removal during the AS process, respectively (Mitch and Sedlak, 2004; Sedlak et al., 2005; Wang et al., 2014). Other precursors such as dimethylformamide and dimethylaminobenzene were less removed by the AS process (e.g., 54%-68% and 57%-72% removal, respectively) (Wang et al., 2014). RNTD was removed variably (20%-91%) during the AS process at different WWTPs (Castiglioni et al., 2006; Gros et al., 2006; Sedlak and Kavanaugh, 2006; Radjenovic et al., 2007; Guerra et al., 2014). Overall NDMA precursor levels that were measured under the FP test could be removed by 5%-98% during the AS process (Mitch and Sedlak, 2004; Sedlak et al., 2005; Wang et al., 2014; Sgroi et al., 2018). Under certain circumstances, NDMA FP even increased after AS treatment (Sedlak et al., 2005). So far, little is known about the deactivation efficiencies of other NDMA precursors (such as amine-based PPCPs) during the AS process, their deactivation pathways (e.g., via biodegradation, biosorption, and volatilization processes), and potential factors affecting their deactivation efficiencies. More importantly, the potential biodegradation mechanism of NDMA precursors (e.g., roles of enzymes) is still largely unknown.

Domestic wastewater influents contain human urine and feces, and laundry, shower, washbasin and kitchen greywaters as major constituents (Friedler et al., 2013; Zeng and Mitch, 2015). However, the relative importance of different sewage components as sources of NDMA precursors before and after AS treatment has not been fully understood. Compared to NDMA precursors, other *N*-nitrosamine (e.g., NDEA, NPYR, *N*-

nitrosopiperidine (NPIP), NMOR, *N*-nitrosodi-n-propylamine (NDPA) and *N*-nitrosodi-n-butylamine (NDBA)) precursors have been much less investigated for their removal during the AS process. At a municipal WWTP equipped with a conventional activated sludge (CAS) process, NPYR FP was removed by 60% and 75% during summer and fall, respectively, and NPIP FP were removed by 75% and 70%, respectively (Krauss et al., 2010). However, higher removal of NPYR FP (i.e., 93%-96%) were found at three WWTPs in Japan, where NPIP FP was removed by 61%-81%, and NMOR FP was removed by 66%-100% (Yoon et al., 2013). For potential improvements of biodegradation efficiencies, addition of exogenous electron donors (EEDs; or biostimulation) has been applied for in-situ bioremediation at contaminated sites with chlorinated solvents (Adams et al., 2015). The application of EEDs, however, has not been fully studied on the removal efficiencies of *N*-nitrosamine precursors during biological wastewater treatment.

Although many previous studies have investigated the formation of *N*-nitrosamines in WWTP influents and effluents, there is much more to learn about the major sources and fates of *N*-nitrosamine precursors during the AS process. Questions such as which sewage components lead to the most *N*-nitrosamine formation, the relationship between *N*-nitrosamine UFC and NDMA FP, removal efficiencies of *N*-nitrosamine precursors varying with precursor sources and treatment conditions, the interactions between biotic and abiotic processes, and the roles of enzyme activities affecting the deactivation efficiencies of *N*-nitrosamine precursors have not been elucidated yet. The major goal of this research was to gain insight into the sources and fates of *N*-nitrosamine precursors during the AS process, especially under different chloramination conditions (i.e., UFC and

FP tests). Specifically, this study focused on (i) the importance of different sewage components as potential sources of *N*-nitrosamine precursors, (ii) comparison of NDMA formation under the UFC and FP protocols, (iii) the potential impacts of biological wastewater treatment on the reactivities of model NDMA precursors, and (iv) the efficiencies of the AS process in deactivating *N*-nitrosamine precursors from different sewage components and the relevant factors.

# CHAPTER II

# LITERATURE REVIEW

## Occurrence of *N*-Nitrosamines in Wastewater

NDMA is a nitrogenous disinfection by-product that may form via the reactions between disinfectants (e.g., chloramines, ozone) and nitrogen-containing organic precursors in drinking water (Mitch et al., 2002, 2003a). NDMA is a potent carcinogen which poses $10^{-6}$ lifetime excess cancer risk at the level of 0.6 ng/L in drinking water (EPA, 2001). Data collected under the second Unregulated Contaminants Monitoring Rule (UCMR2) showed that NDMA was detected in nearly 70% of the chloraminated drinking water systems in the US, with its concentrations near to or above the minimum reporting level (MRL) of 2 ng/L (Russell et al., 2012). Further, in more than one third of the chloraminated water samples in the US, NDMA has been detected (Russell et al., 2012). In California, the public health goal of 3 ng/L and a Notification Level of 10 ng/L have been established for NDMA in drinking water (Cal/EPA, 2006; CDPH, 2010). In Massachusetts, the regulatory limit for NDMA in drinking water was set at 10 ng/L (MassDEP, 2004).

In addition to NDMA, several other *N*-nitrosamines are frequently detected in drinking waters including NMEA, NDEA, NPYR, NPIP, NMOR, NDPA and NDBA (USEPA, 2004). Among these *N*-nitrosamines, NDMA is the most frequently detected *N*-nitrosamine (Krasner et al., 2008; Russell et al., 2012; Uzun et al., 2015). Due to their adverse health risks, six *N*-nitrosamines have been included in the list of US EPA's

UCMR2, including NDMA, NDEA, NMEA, NDPA, NDBA and NPYR (Richardson, 2006). Five *N*-nitrosamines including NDEA, NDMA, NDPA, NPYR and NDPhA have been included in the EPA's Contaminant Candidate List (CCL) 4 for possible regulation in the near future (USEPA, 2016). Further, authorities in many countries and regions, such as those in European Union (EU) and World Health Organization (WHO), have established or are working to establish guidelines to address the health issues related to *N*-nitrosamines in drinking waters (Health Canada, 2010; UK DWI, 2000; Selin, 2011).

Once formed, *N*-nitrosamines are difficult to remove from drinking waters, because of their relatively high solubility and continuous formation in drinking water distribution system due to the slow reactions between chloramines and *N*-nitrosamine precursors (Shen and Andrews, 2011a). A promising strategy for controlling *N*-nitrosamine formation in drinking water is to reduce *N*-nitrosamine precursors present in source waters, especially wastewater-impacted ones. Municipal wastewater effluents are considered one of the major sources of *N*-nitrosamine precursors that can impact downstream source water qualities (Krasner et al., 2013). Although wastewater influents could contain abundant *N*-nitrosamine precursors, the concentrations of *N*-nitrosamines, rather than their precursors, are generally low in wastewater influents (**Table 2.1**). Mean concentrations of NDMA have been reported to range from 5 to 20 ng/L in the primary effluents from 21 full-scale WWTPs in Switzerland, although its peak concentrations reached 1000 ng/L (Krauss et al., 2009). NMOR was found to be relatively abundant in all 21 WWTPs ranging between 5-30 ng/L; other *N*-nitrosamines were detected in a smaller number of WWTPs, but at similar levels to NMOR (i.e., 5-30 ng/L) (Krauss et al., 2009). In the influents from three WWTPs

in China, NPIP, NDMA and NPYR were predominantly detected, with their concentrations ranging from tens to hundreds ng/L (Liu et al., 2019). At nine WWTPs in the US and one WWTP in Australia, NDMA and NOMR were found to be the most prevalent *N*-nitrosamines in the primary effluents (Gerrity et al., 2015). The occurrence levels of NDMA were typically at ~ 25 ng/L in most investigated WWTPs, ranging from nondetectable to 89 ng/L; NMOR concentrations ranged between 50-67 ng/L; NMEA and NDEA were detected in only one WWTP, while NDPA and NDBA were not detected in any of the investigated WWTPs (Gerrity et al., 2015). Similarly, a recent study indicated that NDMA and NMOR were the most frequently detected *N*-nitrosamines in different types (i.e., municipal, domestic, commercial and industrial) of wastewaters, while other *N*-nitrosamines were rarely detected except in metal finishing discharges (Chuang et al., 2019).

**Table 2.1.** Occurrences of *N*-nitrosamines in wastewaters and their removal during biological treatment.

| WWTP[a] location | Number of WWTPs | Influent types | Biological treatment process | *N*-nitrosamines | Concentrations in influents (ng/L) | | Concentrations in secondary effluents (ng/L) | | Removal (%) | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Mean | Range | Mean | Range | Mean[i] | |
| Switzerland | 1 | N.A.[b] | CAS[c] | NDMA | N.A. | 23 | N.A. | 5 | N.A. | 66±35 | Hollender et al., 2009 |
| Switzerland | 20 | Mainly domestic | A/O[d], A/A/O[e], or FBR[f] | | N.D.[h]-89 | 14 | N.D.-33 | 5 | 0-100 | 64 | Krauss et al., 2009 |
| Switzerland | 1 | Mainly domestic | A/A/O | | N.D.-1000 | 22 | <2-188 | 4 | 0-100 | 82 | Krauss et al., 2009 |
| US | 1 | N.A. | CAS | | N.A. | <25 | N.A. | 11 | N.A. | <56 | Gerrity et al., 2015 |
| US | 1 | N.A. | OD[g] | | N.A. | 25 | N.A. | <5 | N.A. | >80 | Gerrity et al., 2015 |
| US | 1 | N.A. | A/A/O | | N.A. | 42 | N.A. | 6.8 | N.A. | 84 | Gerrity et al., 2015 |
| US | 1 | N.A. | CAS | | N.A. | 89 | N.A. | 72 | N.A. | 19 | Gerrity et al., 2015 |
| AUS | 1 | N.A. | CAS | | N.A. | <25 | N.A. | <5 | N.A. | N.A. | Gerrity et al., 2015 |
| US | 2 | N.A. | A/A/O | | N.A. | <25 | N.A. | <5 | N.A. | N.A. | Gerrity et al., 2015 |
| US | 6 | N.A. | CAS, OD, or A/A/O | NMEA | N.A. | <25 | N.A. | <5 | N.A. | N.A. | Gerrity et al., 2015 |
| AUS | 1 | N.A. | OD | | N.A. | <25 | N.A. | <5 | N.A. | N.A. | Gerrity et al., 2015 |
| Switzerland | 21 | Mainly domestic | A/O, A/A/O, or FBR | | N.D. | N.D. | N.D. | N.D. | N.A. | N.A. | Krauss et al., 2009 |
| Switzerland | 1 | N.A. | CAS | NDEA | N.A. | 5 | N.A. | 3 | N.A. | 40 | Hollender et al., 2009 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Switzerland | 20 | Mainly domestic | A/O, A/A/O, or FBR | | N.D.-25 | 5 | N.D.-5 (1 at 24)[j] | <5 | <0-90 | N.A. | Krauss et al., 2009 |
| Switzerland | 1 | Mainly domestic | A/A/O | | 1-20 | 5 | <1-8 | 2 | <0-100 | 60 | Krauss et al., 2009 |
| US | 6 | N.A. | CAS, OD or A/A/O | | N.A. | <50 | N.A. | <10 | N.A. | N.A. | Gerrity et al., 2015 |
| AUS | 1 | N.A. | CAS | | N.A. | <50 | N.A. | <10 | N.A. | N.A. | Gerrity et al., 2015 |
| Switzerland | 1 | N.A. | CAS | NPYR | N.A. | N.D. | N.A. | N.D. | N.A. | N.A. | Hollender et al., 2009 |
| Switzerland | 1 | N.A. | CAS | | N.A. | 9 | N.A. | 6 | N.A. | 40±29 | Hollender et al., 2009 |
| Switzerland | 20 | Mainly domestic | A/O, A/A/O, or FBR | | 3-31 | 15 | 3-26 | 7 | <0-83 | 53 | Krauss et al., 2009 |
| Switzerland | 1 | Mainly domestic | A/A/O | | 3-30 | 7 | 2-24 | 5 | <0-77 | 29 | Krauss et al., 2009 |
| US | 1 | N.A. | CAS | NMOR | N.A. | <50 | N.A. | 12 | N.A. | <76 | Gerrity et al., 2015 |
| US | 1 | N.A. | OD | | N.A. | 67 | N.A. | 21 | N.A. | 69 | Gerrity et al., 2015 |
| US | 2 | N.A. | CAS or A/A/O | | N.A. | <50 | N.A. | <10 | N.A. | N.A. | Gerrity et al., 2015 |
| AUS | 1 | N.A. | CAS | | N.A. | <50 | N.A. | <20 | N.A. | N.A. | Gerrity et al., 2015 |
| US | 2 | N.A. | A/A/O | | N.A. | <50 | N.A. | 11 | N.A. | <78 | Gerrity et al., 2015 |
| Switzerland | 1 | N.A. | CAS | NDPA | N.A. | N.D. | N.A. | N.D. | N.A. | N.A. | Hollender et al., 2009 |
| Switzerland | 20 | Mainly domestic | A/O, A/A/O, or FBR | | N.D. | N.D. | N.D (1 at 12) | N.D. | N.A. | N.A. | Krauss et al., 2009 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Switzerland | 1 | Mainly domestic | A/A/O | | N.D. | N.D. | N.D. | N.D. | N.A. | N.A. | Krauss et al., 2009 |
| US | 6 | N.A. | CAS, OD or A/A/O | | N.A. | <100 | N.A. | <20 | N.A. | N.A. | Gerrity et al., 2015 |
| AUS | 1 | N.A. | CAS | | N.A. | <100 | N.A. | <20 | N.A. | N.A. | Gerrity et al., 2015 |
| Switzerland | 1 | N.A. | CAS | | N.A. | 6 | N.A. | <1 | N.A. | >90 | Hollender et al., 2009 |
| Switzerland | 20 | Mainly domestic | A/O, A/A/O, or FBR | NPIP | N.D.-25 | 9 | <5 | <5 | <0-100 | >45 | Krauss et al., 2009 |
| Switzerland | 1 | Mainly domestic | A/A/O | | 1-20 | 5 | <1-8 | 2 | 67-100 | 40 | Krauss et al., 2009 |
| Switzerland | 1 | N.A. | CAS | | N.A. | 3 | N.D. | <1 | N.A. | >70 | Hollender et al., 2009 |
| Switzerland | 20 | Mainly domestic | A/O, A/A/O, or FBR | | N.D.-25 | 6 | <5 (1 at 19) | <5 | <0-100 | >17 | Krauss et al., 2009 |
| Switzerland | 1 | Mainly domestic | A/A/O | NDBA | N.D. | N.D. | N.D. | N.D. | <0-71 | N.A. | Krauss et al., 2009 |
| US | 6 | N.A. | CAS, OD or A/A/O | | N.A. | <100 | N.A. | <20 | N.A. | N.A. | Gerrity et al., 2015 |
| AUS | 1 | N.A. | CAS | | N.A. | <100 | N.A. | <20 | N.A. | N.A. | Gerrity et al., 2015 |

[a]: Wastewater treatment plants. [b]: Not available. [c]: Conventional activated sludge process. [d]: Anaerobic/oxic process. [e]: Anaerobic/anoxic/oxic process. [f]: Fluidized bed reactor. [g]: Oxidation ditch. [h]: Not detected. [i]: Estimated based on the mean concentrations measured in primary effluents and secondary effluents at WWTPs. [j]: One sample was detected with 24 ng/L NDEA.

So far, the main sources of *N*-nitrosamines present in wastewater influents are still largely unknown. Zeng and Mitch (2015) estimated the occurrence levels of *N*-nitrosamines in different sewage components (i.e., urine and feces blackwaters, laundry, shower, bathroom washbasin and kitchen greywaters). NDMA was detected in all greywaters, with mean concentrations of 9, 5, 5 and 3 ng/L in laundry, shower, bathroom washbasin and kitchen greywaters, respectively. NDEA were detected only in shower (i.e., 4 ng/L) and bathroom washbasin (i.e., 5 ng/L) greywaters, NMOR detected only in laundry (i.e., 15 ng/L) and shower (i.e., 4 ng/L) greywaters, and NPYR detected only in urine (i.e., 3 ng/L) and feces (i.e., 3 ng/L) blackwaters. These levels were at the lower ends of those reported in wastewater influents. Human urine excretion was considered to account for <5 ng/L of NDMA and <1 ng/L of other *N*-nitrosamines in wastewaters, while other components (i.e., feces blackwater and greywaters) may contribute <5 ng/L of NMOR (Krauss et al., 2009). Levels above these are presumably contributed by non-domestic discharges.

Industrial discharge might be a more importance source of *N*-nitrosamines than domestic sewage. Total *N*-nitrosamines were found to be significantly ($p$ <0.05) higher in industrial impacted WWTPs than domestic only WWTPs (Lee and Oh, 2016). Extremely high NPIP (~ 300 ng/L) and NMOR (~ 400 ng/L) concentrations were found in WWTPs located in industrial areas in Japan, while in domestic WWTPs, their concentrations were <20 and <150 ng/L, respectively (Yoon et al., 2013). Rubber, pharmaceuticals, dye, textile and tire manufacturing processes are known to involve the applications of chemicals

containing or producing *N*-nitrosamines that can be released to industrial discharges (Glover et al., 2019).

## ***N*-Nitrosamine Precursors from Wastewater**

To determine the level of *N*-nitrosamine precursors in water and wastewater samples, the FP and UFC tests have been used. The FP test uses an excessive monochloramine dosage (i.e., 100-140 mg $Cl_2$/L) and a sufficiently long reaction time (i.e., 5-10 d) to convert all precursors to *N*-nitrosamines (Krasner et al., 2013). Therefore, *N*-nitrosamine FP can be an indicator of the total amounts of *N*-nitrosamine precursors. In contrast, the UFC test adopts less of a monochloramine dosage (i.e., 2-5 mg $Cl_2$/L) and a shorter reaction time (i.e., 1-3 d), aiming to simulate the typical chloramination conditions practically used in water and wastewater treatment utilities. Differing from the FP test, the *N*-nitrosamine UFC test reflects the formation of *N*-nitrosamines under chloramination conditions typically found in water distribution systems or wastewater treatment facilities. The details of chloramination conditions adopted for *N*-nitrosamine FP and UFC tests as reported in previous studies are summarized in **Table 2.2** and **2.3**, respectively.

**Table 2.2.** Chloramination conditions applied for NDMA FP tests.

| *N*-nitrosamine precursors | pH | Monochloramine dose (mg $Cl_2$/L) | Contact time (d) | Study |
|---|---|---|---|---|
| 20 PPCPs[a] | 7.0 | 28.4 | 1 | Shen and Andrews, 2011a |
| 3 NOM concentrates | 6-9 | 71 | 5 | Chen and Valentine, 2006 |
| 21 amines | 7.5 | 100 | 5 | Selbes et al., 2013 |
| 12 amines & 3 polymers | 7.5 | 100 | 5 | Selbes et al., 2014 |
| 12 surface waters | 7.8 | 100 | 5 | Uzun et al., 2015 |
| 43 surface waters | 7.0 | 140 | 10 | Zeng et al., 2016 |
| 13 compounds, 8 WW constituents, 4 organisms | 6.9 | 140 | 10 | Mitch and Sedlak, 2004 |
| Wastewater influents and effluents, 4 surface waters | 6.1-9.5 | 140 | 7-14 | Mitch et al., 2003 |
| 8 compounds | 4-5.5, 8.5, 10 | 200-300 | 5 | Le Roux et al., 2011 |

[a]: Pharmaceuticals and personal care products.

**Table 2.3.** Chloramination conditions applied for NDMA UFC tests.

| *N*-nitrosamine precursors | pH | Monochloramine dose (mg $Cl_2$/L) | Contact time (d) | Study |
|---|---|---|---|---|
| 2 surface waters, 4 wastewaters | 8 | 2.0[a] | 3 | Beita-Sandi and Karanfil, 2017 |
| 20 PPCPs | 7.0 | 2.5 | 1 | Shen and Andrews, 2011a, b |
| 2 pharmaceuticals | 6-9 | 2.5 | 3-5 | Shen and Andrews, 2013b |
| 7 drinking waters, 2 river waters | 8 | 2.5[b] | 3 | Shah et al., 2012 |
| NOM fractions | 7.0 | 3.5 | <5 | Chen and Valentine, 2007 |
| 43 surface waters | 8 | 5 | 3 | Zeng et al., 2016 |
| 13 compounds, 8 WW constituents, 4 organisms | 6.9 | 7 | 4 h | Mitch and Sedlak, 2004 |

[a]: Monochloramine residual after 3-d contact time. [b]: Monochloramine residual after 3 min.

In primary effluents of seven different WWTPs in California, NDMA FPs ranged between 2497-17950 ng/L with a mean level of 6383 ng/L (Sedlak and Kavanaugh, 2006). In the influents of twelve different WWTPs in Japan, NDMA FPs ranged between 1070-8230 ng/L, with a mean level of 3953 ng/L (Yoon et al., 2011). The FPs of other *N*-nitrosamines were found to be much lower than NDMA FPs. In a municipal WWTP in Japan, NDEA FPs and NPYR FPs were found to be ~500 and ~600 ng/L, respectively, which are significantly lower than NDMA FPs (i.e., ~2000 ng/L), while NPIP FPs, NMOR FPs and NDBA FPs were all <100 ng/L (Yoon et al., 2013). In the primary effluents of a municipal WWTP in Belgium, NDMA FPs ranged between 2500-4500 ng/L, NPYR FPs 150-250 ng/L, NPIP FPs 50-100 ng/L, while the FPs of other *N*-nitrosamines were below their detection limits (i.e., 1-4 ng/L) (Krauss et al., 2010).

NDMA precursors in wastewaters are considered to come from anthropogenic sources, including domestic sewage (e.g., blackwaters and greywaters), industrial discharges (e.g., tannery, textile and tire wastewaters) and PPCPs (Shen and Andrews, 2011b; Zeng et al., 2016; Zeng and Mitch, 2015). Domestic sewage and commercial sewage were considered to be major sources of NDMA precursors present in wastewater influents, while industrial discharges may not be significant sources of NDMA precursors (Chuang et al., 2019). However, extremely high NDMA FPs (i.e., 81000±5400 ng/L) were found in a WWTP influent impacted by industrial discharges, presumably because of dimethylamides present in pesticide (e.g., diuron), solvent (e.g., dimethylformamide) and rubber (e.g., thiram) manufacturing wastewaters that can be hydrolyzed to DMA (Sedlak and Kavanaugh, 2006). Further, exceptionally high NDMA FPs in secondary effluents (i.e.,

27100-28900 ng/L) were observed at a WWTP using cationic polymers as flocculants in a secondary clarifier, which served as NDMA precursor sources (Sedlak and Kavanaugh, 2006). This may suggest that amine-based polymers could be important sources of NDMA precursors present in wastewater effluents.

Under realistic chloramination conditions (i.e., UFC test), *N*-nitrosamine formation from different sewage components (i.e., blackwaters and greywaters) were monitored, and their relative importance as sources of *N*-nitrosamine precursors were evaluated based on their volume fractions in domestic sewage. Among the different sewage components tested, laundry greywater was found to be the major source of *N*-nitrosamine precursors, followed by shower greywater and urine blackwater (Mitch and Sedlak, 2015). However, the UFC test provides negligible information regarding the total amounts of *N*-nitrosamine precursors present in domestic sewage. Instead, the UFC test is more related to the *N*-nitrosamine formation under the typical chloramination condition used in water and wastewater utilities. The amounts of *N*-nitrosamine precursors present in sewage components need to be evaluated by conducting FP tests under an excessive monochloramine dosage (i.e., 100-140 mg $Cl_2$/L) and a prolonged reaction time (i.e., 5-10 d).

DMA and TMA are typical NDMA precursor compounds present in human urine and feces. The concentrations of DMA and TMA in human urine have been estimated to be ~200 and ~2 μM, respectively (Tsikas et al., 2007; Svensson et al., 1994; Lee et al., 2010). According to the volume fraction (i.e., 22%) of urine blackwater (i.e., raw urine diluted in 6-L toilet flush) in domestic sewage (Friedler et al., 2013), ~45 μg/L of DMA

and ~0.6 µg/L of TMA may be present in domestic sewage. As shown in the primary effluents of seven WWTPs in California, DMA concentrations ranged between 43-120 µg/L, with a mean level of 81 µg/L (Sedlak and Kavanaugh, 2006). Based on the reported NDMA FP yields from DMA and TMA (i.e., 1.2% and 1.9% on a molar basis, respectively; Selbes et al., 2013) and their mean concentrations reported in primary effluents, DMA is estimated to contribute 900 ng/L NDMA FP in domestic sewage, while TMA contributed only 14 ng/L NDMA FP. The contributions of DMA and TMA to the total NDMA FP in wastewater influents were estimated to be only 11%-21% and 4%-8%, respectively (Mitch and Sedlak, 2004; Wang et al., 2014). After biological wastewater treatment, DMA was found to still contribute 14%±3% NDMA FP in secondary effluents at some WWTPs (Mitch and Sedlak, 2004).

Many pharmaceuticals that contain one or more DMA moieties in their chemical structures are potential NDMA precursors, such as RNTD, AZM, CLA, CTC, ERY, ROX and TCN. The concentrations of these compounds in wastewater influents are summarized in **Table A-1** in **Appendix A**. RNTD is a potent NDMA precursor exhibiting 90% NDMA FP yields on a molar basis (Selbes et al., 2013). RNTD concentrations in wastewater influents varied between undetectable and 1.5 µg/L, with a mean concentration of less than 0.61 µg/L at most WWTPs (Gros et al., 2012). However, exceptionally high RNTD concentrations (i.e., 11-12 µg/L) were detected in two municipal WWTPs in UK (Kasprzyk-Hordern et al., 2009). Other amine-based pharmaceuticals were also detected in these two WWTPs at relatively high levels, including tramadol (i.e., 86-89 µg/L), doxycycline (6.8-10.0 µg/L), amitriptyline (5.1-6.7 µg/L), and diltiazem (3.2-5.3 µg/L)

(Kasprzyk-Hordern et al., 2009). As shown in **Table A-1**, the concentrations of AZM in WWTP influents ranged from undetectable to 3.0 µg/L, with its mean concentration less than 2.0 µg/L (Tran et al., 2016). Similarly, the concentrations of CLA ranged between 0.005 and 1.9 µg/L, with its mean concentration ranging between 0.2-1.5 µg/L measured at different WWTPs (Castiglioni et al., 2006; Senta et al., 2013; Tran et al., 2016). ERY is among the most frequently detected NDMA precursors in wastewater influents, with its concentrations generally ranging between undetectable and 2.7 µg/L, and its mean concentration <0.8 µg/L at different WWTPs (Radjenovic et al., 2009). Other frequently detected amine-based pharmaceuticals such as CTC, TCN, ROX and OXY ranged between undetectable and 1.0 µg/L, with their mean concentrations <0.5 µg/L (Ben et al., 2018; Zhang et al., 2018; Guerra et al., 2014; Kim et al., 2013; Gao et al., 2012). One exception is a municipal WWTP located in Singapore, where relatively high concentrations of CTC (i.e., 2.3-15.9 µg/L, mean 6.4 µg/L), OXY (i.e., 1.6-30.1 µg/L, mean 4.9 µg/L) and TCN (i.e., 1.2-12.3 µg/L, mean 3.6 µg/L) were detected in its influents (Tran et al., 2016). Although these compounds were potential NDMA precursors present in wastewater influents, their mean concentrations are generally low (i.e., <5 µg/L). Based on their chemical structures, their NDMA yields are likely to be low (i.e., <1 % on a molar basis), because none or two carbon atoms are present between a ring structure and the DMA moiety, which has been shown to cause less NDMA formation during chloramination (Selbes et al., 2013).

## Biological Removal of *N*-Nitrosamine Precursors

Global climate change (e.g., extreme draughts) and population growth have caused increasing water shortage worldwide (US EPA, 2017). To address this challenge, direct potable reuse (DPR) of wastewater is increasingly a part of drinking water supply (US EPA, 2017). The presence of *N*-nitrosamines and their precursors in wastewater effluents used as influents for DPR may however pose potential health risks. NDMA concentrations were found to frequently equal or exceed the California Notification Level of 10 ng/L (CDPH, 2010) in wastewaters for potable reuse (Zeng et al., 2016b; Fujioka et al., 2013; Takeuchi et al., 2018). Other *N*-nitrosamines such as NDEA, NPYR and NMOR may exceed their $10^{-6}$ cancer risk levels (Zeng et al., 2016b; Takeuchi et al., 2018). Because the advanced wastewater treatment (i.e., $H_2O_2$/UV, membrane filtration process) for DPR cannot completely remove *N*-nitrosamines or their precursors, especially for low-molecular-weight NDMA (Zeng et al., 2016b), a better control of *N*-nitrosamine precursors from secondary wastewater effluents would be important.

WWTP effluents have been known as major sources of NDMA precursors which can negatively impact the water qualities in downstream utilities (Krasner et al., 2013; Shah and Mitch, 2012; Gerecke and Sedlak, 2003). At WWTPs, NDMA precursors from domestic sewage are subject to primary, secondary and occasionally tertiary treatment. Primary treatment mostly removes particle-associated precursors. Secondary biological treatment (usually the AS process) has shown different removal efficiencies of various NDMA precursors (Mitch and Sedlak, 2004; Sedlak et al., 2005; Yoon et al., 2011; Wang et al., 2014). Precursors such as DMA and TMA were readily removed (>75% and >71%

removal, respectively) during biological treatment (**Table 2.4**). Less removal was observed for dimethylformamide (54%-68%) and dimethylaminobenzene (57%-72%) (Wang et al., 2014). RNTD which exhibits a relatively high (e.g., 90%) NDMA molar yield under FP chloramination condition (Selbes et al., 2013) was removed variably at different WWTPs, with its removals ranging from <0% to 100% (**Table 2.5**). Other PCPPs such as AZM, CLA, CTC, ERY, ROX and TCN were also removed variably during biological treatment at WWTPs (**Table A-1**). The overall removal of NDMA precursors which were determined by the changes of NDMA FPs, ranged from 10% to 98% at various WWTPs (Mitch and Sedlak, 2004; Sedlak et al., 2005; Wang et al., 2014; Yoon et al., 2011). Under certain circumstances, NDMA FP even increased after biological wastewater treatment (Gros et al., 2006; Yu et al., 2012). The reasons for such inconstant removal efficiencies of NDMA precursors during biological wastewater treatment are still largely unknown.

Precursors of other *N*-nitrosamines in wastewaters were removed at different levels by biological wastewater treatment. Krauss et al. (2010) reported that ~65% and ~75% of NPYR precursors were removed by the AS process at a municipal WWTP, and ~85% and ~75% of NPIP precursors were removed. Yoon et al. (2013) reported ~20% of NDEA precursors, >95% of NPYR precursors, >60% of NPIP precursors, >65% of NMOR precursors and >90% of NDBA precursors were removed by the AS process at three WWTPs. Despite these studies, knowledge about the removal of *N*-nitrosamine precursors other than NDMA is still limited.

**Table 2.4.** Reported removal of dimethylamine (DMA) and trimethylamine (TMA) during biological wastewater treatment.

| Compound | WWTP location | Influent type/ industrial impact[a] | Biological treatment process | Season | Removal (%) | References |
|---|---|---|---|---|---|---|
| DMA | California, US | Municipal | CAS | winter | 98 | Mitch and Sedlak, 2004 |
| | California, US | | | spring | 87.2 | |
| | California, US | | | winter | >87.3 | |
| | Nevada, US | | | winter | >96 | |
| | California, US | | | spring | 98.4 | |
| | California, US | | | spring | >98.5 | |
| DMA | California, US | <2% | CAS | spring | 100 | Sedlak et al., 2005 |
| | California, US | 4% | | spring | 40; 50 | |
| | California, US | 18% | | winter; spring | >95, >90 | |
| | California, US | Municipal | | autumn | 100 | |
| DMA | California, US | Municipal | Trickling filter | winter | >84.6 | Mitch and Sedlak, 2004 |
| | California, US | | | spring | >96.5 | |
| DMA | Laboratory test | Synthetic wastewater | Respirometric batch test | winter | 100 | Zhang et al., 2014 |
| | | Municipal | | spring | 94 | |
| | | Textile | | spring | 64 | |
| DMA; TMA | Shanghai, China | 10% | CAS | spring | 96[b]; 80[c] | Wang et al., 2014 |
| | | 0% | A/O | | 90; 73 | |
| | | 50% | A/A/O | | 80; 71 | |
| | | 30% | Carrousel oxidation ditch (OD) | | 93; 75 | |
| | | 70% | Modified sequencing batch reactor (MSBR) | | 80; 75 | |
| DMA; TMA | Laboratory test | Aqueous solution | Aerated biofilter (BF) | N.A. | >75; >90 | Hwang et al., 1994 |

[a]: Percentage of industrial inflow (%) in WWTP influent. [b]: Removal of DMA. [c]: Removal of TMA.

**Table 2.5.** Reported removal of RNTD during biological wastewater treatment.

| WWTP location | Influent Type | Biological treatment Processes | HRT[a] (h) | SRT[b] (d) | Concentrations in influents (µg/L) | | Removal (%) | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Mean | | |
| South Africa | Municipal/industrial | A/O and MLE[c] | N.A. | N.A. | 0.05-0.24 | N.D. | 100 | Archer et al., 2017 |
| Spain | Municipal/industrial | MBR[d] | 14 | N.A. | 0.11-0.80 | 0.30 | 95 | Radjenovic et al., 2007 |
| Spain | Municipal | CAS | 32 | N.D. | N.D.-0.29 | 0.19 | 91 | Gros et al., 2006 |
| UK | Municipal | CAS and OD | N.A. | N.A. | <0.01-11.66 | 1.73 | 90 | Kasprzyk-Hordern et al., 2009 |
| Italy | Municipal | N.A. | N.A. | N.A. | N.A. | N.A. | 84 (summer) | Castiglioni et al., 2006 |
| Spain | N.A. | N.A. | N.A. | N.A. | N.A. | 0.61 | 81 | Gros et al., 2012 |
| Spain | Municipal | CAS | 32 | N.A. | N.A. | N.A. | 78 | Gros et al., 2010 |
| | | A/A/O | 40 | 16 | N.D.-0.33 | 0.13 | 75 | Jelic et al., 2011 |
| Spain | Municipal | CAS | 33 | | | | 70 | Gros et al., 2006 |
| Spain | N.A. | N.A. | N.A. | N.A. | N.A. | 0.59 | 70 | Gros et al., 2012 |
| US | Municipal | CAS | 10 | N.A. | 0.04 | N.A. | 57 | Blair et al., 2015 |
| Switzerland | N.A. | A/O | 17 | 16.5 | N.A. | 0.42±0.16 | 55 | Kern et al., 2010 |
| Spain | Municipal/industrial | CAS | 8 | | | | 54 | Gros et al., 2006 |
| Spain | Municipal | CAS | 8 | | | | 52 | Gros et al., 2006 |
| Spain | Municipal/industrial | CAS | 25 | | | | 50 | Gros et al., 2006 |
| Spain | Municipal | CAS | 8.5 | N.A. | N.A. | N.A. | 46 | Gros et al., 2010 |
| Spain | Municipal/industrial | CAS | 12 | 3 | 0.11-0.80 | 0.30 | 42 | Radjenovic et al., 2007 |
| Spain | Municipal | CAS and tertiary treatment | 33 | 10 | 0.05-0.55 | 0.2 | 40 | Jelic et al., 2011 |
| UK | Municipal/industrial | Biofiltration | N.A. | N.A. | 2.01-11.15 | 5.06 | 40 | Kasprzyk-Hordern et al., 2009 |
| Spain | Municipal/industrial | MF[e] MBR | 7.2 | N.A. | 0.07-0.54 | 0.35 | 44±30 | Radjenovic et al., 2009 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Italy | Municipal | N.A. | N.A. | N.A. | N.A. | N.A. | 39 (winter) | Castiglioni et al., 2006 |
| Spain | Municipal | Biological filters | 18 | N.A. | N.D.-0.29 | 0.19 | 37 | Gros et al., 2006 |
| Spain | Municipal/industrial | A/A/O | N.A. | N.A. | N.D.-1.47 | 0.52 | 31 | Rosal et al., 2010 |
| Spain | Municipal/industrial | UF[f] MBR | 15 | N.A. | 0.07-0.54 | 0.35 | 30±48 | Radjenovic et al., 2009 |
| Spain | Municipal/industrial | A/O | 11.5 | 10 | 0.07-0.54 | 0.35 | 25±45 | Radjenovic et al., 2009 |
| | | Laboratory batch test | N.A. | N.A. | 2000 | | 22[g] | Carucci et al., 2006 |
| Spain | Municipal | CAS | 20 | 6 | N.D.-0.45 | 0.12 | 20 | Jelic et al., 2011 |
| Spain | Municipal/ hospital | Tricking filter | N.A. | N.A. | 0.068-0.27 | 0.18 | 11±762 | Santos et al., 2013 |
| | | Laboratory batch test | 8 | 14 | 2000 | | 0 | Carucci et al., 2006 |
| Spain | Municipal | CAS | 10 | N.A. | N.D.-0.29 | 0.19 | -33[h] | Gros et al., 2006 |
| Hong Kong | N.A. | A/O | 11 | N.A. | N.A. | 0.02 | -347 | Yu et al., 2012 |
| Hong Kong | N.A. | A/O | N.A. | N.A. | N.A. | 0.18 | -1138 | Yu et al., 2012 |

[a]: Hydraulic retention time. [b]: Solid retention time. c: Modified Ludzack-Ettinger process. [d]: Membrane biological reactor. [e]: Microfiltration. [f]: Ultrafiltration. [g]: Removal by laboratory batch test which was not measured at WWTPs. [h]: Negative removal indicates an increase in RNTD concentration after treatment.

The removal of specific *N*-nitrosamine precursors (such as model compounds) has been also investigated at WWTPs. As shown in **Table A-1**, among the tens of different NDMA precursor compounds surveyed in wastewater influents and effluents, AZM, CTC, CLA, desvenlafaxine (DVS), doxycycline (DOX), ERY, oxytetracycline (OTC), ROX, TCN, tramadol (TRA), tylosin (TYL) and venlafaxine (VLFX) were the most frequently detected. Most of these compounds exhibited widely varying removals from <0% to 75%-100% during biological wastewater treatment, except for DVS (i.e., <0%-29%), TRA (i.e., <0%-40%), VLFX (i.e., <0%-55%) and TYL (i.e., 87%; single measurement) which showed more consistent removal. The reasons causing large variations in the removal of most reported NDMA precursors are still largely unclear, suggesting a necessity to investigate the factors affecting biological removal of NDMA precursors.

Some factors were found to possibly affect the removal efficiencies of *N*-nitrosamine precursors during biological wastewater treatment, such as treatment processes (e.g., CAS, membrane bioreactor (MBR)) and nitrification performance. Compared with CAS process, removal of PPCPs were considered slightly higher in an MBR due to the increased sludge age and complete interception of suspended solids from effluents (Siegrist and Joss, 2012). At WWTPs equipped with an effective nitrification process, the occurrences of NDMA precursors determined by FP tests of secondary effluents were lower than those of WWTPs with a poor nitrification process (Krasner et al., 2008). Similarly, in an MBR with a fully nitrifying system, NDMA FPs were reduced by >94%, while this removal was reduced to 72% when nitrification was minimized (Mamo et al., 2016). For the selected model NDMA precursors, average removal also decreased from

68% to 59% for AZM, 31% to 17% for citalopram, 35% to 15% for VLFX, and 61% to 16% for ERY after the nitrification process in the MBR was minimized (Mamo et al., 2016). However, removal of CLA, o-desmethylvenlafaxine and RNTD were found to be less affected by nitrification inhibition (Mamo et al., 2016). These results suggest that nitrification may pose different effects on the removal of different NDMA precursors.

In addition, the effects of WWTP operational conditions on the removal of NDMA precursors may depend on specific compounds. In a survey conducted for 73 PPCPs in seven WWTPs in Spain, the removal efficiencies of compounds showed three distinct patterns during wastewater treatment, including (i) increased after treatment, (ii) insignificantly reduced or moderately removed, and (iii) readily removed after treatment (Gros et al., 2010). NDMA precursors such as TCN and RNTD were found to belong to the second category (i.e., moderately removable), with their average removals ranging between 40%-70% during wastewater treatment (Gros et al., 2010). For compounds exhibiting negligible or high removal during wastewater treatment, increasing hydraulic retention time (HRT) may not affect their removal efficiencies (Gros et al., 2010). For moderately removable compounds (such as TCN and RNTD), however, increasing HRT could increase their removal efficiencies at WWTPs (Gros et al., 2010). These findings further demonstrated that the effects of certain factors could be highly compound specific.

## Biodegradation Pathways of *N*-Nitrosamine Precursors

Aromatic NDMA precursors are among the most persistent pollutants, with their stability arising from the delocalization of π electrons (resonance structures) (Diaz, 2008). Monooxygenase and dioxygenase insert one and two atoms of oxygen into the aromatic rings, respectively, and the subsequent ring cleavage step is catalyzed by ring-cleaving oxygenases. As a result, a wide variety of aromatic compounds are transformed to central metabolites (catechols, gentisates and hydroquinones) by a limited number of enzymes, and then to metabolites that can be incorporated into the tricarboxylic acid (TCA) cycle (Diaz, 2008). Several bacterial/fungal strains, including five heterotrophs (i.e., *Pseudomonas putida (P. putida), Rhodococcus sp.* Strain YU6, *Arthrobacter sp.* JS443, *Bacillus pumilus* and *Phanerochaete chyrsosporium*) and one autotroph (i.e., *Nitrosomonas europaea (N. europaea)*) are known to express non-specific oxygenase cleaving ring structures in different aromatic compounds, and/or oxidizing long-chain aliphatic compounds (Wackett et al., 1988; Harayama et al., 1991; Nakagawa and Takeda, 1962; Gerginova et al., 2007; Kobayashi et al., 1989; Eaton and Chapman, 1992; Seeger et al., 1995; Small and Ensign, 1995; Beilen et al., 1994; Jang et al., 2005; Jain et al., 1994; Moody et al., 2001; Hammel et al., 1992; Caranto and Lancaster, 2017). Many ring-cleaving oxygenases such as toluene dioxygenase, phenol monooxygenase and ammonia monooxygenase are known to non-specifically catalyze biodegradation of various halogenated and nonhalogenated aromatics, heterocyclic aromatics, and nitroaromatics (Arciero et al., 1989; Chen et al., 2008; Robertson et al., 1992; Semak et al., 2012; Chang

et al., 2002). The reported biodegradation pathways of aromatic compounds catalyzed by ammonia monooxygenase and benzoate dioxygenase are illustrated in **Figures 2.1**.

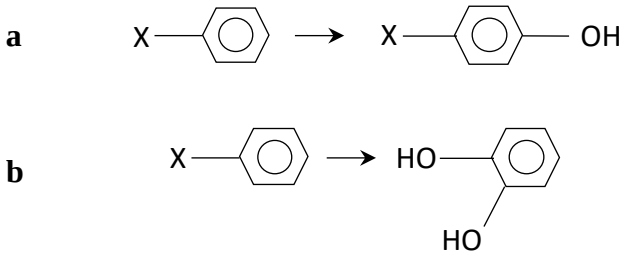



**Figure 2.1.** Proposed biodegradation pathways of aromatic compounds catalyzed by (a) ammonia monooxygenase and (b) benzoate dioxygenase.

So far, few studies have investigated the biodegradation pathways for NDMA precursors. The formation of DMA has been reported during biological treatment of cationic surfactants and some dyes (e.g., methyl orange, methyl violet) (Friedli, 2001; Van Ginkel, 1995; Chen et al., 2008; Kalyani et al., 2009; Mansour et al., 2009; Ayed et al., 2010; Yan et al., 2009; Chen et al., 2010; Parshetti et al., 2010; Oh et al., 2014; Cheng et al., 2012; Nouren and Bhatti, 2015), as illustrated in **Figure 2.2**. Further, based on the known metabolic pathways of aromatic compounds catalyzed by some typical oxidizing enzymes (i.e., ammonia monooxygenase and benzoate dioxygenase), more biodegradation pathways of selected NDMA precursors with relatively simple chemical structures have been proposed in **Figures 2.3** and **2.4**. For compounds with more complex chemical structures, their biodegradation pathways could be preliminarily predicted by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) - Biocatalysis/Biodegradation Database (BBD) Pathway Prediction System. As an example,

the predicted potential biodegradation pathways of RNTD (e.g., via demethylation, hydroxylation followed by oxidation) are shown in **Figure 2.5**.

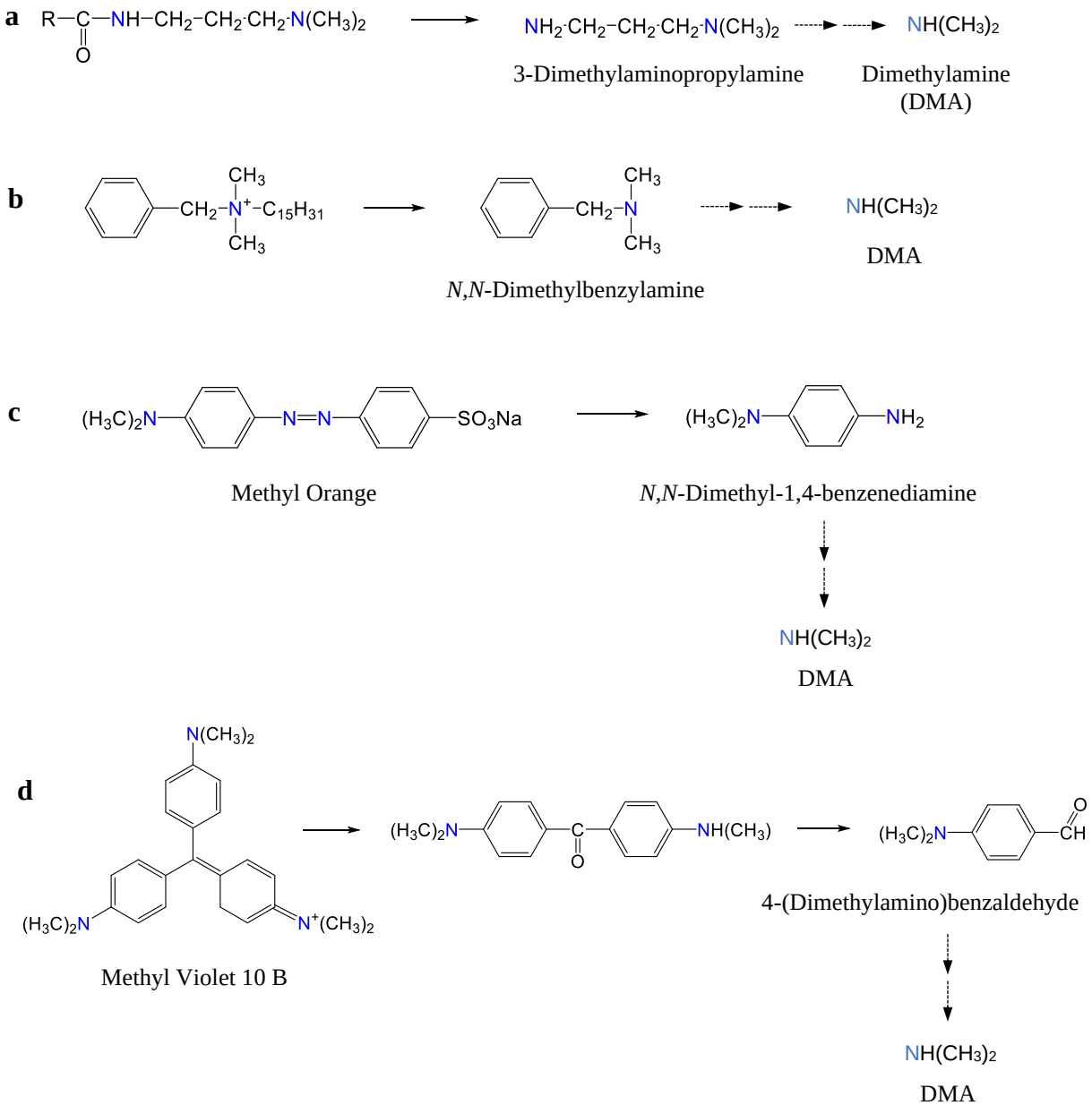



**Figure 2.2.** Proposed biotransformation pathways of some typical ingredients (i.e., surfactants and synthetic dyes) of personal care products during microbial biodegradation. (a) and (b): surfactants; (c) and (d): synthetic dyes.

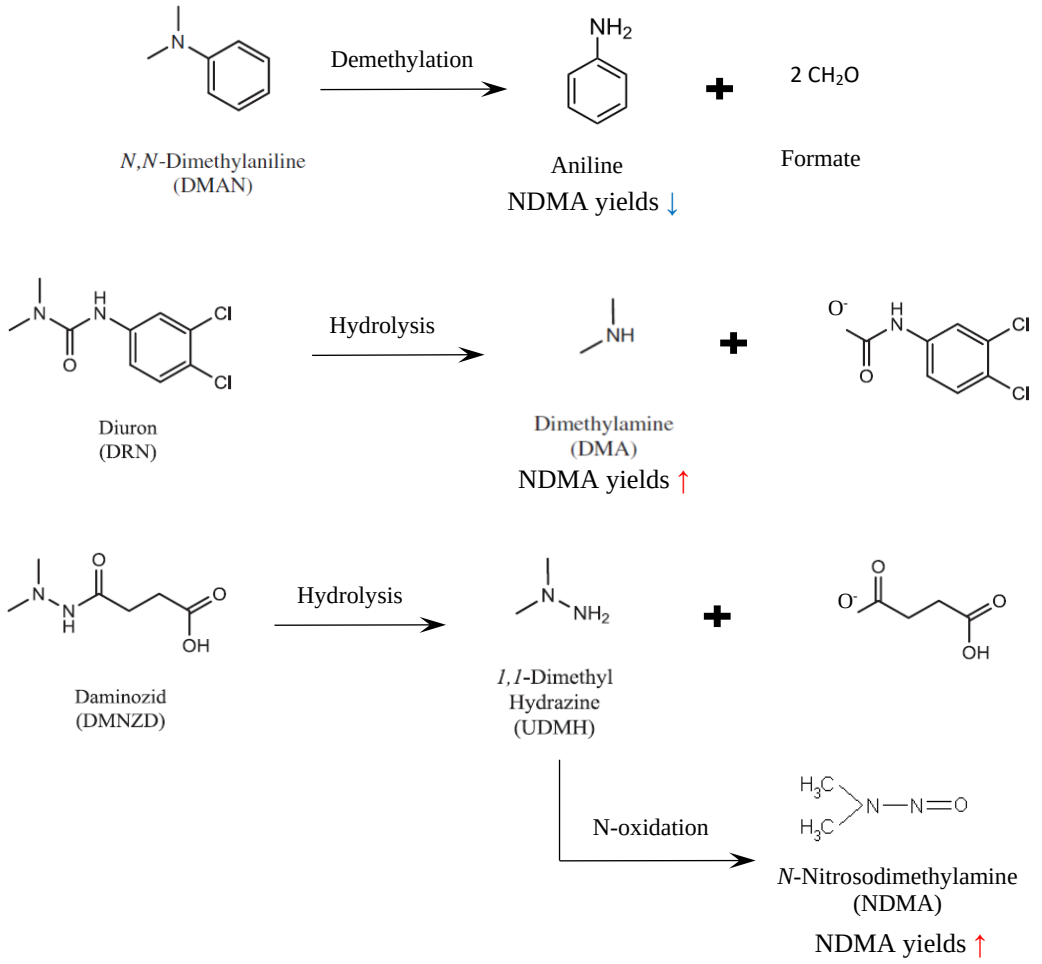



**Figure 2.3.** Proposed biodegradation pathways of selected NDMA precursors catalyzed by ammonia monooxygenase. Blue arrows indicate decreases in NDMA yields, and red arrows indicate increases in NDMA yields after biodegradation of compounds.

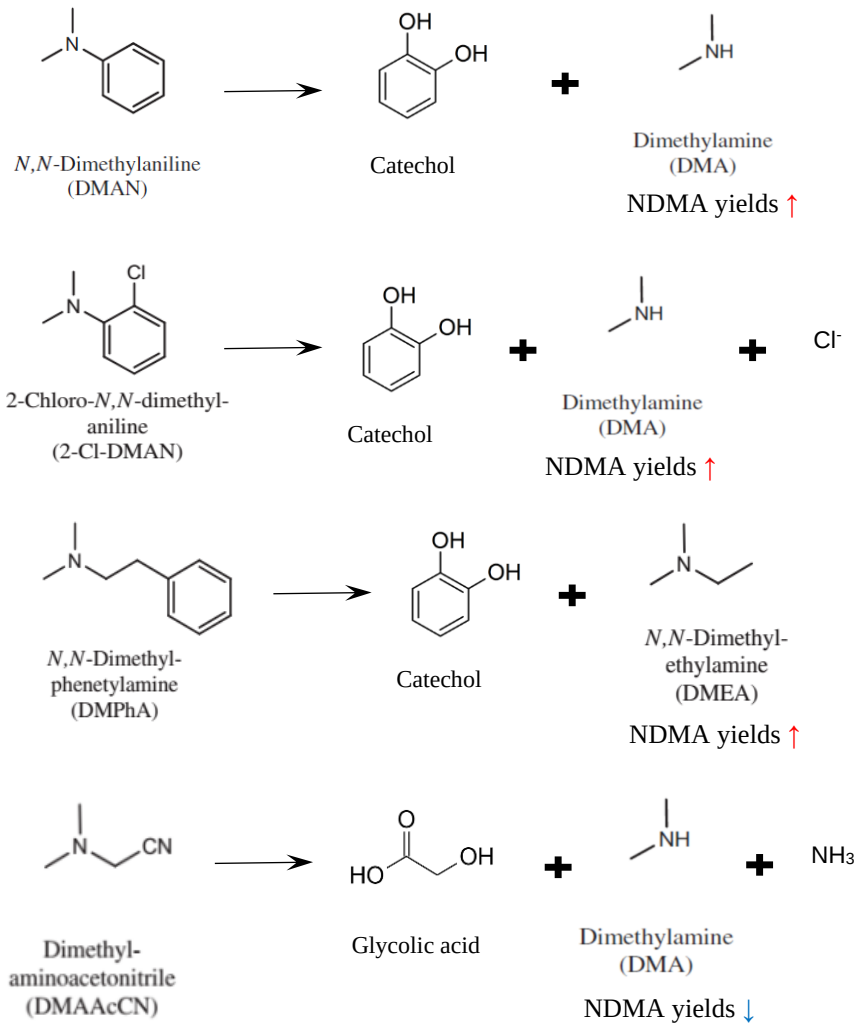



**Figure 2.4.** Proposed biodegradation pathways of selected NDMA precursors catalyzed by benzoate dioxygenase. Blue arrows indicate decreases in NDMA yields, and red arrows indicate increases in NDMA yields after biodegradation of compounds.

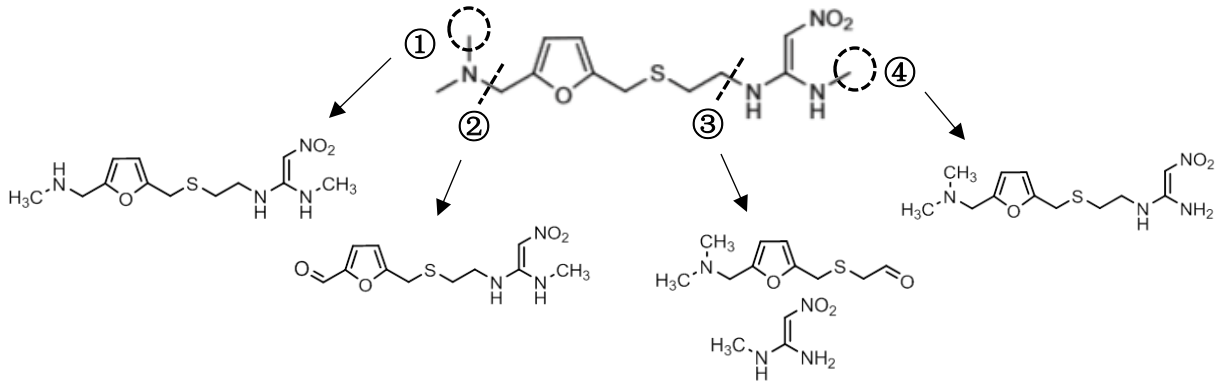


**Figure 2.5.** Biodegradation pathways of ranitidine (RNTD) predicted by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) -Biocatalysis/Biodegradation Database (BBD) Pathway Prediction System. The dotted lines or circles indicate the potential bond breaks occurring during biodegradation of RNTD, and the numbers "1-4" indicate the types of biodegradation pathways of RNTD.

Though applied for decades, biological treatment at WWTPs are still a 'black box' due to the extremely complex and uncertain composition of wastewater influents, and various processes occurring simultaneously within an aeration basin (Gunther et al., 2012; Bailon-Salas et al., 2017). Improvements to biological treatment of NDMA precursors require a fundamental understanding of the biotic (i.e., biodegradation) and abiotic (i.e., biosorption, volatilization) processes, metabolic and/or co-metabolic biodegradation pathways and their roles, potential microbial degraders and their performances within the matrix of microbial community dynamics varying with influents' composition and other factors such as treatment processes (e.g., CAS vs anaerobic/oxic (A/O) or anaerobic/anoxic/oxic (A/A/O)), operational parameters (e.g., HRT, SRT, organic loadings) and environmental factors (e.g., temperature, rainfall, etc.).

# CHAPTER III

# OBJECTIVES, APPROACHES, AND EXPERIMENTAL DESIGNS

## Objectives

Despite the significant efforts devoted to investigating the formation of *N*-nitrosamines in WWTP influents and effluents, the fates of *N*-nitrosamine precursors during biological wastewater treatment (especially the AS process) is still poorly understood. In this research, the main goal was to comprehensively examine the source, fate and removal of *N*-nitrosamine precursors in wastewaters during the AS process, which is especially important because of i) the potential health risks of *N*-nitrosamines, ii) the increasing impact of wastewater effluents on source water qualities, and iii) the expanding applications of wastewater potable reuse as alternative drinking water sources. The followings are the specific objectives in this study:

1. To examine the formation of *N*-nitrosamines in different sewage components (i.e., blackwaters and greywaters) under UFC and FP chloramination protocols.
2. To investigate the effects of selected factors (i.e., pH, $Br^-$, dissolved organic carbon (DOC), specific ultraviolet absorbance at 254 nm ($SUVA_{254}$)) on the formation of NDMA under UFC and FP chloramination protocols.
3. To determine the deactivation efficiencies, pathways, and mechanisms of NDMA precursors during the AS process.
4. To evaluate the removal of *N*-nitrosamine precursors from different sewage components during the AS process and relevant factors.

## Approaches and Experimental Designs

- ***Objective 1*: Examination of the formation of *N*-nitrosamines from different sewage components (i.e., blackwaters and greywaters) under UFC and FP chloramination protocols.**

  *Approach:* Four blackwaters (including two sources of urine blackwaters and two sources of feces blackwaters) and eight greywaters (including two laundry greywaters, three shower greywaters, a bathroom washbasin greywater, and two kitchen greywaters) were selected based on their large volume fractions (i.e., each >9%) in domestic sewage. UFC and FP protocols were employed to measure the formation of *N*-nitrosamines from these sewage components (**Figure 3.1**).

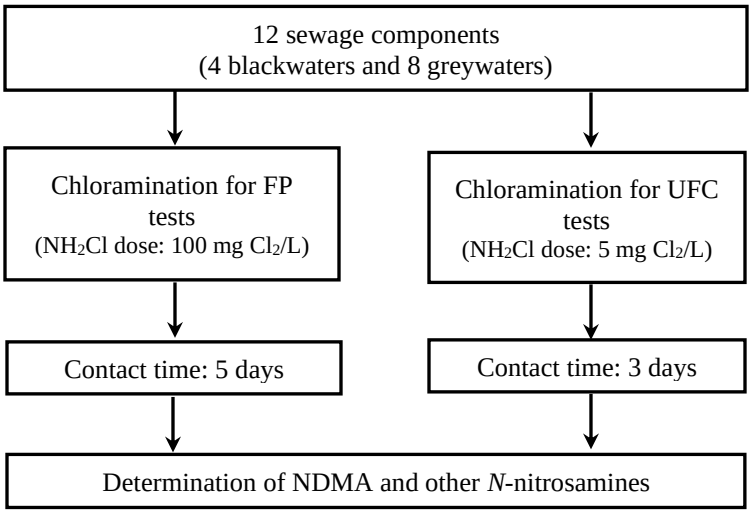



**Figure 3.1.** Experiments conducted for Objective 1.

- ***<u>Objective 2</u>: Investigation of (i) the effects of selected factors (i.e., pH, $Br^-$, DOC, $SUVA_{254}$) on NDMA UFC from selected model compounds and surface waters, (ii) effects of these selected factors on NDMA FP, and (iii) correlations between NDMA UFC and NDMA FP.***

  *<u>Approach:</u>* To explore the effects of pH on NDMA formation, 16 model NDMA precursor compounds were selected based on their chemical structures and pKa values, dosed in DDW and chloraminated at four pH levels (i.e., 6.0, 6.8, 7.8 and 8.8) (**Figure 3.2**). In addition, five raw surface waters and four treated surface waters were also collected and chloraminated to examine NDMA formation at pH 6.8, 7.8 and 8.8. The effects of DOC and $SUVA_{254}$ were investigated by measuring NDMA UFC and NDMA FP from (i) four selected model compounds dosed in the influent and effluent from Myrtle Beach water treatment plant, SC, with adjusted DOC (i.e., 1.0 and 2.5 mg/L), (ii) five raw surface waters with adjusted DOC (i.e., 1, 2.5, 5 and 9-25 mg/L) and four treated surface waters, which exhibited different $SUVA_{254}$ (i.e., 1.7-5.1 $L \cdot mg^{-1} \cdot m^{-1}$). To investigate the effects of $Br^-$, seven selected model compounds were dosed in DDW in the presence and absence of a high level of $Br^-$ (i.e., 1000 µg/L), chloraminated and examined for their NDMA UFC. Finally, the relationships between NDMA UFC and NDMA FP were examined by conducting linear regressions between NDMA UFC and NDMA FP measured in different surface waters.

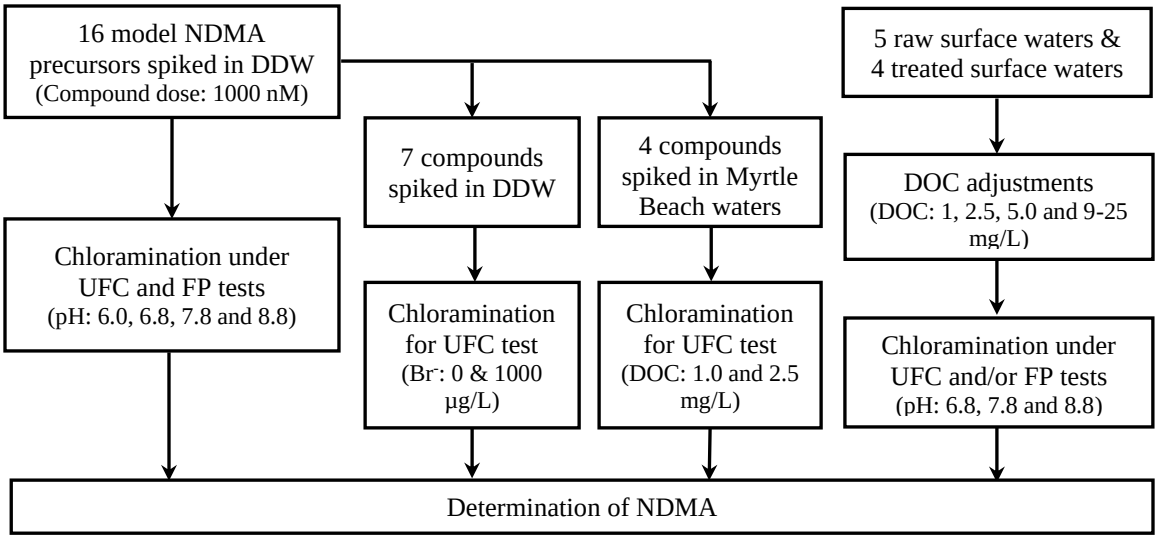



**Figure 3.2.** Experiments conducted for Objective 2.

- ***<u>Objective 3</u>: Determination of (i) the deactivation efficiencies of model NDMA precursors during the AS process, (ii) the factors (i.e., AS sources, incubation time or HRT, SRT of AS, seasonal variations in AS activity) that influence the removal of model NDMA precursors, and (iii) the different deactivation pathways (i.e., biodegradation, biosorption and volatilization) of NDMA precursors, and (iv) the roles of biostimulation and non-specific oxygenase affecting the deactivation efficiencies for model NDMA precursors.***

  <u>*Approach:*</u> TMA and three amine-based pharmaceuticals (i.e., RNTD, minocycline (MNCL), and sumatriptan (SMTR)) were selected based on their wide uses and relatively high NDMA yields (i.e., >1% on a molar basis under the FP test). Their NDMA UFC and NDMA FP were monitored before and after treatment with four sources of AS (i.e., domestic rural, domestic urban, textile

and lab-grown AS). The effects of selected factors (i.e., incubation time or HRT, SRT, seasonal variation in AS activity) were examined on the deactivation of NDMA precursors (**Figure 3.3)**. The impact of each deactivation pathway (i.e., biodegradation, biosorption, or volatilization) on the reduction in NDMA formation of model precursors during an AS process was further evaluated via a series of experiments. The role of biostimulation was examined by adding different types of biostimulants (i.e., benzoate, ammonia, acetate and ammonia, glucose and ammonia, ethanol and ammonia) during an AS treatment of a representative NDMA precursor (i.e., RNTD). To examine the role of non-specific oxygenase, the NDMA FP of RNTD was monitored during treatment with a model microbial species (i.e., *P. putida*) under aerobic conditions with and without the presence of an oxygenase inhibitor (i.e., acetylene), and under anaerobic conditions (i.e., in the absence of oxygen).

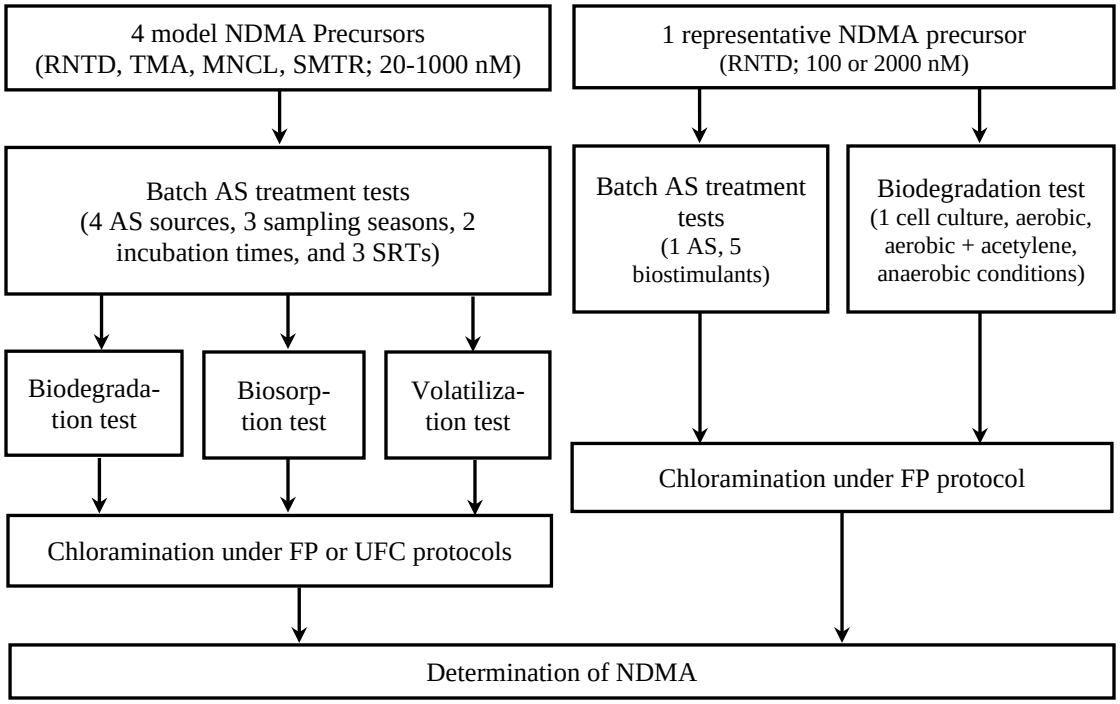



**Figure 3.3.** Experiments conducted for Objective 3.

- ***<u>Objective 4</u>: Evaluation of (i) the deactivation efficiencies of N-nitrosamine precursors from different sewage components and WWTP influents; (ii) the differences in deactivation efficiencies measured under the UFC and FP tests, and (iii) the effects of biostimulation (i.e., adding EEDs) and other selected factors (i.e., AS types, incubation time or HRT) on the deactivation efficiencies of N-nitrosamine precursors from sewage components.***

  <u>*Approach:*</u> Twelve sewage components (i.e., four blackwaters and eight greywaters) and four WWTP influents were collected and their *N*-nitrosamine formations (i.e., FP and UFC) were monitored before and after treatment with three types of AS (i.e., domestic rural, domestic urban, and textile AS) (**Figure**

**3.4**). Glucose (144 mg/L) and yeast extract (133 mg/L) were added as EEDs during AS treatment of sewage components to examine the effects of biostimulation on the deactivation efficiencies of *N*-nitrosamine precursors.

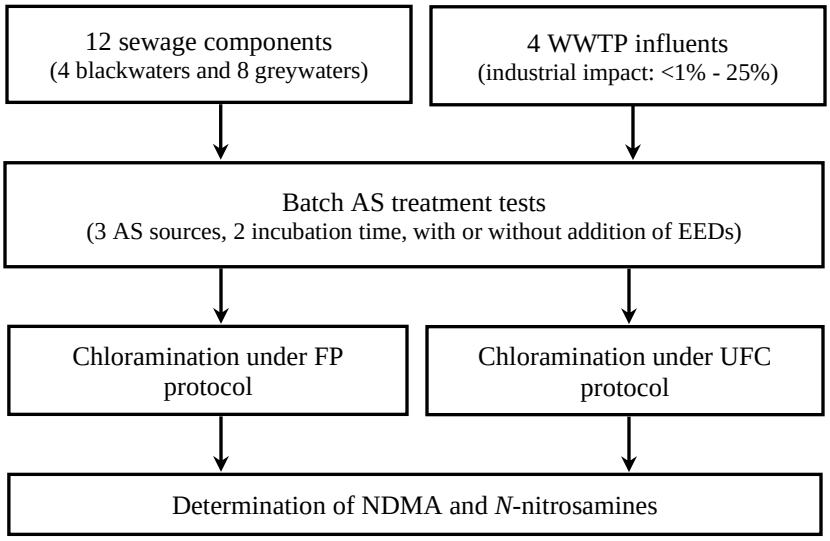



**Figure 3.4.** Experiments conducted for Objective 4.

The following chapter (IV) describes the materials and methods used in this research. Chapters V, VI, VII, and VIII present the results that address objectives 1, 2, 3 and 4, respectively. Chapter IX provides a comprehensive set of conclusions and recommendations.

# CHAPTER IV

# MATERIALS AND METHODS

In this chapter, an overall description of the experimental materials and methods used in this study is provided. Because different samples and methods were used in each phase of this research, there is a short materials and methods section in each chapter to illustrate the specific precursors used and the experimental matrix conducted.

## Glassware, Reagent Water, and Chemical Reagents

Glassware was cleaned by tap water with a detergent, sonicated for 30 min, and rinsed five times with distilled water followed by DDW for five times. The glassware was dried overnight at a temperature of 105°C and baked in an oven at 400°C for at least 1 h to remove any organic contaminants. DDW, Type I water with a resistivity of 18MΩ·cm, produced by a Millipore water purification system was used. All chemicals, except for nitrosamine precursors, were American Chemical Society (ACS) reagent grade. All solvents used were in high purity. All stock solutions and buffers were prepared at the use time, stored at 4°C for up to one week before used.

## Model NDMA Precursors

All precursors were purchased from certified vendors (e.g., Sigma-Aldrich, TCI, Matrix Scientific, Santa Cruz Biotechnology) with purities of 98.0%-99.5%. Some precursors such as TMA were purchased as an aqueous solution with the mass

concentrations ranging from 20.0% to 45.0%. All precursors were chosen because of their diverse chemical structures (e.g., aliphatic amines with different chain lengths, aromatic amines with a different number of carbon atoms between the DMA group and ring structure), and/or their frequent occurrences in wastewater. A stock solution was prepared for each precursor in methanol or DDW and stored at 4°C until used.

## Domestic Sewage Components Collection and Preservation

The major components of domestic sewage, including blackwaters (i.e., human urine and feces blackwaters) and greywaters (i.e., laundry, shower, bathroom washbasin and kitchen greywaters) were collected for the investigation of their *N*-nitrosamine formation. Raw human urine and feces were collected by Dr. William A Mitch's group from Stanford University, filtered through 0.7-µm fiber glass membrane filter, then frozen and shipped to Clemson University. Laundry greywaters were collected from the discharges of a washing machine at Stanford University, filtered and shipped to Clemson University in coolers with ice packs. Shower, bathroom washbasin and kitchen greywaters were all collected from a local residence, Clemson, SC. More details about the collection and processing of blackwater and greywater samples are provided in Chapter V. The collected samples were filtered through 0.45-µm cellulose nitrate membrane (Whatman, Maidstone, UK) before stored at 4°C or -20°C. Urine and feces blackwaters and kitchen greywater which contain easily biodegradable organic substances were stored at -20°C to minimize microbial activities, while other greywaters which contain relatively bio-refractory constituents (e.g., detergents, personal care products) were stored at 4°C until

used. All the experiments with human urine and feces samples, starting from thawing until chloramination, were conducted in a biosafety cabinet (Class II, Labconco, US).

## Surface Waters and Wastewater Influents

The effects of pH and NOM on NDMA UFC and NDMA FP were investigated in surface water samples with distinct water quality characteristics (e.g., DOC, $SUVA_{254}$). The effects of different factors on NDMA formation in surface waters may be applicable to wastewaters. In addition, surface waters are more related to NDMA formation in water distribution systems and residential taps, rather than wastewater influents or effluents. Therefore, surface waters were selected to examine the effects of different factors on NDMA UFC and NDMA FP. During surface water sample collection, 20 to 40 L of surface water were taken as grab samples from five watersheds in South Carolina near the intakes of drinking water treatment plants. From these plants, treated water samples were also collected after conventional treatment processes (i.e., coagulation, flocculation, and sedimentation). The watersheds and water treatment plants were selected to provide a variation in water qualities (e.g., DOC, $SUVA_{254}$). The collected samples were transported to the lab, immediately filtered through 0.45-μm membrane filter, and stored at 4°C until used.

The deactivation of *N*-nitrosamine precursors was examined in four wastewater influents with distinct industrial impacts (i.e., contributions of industrial discharge in influents) of <1%, 8-15%, 8-15% and 25%, respectively. During sample collection, ~10 L primary effluents were taken as grab samples from the inlets of aeration basins at four local

WWTPs in SC. The WWTPs were selected to provide variations in influent types and treatment processes, with details provided in Chapter VII. The collected wastewater samples were transferred to the lab within two hours of collection, filtered through 0.45-μm membrane filter (Whatman, GE Healthcare Life Sciences, US), and stored at 4°C until used.

## Batch Biological Treatment Test Methods

A modified version of an EPA standard test method, (Office of Prevention, Pesticides & Toxic Substances (OPPTS) 835.3280 -314B,) (US EPA, 2008) was employed to assess the deactivation efficiencies of selected *N*-nitrosamine precursors during an AS process. The modifications made to the standardized batch test are listed as follows.

(i) Modifications to the AS pretreatment

The EPA batch test method uses freshly collected AS liquor to treat target organic substances without any pretreatment (EPA 2008). The modified method consisted of washing the AS with a mineral salts solution three times before batch tests to reduce the amount of exogenous NDMA precursors introduced by the AS liquor. Based on preliminary testing, the impact of washing AS on the biological removal of model precursors was insignificant. The overall removal of the NDMA FP of RNTD after 24-hr incubation was 97% and 94% when treated by a domestic AS collected from a local WWTP (i.e., AS 1) before and after AS washing, respectively.

(ii) Modifications to the biosorption test

The EPA test method uses $^{14}C$-labeled compounds to track the fractions that were

ultimately biodegraded, primarily biodegraded, adsorbed onto AS solids and/or volatilized (EPA 2008). Due to the high costs of $^{14}C$-labeled compounds, the biosorption test was conducted using AS deactivated with sodium azide ($NaN_3$).

(iii) Modifications to the volatilization test

To assess the impact of volatilization, the EPA batch test uses a trap to adsorb $^{14}C$-labeled volatile compounds that escape into the gas phase (EPA 2008). This was modified by using a mineral solution in the absence of AS and under a constant aeration condition, making it possible to evaluate compound losses attributable to volatilization and other possible abiotic processes such as hydrolysis.

Before the tests, AS samples were collected from the mixing zone of aeration basins at three WWTPs (the domestic rural, domestic urban, and textile WWTPs), or a lab-scale sequencing batch reactor (SBR) fed with synthetic wastewater. The wastewater treatment facilities were selected to provide variations in treatment capacities, treatment processes and conditions (i.e., HRT 6-24 h, SRT 6-26 d; more details are provided in Chapter VII). The AS samples were transported to the lab within 1 h of collection and aerated at 23±2 ºC for less than 12 h before use. In addition, before use, each collected AS sample was washed three times with a mineral solution (OECD 2003) based on the following protocols: (i) AS liquor was centrifuged (2000×g, 5 min), (ii) the supernatant was decanted, and the solids were resuspended in a mineral solution (pH = 7.4 buffered with 4 mM phosphate; OECD, 2003) to a concentration of ~6 g/L, and (iii) the same procedure was repeated three times to remove background NDMA precursors from the AS liquor.

During the batch tests, the washed AS (500 mL) was placed in a 1-L glass bottle, dosed with NDMA precursor under vigorous mixing conditions using a magnetic stir bar, and constantly aerated using compressed air (150 L/m$^3$·min) for 6 or 24 h at 25±2 ºC. The aeration rate was set as close as possible to typical aeration rates (20-90 L/m$^3$·min) used in aeration basins of WWTPs (Grady et al., 2011). The incubation duration (i.e., 6 and 24 h) was selected as the low and upper ends of typical HRTs used at municipal WWTPs. At the end of incubation, 250 mL of AS liquor were collected from each bottle and centrifuged at ~2000×*g* for 5 min. The supernatant was collected and filtered through 0.45-µm cellulose nitrate membranes (Whatman, GE Healthcare Life Sciences, US) and chloraminated using the FP or UFC protocol. The test procedures are shown in **Figure 4.1**.

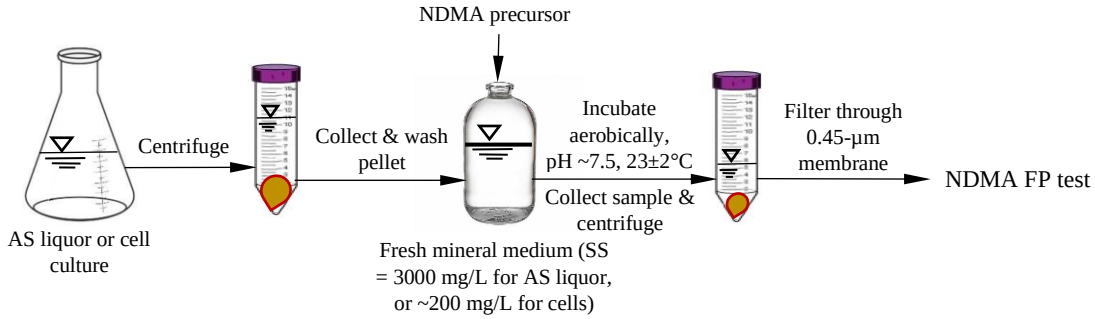



**Figure 4.1.** Diagram of batch biodegradation tests.

Similarly, for conducting the batch tests with *P. putida*, cells were harvested at exponential growth phase , washed and resuspended in a mineral solution (OECD 2003) with the MLSS adjusted to ~200 mg/L. Approximately 80 mL of the washed and resuspended cells were transferred to a 160-mL serum bottle, dosed with RNTD as a model

NDMA precursor, and incubated under aerobic conditions (i.e., with sterile sponge plugs in the neck of the bottle to maintain aseptic conditions) on a shaker table (i.e., 200 rpm) at 23±2°C for up to 20 days. To conduct biodegradation test under aerobic conditions in the presence of the oxygenase inhibitor, acetylene, ~80 mL of the washed and resuspended cells were transferred to 160-mL serum bottle, sealed with septa and crimp caps, and then ~20 mL of acetylene gas were injected with a syringe. To conduct the biodegradation test under anaerobic conditions, ~80 mL of the washed and resuspended cells in 160-mL serum bottle were transferred to an anaerobic glove box and exposed to a mixture of nitrogen and hydrogen gases for at least 2 hours. The serum bottles were sealed with caps inside the glove box, and then transferred to a shaker table to incubate anaerobically for up to 20 days. At the end of the incubation period, 20 mL of liquor were harvested from each bottle, filtered through a 0.45-µm membrane filter, and the filtrates were chloraminated for the NDMA FP test.

## ***N*-Nitrosamine Formation Tests**

The FP test as described by Selbes et al. (2013) was used to determine the total amount of *N*-nitrosamine precursors in the samples. During FP test, the formation of *N*-nitrosamines was measured after a 5-d contact time with an excessive amount (100 mg $Cl_2$/L) of pre-formed $NH_2Cl$. The $NH_2Cl$ stock solution was prepared by adding a sodium hypochlorite solution (NaClO, 4000 mg $Cl_2$/L, pH adjusted to ~9.0) drop by drop into an ammonium chloride solution ($NH_4Cl$, 1000 mg N/L, pH adjusted to ~9.0) under vigorous

mixing conditions to minimize the formation of $NHCl_2$. The Cl:N mass ratio was set at 4:1 to mimic the practical chloramination applied in drinking water utilities.

The procedure for conducting the *N*-nitrosamine FP test is illustrated in **Figure 4.2**. Briefly, 250 mL of AS filtrate (0.45µm-filtered) were buffered with 20-mM phosphate (pH = 7.8±0.2) and chloraminated by adding a predetermined volume of $NH_2Cl$ stock solution (~2000 mg $Cl_2$/L) to obtain a target $NH_2Cl$ concentration of 100 mg $Cl_2$/L. After a 5-d contact time at 23±2ºC in the dark, the residual $NH_2Cl$ was quenched by adding $Na_2S_2O_3$ prior to *N*-nitrosamine extraction.

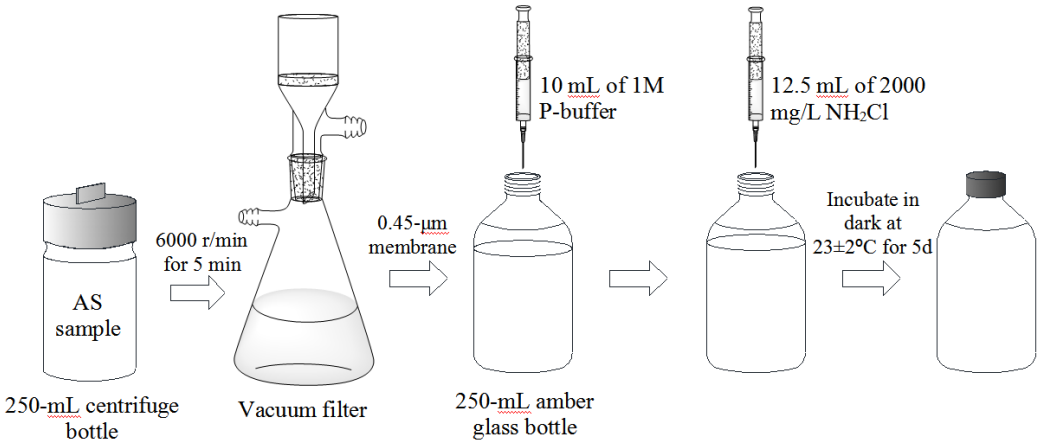



**Figure 4.2.** Procedures for conducting NDMA FP tests.

The *N*-nitrosamine UFC test is designed to monitor *N*-nitrosamine formation in a typical US distribution system where a realistic chloramine dose (~5 mg $Cl_2$/L of $NH_2Cl$, 3-d contact time and 1.0±0.4 mg $Cl_2$/L of $NH_2Cl$ residual at the end of contact) was applied. Similar to the FP test, the AS filtrates were phosphate-buffered, chloraminated and

incubated at 23±2ºC for 3 days in the dark. After incubation, residual $NH_2Cl$ was quenched by adding $Na_2S_2O_3$. Quenched samples were stored at 4ºC for less than 14 days before *N*-nitrosamine extraction.

## Analytical Methods

A summary of the parameters, analytical methods, instruments and MRL is provided in **Table 4.1**. These methods followed either Standard Methods (APHA et al., 2005) or US EPA Methods. All experiments were conducted in duplicate and the results shown in tables and figures represent the averages of duplicate measurements.

**Table 4.1.** Summary of analytical methods.

| Water parameters | Unit | Analytical Methods | Instruments | MRL[k] or Accuracy |
|---|---|---|---|---|
| DOC[a] | mg C/L | SM[f] 5310B | Shimadzu TOC-$V_{CHS}$ & TNM-1 | 0.1 |
| DN[b] | mg N/L | High-temperature combustion | | 0.1 |
| COD[c] | mg/L | SM 5520 | Accu-Test COD System, Bioscience Inc. | 10 |
| $UV_{254}$[d] | $cm^{-1}$ | SM 5910 | Varian Carry 50 | ±0.004[l] |
| $Br^-$ | µg/L | EPA Method 300 | ICS 2100, Dionex Corp. | 10 |
| $NO_3^-$ | | | | 15 |
| $NO_2^-$ | | | | 20 |
| pH | | SM 4500-$H^+$ | VWR Symphony | ±0.01[m] |
| $NH_3$-N[e] | mg N/L | Salicylate method | HACH Test Kit | 0.02 |
| Free and combined chlorine | mg $Cl_2$/L | SM 4500-Cl F | N/A[g] | 0.05 |
| Trichloroethylene (TCE) | mg/L | EPA Method 8015 | GC-FID[h] | N.A. |
| Oxygen | mg/L | EPA Method 3C | GC-TCD[i] | N.A. |
| *N*-nitrosamine | ng/L | EPA Method 521 | Varian GC/MS/MS[j] | Shown in **Table 4.3** |

[a]: Dissolved organic carbon. [b]: Dissolved nitrogen. [c]: Chemical oxygen demand. [d]: Specific ultraviolet absorbance at 254 nm. [e]: Ammonia nitrogen. [f]: Standard Methods (APHA et al., 2005). [g]: Not applicable. [h]: Gas chromatograph (GC) equipped with flame ionization detector (FID). [i]: GC equipped with thermal conductivity detector (TCD). [j]: GC - tandem mass spectrometer (MS/MS). [k]: Minimum reporting level. [l]: Accuracy of $UV_{254}$. [m]: Accuracy of pH.

## Chloramines Measurements

Monochloramine ($NH_2Cl$) and dichloramine ($NHCl_2$) concentrations were measured using an *N,N*-diethyl-p-phenylenediamine (DPD) method (SM 4500-Cl F). The samples were diluted in DDW based on their expected residual chloramine concentrations to the range of 0-5 mg $Cl_2$/L. 100 mL of diluted samples were poured into a flask containing 5-mL DPD solution and 5-mL phosphate buffer. The sample was then titrated using a ferrous ammonium sulfate (FAS) solution to the end point until pink color disappeared. The titrant volumes were recorded and used to calculate the concentrations of chloramines.

## *N*-Nitrosamine Measurements

Aliquots (1-mL) of NDMA-$d_6$ primary dilution solution (PDS; 50 μg/L) in DDW were added to 250-mL quenched samples to obtain 200 ng/L of NDMA-$d_6$ prior to solid phase extraction (SPE). Cartridges used for SPE were packed with 2-g coconut charcoal (UTC, Farmington, US), pre-conditioned by sequentially passing through ~6-mL dichloromethane (DCM), ~9-mL methanol and ~15-mL DDW driven by a vacuum system, followed by passing through 250-mL water samples. Cartridges were then dried under a mild vacuum for ~30 min and eluted by passing through ~12-mL DCM. Eluates were collected in a 15-mL centrifuge glass tube, passed through a drying column packed with 6-g baked (~105ºC, overnight) and cooled anhydrous sodium sulfate ($Na_2SO_4$) powder, and finally concentrated to ~1 mL under a gentle nitrogen gas flow. Concentrated extracts were then spiked with 20-μL NDPA-$d_{14}$ PDS as internal standard (80 ng/L of NDMA-$d_{14}$) and

transferred to 2-mL amber gas chromatograph (GC) vials for the GC-tandem mass spectrometer (GC/MS/MS) analysis.

Two different types of GC/MS/MS instruments were used to analyze *N*-nitrosamines, including GC (Varian 3800, Agilent, US)/MS/MS (Waters 4000, Agilent, US) equipped with an RTX-5MS (Restek 30 m × 0.25 mm × 0.25 μm) capillary column, and GC/MS/MS ( 7000 QQQ, Agilent, US) equipped with DB-1701 (30 m × 0.25 mm × 0.25 μm) capillary column. The GC/MS/MS analysis parameters for *N*-nitrosamine measurements are shown in **Table 4.2**. *N*-nitrosamine were analyzed by MS/MS under the chemical ionization (CI) mode. The GC inlet temperature programming was set as follows: the injection temperature was 35°C and held for 0.8 min, then increased to 260°C at 200°C/min and held for 2.08 min. The column temperature program was 35°C held for 5 min, increased to 70°C at 5°C/min, increased to 87°C at 3°C/min, increased to 120°C at 5°C/min and increased to 250°C at 40°C/min and then held for 2.48 min. The injection volume was set at 8 μL for GC (Varian 3800, Agilent, US) coupled with MS/MS (Waters 4000, Agilent, US), or 1 μL for Agilent 7000 QQQ GC/MS/MS. Duplicate samples were injected for *N*-nitrosamine analysis. The mean concentration of duplicate injections was calculated as reporting values for each test condition. A set of calibration standards consisting of nine points were prepared using PDS of NDMA, NDPA-$d_6$ and NDPA-$d_{14}$ in DCM and analyzed every 10-20 samples on the GC/MS/MS.

The detection limit (DL) of *N*-nitrosamines was determined by a consecutive analysis of eight spiked samples (in DDW) containing 5-ng/L *N*-nitrosamines and calculated based on the following equation:

$$DL = S \times t_{(n-1,\ 1-\alpha)} \qquad \textbf{Equation 4.1}$$

where S is the standard deviation of eight replicated analysis, t ($_{(n-1,\ 1-\alpha)}$) is the student-t value for a 1-α confidence with n-1 degrees of freedom (e.g., $t_{(7,\ 0.99)} = 2.998$ for 8 replicates at a 99% confidence level), n is the number of replicated samples (i.e., eight), and 1-α is the confidence level (0.99, α = 0.01). The MRL of *N*-nitrosamines was established to be 3 times of DL. The DL and MRL determined for *N*-nitrosamines on GC (Varian 3800, Agilent, US) coupled with MS/MS (Waters 4000, Agilent, US) were shown in **Table 4.3**. For simplicity and convenience, the MRL for all *N*-nitrosamine species was set at 3.0 ng/L. However, a relatively lower MRL of NDMA was identified on Agilent 7000 QQQ GC/MS/MS, which was 0.5 ng/L.

Recoveries of *N*-nitrosamines were evaluated by analyzing *N*-nitrosamine concentrations in two surface water samples before and after spiking of 10 ng/L *N*-nitrosamine standards. Recoveries (R) of *N*-nitrosamines were calculated based on the following equation:

$$R\ (\%) = (A-B) / C \qquad \textbf{Equation 4.2}$$

where A is the measured *N*-nitrosamine concentration (ng/L) in spiked samples, B is the measured *N*-nitrosamine concentration (ng/L) in unspiked samples, and C is the *N*-nitrosamine concentration spiked (i.e., 10 ng/L). The measured *N*-nitrosamine concentrations using GC (Varian 3800, Agilent, US) coupled with MS/MS (Waters 4000, Agilent, US) and the calculated recoveries are shown in **Table 4.3**.

**Table 4.2.** Detection information of *N*-nitrosamine analysis on GC/MS/MS.

| *N*-nitrosamines | Molecular Weight (g/mol) | Quantification ion mass (m/z) | Confirmation ion mass (m/z) | Retention time (min) |
|---|---|---|---|---|
| NDMA | 74 | 75.0 | 43.3, 47.3 | 6.9 |
| NDMA-$d_6$ | 80 | 81.1 | 50.3, 49.3 | 6.9 |
| NMEA | 88 | 89.0 | 61.1, 43.2 | 9.5 |
| NDEA | 102 | 103.1 | 103.9, 75.0 | 11.7 |
| NPYR | 100 | 101.1 | 55.1, 102.1 | 18.0 |
| NDPA-$d_{14}$ | 144 | 145.2 | 97.2, 146.3 | 18.0 |
| NDPA | 130 | 131.2 | 89.1, 132.1 | 18.3 |
| NMOR | 116 | 117.2 | 101.2, 87.0 | 18.2 |
| NPIP | 114 | 115.1 | 69.1, 116.2 | 19.6 |
| NDBA | 158 | 159.1 | 160.2, 103.1 | 24.7 |

**Table 4.3.** Detection limits (DL) and MRL of *N*-nitrosamines.[a]

| *N*-nitrosamines | Measured mean concentrations[b] (ng/L) | RSD[c] (%) | DL[d] (ng/L) | MRL (ng/L) |
|---|---|---|---|---|
| NDMA | 4.8 | 5.2 | 0.7 | 2.2 |
| NMEA | 5.1 | 5.7 | 0.9 | 2.6 |
| NDEA | 5.0 | 4.4 | 0.7 | 2.0 |
| NPYR | 5.2 | 4.9 | 0.8 | 2.3 |
| NDPA | 5.5 | 5.6 | 0.9 | 2.8 |
| NMOR | 5.0 | 6.7 | 1.0 | 3.0 |
| NPIP | 4.5 | 6.1 | 0.8 | 2.4 |
| NDBA | 4.5 | 6.7 | 0.9 | 2.7 |

[a]: Determined at a target concentration of 5 ng/L. [b]: The mean concentration of duplicate samples. [c]: Relative standard deviation of measured mean concentrations. [d]: Detection limit.

## Dissolved Organic Carbon and Dissolved Nitrogen Measurement

DOC and DN were measured using a Shimadzu TOC-VCHS high temperature combustion analyzer equipped with a TN module. The TOC standards were prepared by diluting a PDS of potassium hydrogen phthalate (PHP; 1000 mg/L) solution in DDW, to achieve the target TOC concentration range of 0.2-15 mg/L. The TN standards were prepared by diluting a PDS of potassium nitrate solution (1000 mg/L) in DDW, to obtain the concentration range of 0.2-5 mg/L.

## Ammonia Measurement

The $NH_3$-N concentration was measured using salicylate method with HACH kits. Prior to measurements, salicylate reagent was added to 10 mL water sample, mixed and contacted for 3 min. And then cyanurate reagent was added, mixed, and contacted for 15 min. The $NH_3$-N concentration was the measured with a HACH DR/820 colorimeter.

## $UV_{254}$ Absorbance

The UV absorbance at 254 nm wavelength ($UV_{254}$) was measured using a Cary 50 UV Vis spectrophotometer (Varian). During measurements, water sample was poured in a 1-cm quartz cuvette and then measured at a wavelength of 254 nm. The spectrophotometer was zeroed by measuring the absorbance of DDW. The method performance was periodically monitored by measuring the absorbance of DOC standards.

## pH

The pH of water samples was measured using a SM 4500-$H^+$ pH electrode with a Symphony pH meter (VWR, Radnor, US). Before measurements, the pH meter and electrode were calibrated using standard buffer solutions with pH 2, 4, 7 and 10.

## Bromide Measurement

Bromide ($Br^-$) concentration was measured using an ion chromatography (IC) system (Dionex ICS-2100) equipped with A Dionex AS-HC9 column (coupled with an AG-HC9 guard column) and an AAES suppressor. A $Na_2CO_3$ solution was used as the

mobile phase. The sample injection volume was 250 µL. A calibration curve of $Br^-$ was obtained by measuring a series of standard solutions prepared using NaBr (>99.9%, Sigma).

## Trichloroethylene and Oxygen Measurements

The concentrations of TCE were analyzed using a Hewlett-Packard 5890 Series II GC equipped with a flame ionization detector (FID) and a column packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco Inc., St. Louis, US). High purity nitrogen was used as the carrier gas. The temperature program was set at 60°C for 2 min, ramp at 20°C/min to 150°C, then ramp at 10°C/min to 185°C and hold for 5 min. A series of TCE standards were prepared by adding different amounts (i.e., 0.05-1 mL) of TCE-saturated water into 40 mL DDW in a 70 mL serum bottle to achieve the aqueous concentrations of TCE at 1-20 mg/L. A headspace sample (0.5 mL) was collected using 1-mL syringe and analyzed with a GC-FID.

Oxygen concentrations were monitored in headspace samples (0.5 mL) on a Hewlett Packard 5890 Series II GC equipped with a thermal conductivity detector (TCD) in conjunction with a MS-5A 60/80 Mesh Molecular Sieve column (Alltech, Nicholasville, US). Nitrogen gas served as the carrier gas. The temperature program was set at 60 °C for 5 min. The injector temperature was set at 150°C, and the detector temperatures was set at 100 °C. The response from the GC was calibrated using the room air (i.e., 20% volume fraction).

# CHAPTER V

# *N*-NITROSAMINE FORMATION FROM SEWAGE COMPONENTS

## Introduction and Objective

*N*-Nitrosamines are a group of probable human carcinogens that can be frequently detected in wastewaters and wastewater-impacted drinking waters (Mitch and Sedlak, 2002; US EPA, 2001). *N*-Nitrosamines are formed via reactions between disinfectants (e.g., chloramines, ozone) and organic precursors (i.e., secondary, tertiary and quaternary amines; Mitch et al., 2003; Oya et al., 2008). Low ng/L of *N*-nitrosamines (i.e., 0.2-15 ng/L) could be associated with $10^{-6}$ lifetime cancer risks in drinking waters (US EPA, 2001). Due to their adverse health risks, six *N*-nitrosamines have been included in the UCMR promulgated by the United States Environmental Protection Agency (US EPA), including NDMA, NDEA, NMEA, NDPA, NDBA and NPYR (Richardson, 2006). Five *N*-nitrosamines including NDMA, NDEA, NDPA, NPYR and NDPhA were also included in the US EPA CCL 4 for possible regulation in near future (US EPA, 2016). In California, 10 ng/L Notification Levels have been established for NDMA, NDEA and NDPA in drinking waters (CDPH, 2010).

Among the eight commonly reported *N*-nitrosamines, NDMA was always the predominant species in drinking waters, with other *N*-nitrosamines detected with much less frequencies (i.e., <10%) (Krasner et al., 2008; Boyd et al., 2011; Wang et al., 2011; Russell et al., 2012; Uzun et al., 2015; Zeng et al., 2016a). In wastewater-impacted surface waters, elevated concentrations of *N*-nitrosamines were detected (Boyd et al., 2011; Ma et al.,

2012; Zeng et al., 2016a). In total seven *N*-nitrosamines (including NDMA) were detected above their MRLs (i.e., 1-3 ng/L) in >23% of surface water samples around industrial polluted areas, among which five *N*-nitrosamines were >5 ng/L. In wastewaters for potable reuse, NDMA FP frequently equaled or exceeded its California Notification Level of 10 ng/L (Zeng et al., 2016b; Takeuchi et al., 2018), while other *N*-nitrosamine (e.g., NDEA, NPYR, NMOR) FP could exceed their $10^{-6}$ cancer risk levels (Zeng et al., 2016b; Takeuchi et al., 2018; Sgroi et al., 2018). As application of wastewater reuses increases to provide alternative water sources, and advanced wastewater treatment cannot completely remove all *N*-nitrosamines and their precursors, especially for low-molecular-weight NDMA, controlling *N*-nitrosamines and their precursors at secondary effluents could be important (Casey, 2015; Zeng et al., 2016b; Sgroi et al., 2018).

Municipal wastewater effluents are considered one of the major sources of *N*-nitrosamine precursors that can negatively impact downstream source water qualities (Krasner et al., 2013). Domestic wastewater influents contain human urine and feces, laundry, shower, washbasin and kitchen greywaters as major constituents (Friedler et al., 2013; Zeng and Mitch, 2015). Among all these sewage components, laundry greywater was found to be the most significant contributor to NDMA precursor pools when NDMA formation was measured under the practical chloramination condition (i.e., UFC test; 2.5 mg $Cl_2$/L and 3-d reaction time) (Zeng and Mitch, 2015). Among the two different methods (i.e., UFC and FP tests) used for measuring *N*-nitrosamine formation, the UFC test is more related to NDMA formation found in water and wastewater treatment utilities (Krasner et al., 2013). However, the UFC test provides negligible information regarding the amounts

of NDMA precursors present in water and wastewater samples. Instead, the amounts of NDMA precursors are evaluated by measuring NDMA formation under extreme chloramination conditions conducted in laboratory tests, e.g., using excessive chloramine dosage (i.e., 100-140 mg $Cl_2$/L) and prolonged reaction time (i.e., 5-10 d). Conducting both the FP and UFC tests with the same water and wastewater samples can be meaningful to address the formation and precursor controls of *N*-nitrosamines. Moreover, it was considered that *N*-nitrosamine precursors, rather than *N*-nitrosamines present in wastewater effluents, are mainly attributable to *N*-nitrosamine formation in downstream drinking water utilities. The evaluation of *N*-nitrosamine precursors (i.e., *N*-nitrosamine FP) from sewage components could be important for identifying the sources of contamination and developing potential mitigation strategies.

The major objective of this study is to evaluate both *N*-nitrosamine UFC and FP from different sewage components (i.e., blackwaters and greywaters) that can contribute to wastewater influents. The relative importance of different sewage components as the sources of *N*-nitrosamine precursors (i.e., *N*-nitrosamine FP) was further evaluated. The ultimate goal is to improve our understanding of the formation and precursor sources of *N*-nitrosamines from wastewaters.

## Materials and Methods

### *Sewage Components*

Urine and feces blackwaters and greywaters including laundry, shower, bathroom washbasin, and kitchen greywaters were selected as the representative components of domestic sewage. Raw human urine and feces samples were collected from a volunteer before (U and F, pre-RNTD) and after (UR and FR, post-RNTD) taking a Zantac 150 tablet which contains 150 mg of RNTD as active ingredient. Laundry greywater samples were collected from washing machine discharges after a medium-size load of white and colored clothes were washed using only detergent (LD) and detergent plus fabric softener (LF), respectively. Urine, feces, and laundry greywater samples were collected and frozen at Stanford University and shipped to Clemson, South Carolina. More details about collecting and processing urine, feces and laundry greywater samples are described elsewhere (Zeng and Mitch, 2015).

Shower greywater samples were collected from a bathtub after a volunteer took a hot shower not using any personal care products (S), using shampoo only (SS), and using body wash only (SB), respectively. A bathroom washbasin greywater (W) was collected by grabbing a mixed liquor of hand-washing, tooth-brushing, and face-cleaning wastewaters after the volunteer conducted a routine morning wash, with each step of activities using a single brand of personal care product. Kitchen greywater samples were collected from a stoppered kitchen sink after a manual wash of clean dishes using detergent (KD), and after food materials (i.e., raw and cooked vegetables, grains, meats and seafood, each ~50 g/L) were soaked (for 30 min) and boiled (for 2 min) in tap water, respectively.

The soaking tap water and boiled soup were then mixed in 1:4 volume ratio (KF) to mimic an ordinary dietary consisting of raw and cooked food. All collected blackwaters and greywaters were filtered through 0.45-μm membrane (Whatman, GE Healthcare Life Sciences, US) before stored or used. Urine and feces blackwaters, KF greywater were stored at -20ºC, while other greywaters were stored at 4ºC until used. The selected water quality parameters of filtered blackwater and greywater samples were measured based on Standard Methods (APHA et al., 2005) and shown in **Table 5.1**.

**Table 5.1.** Selected water quality parameters measured in sewage components (after dilution).

| Category | Sample[a] | Dilution factor | COD (mg/L) | DOC (mg/L) | $SUVA_{254}$ | $NH_3$-N (mg N/L) | DN (mg N/L) |
|---|---|---|---|---|---|---|---|
| Urine blackwaters | U | 250 | 18 | 2.2 | 1.2 | 1.4 | 2.5 |
| | UR | 250 | 14 | 3.4 | 1.2 | 1.6 | 3.9 |
| Fecal blackwaters | F | 150 | 15 | 3.3 | 1.1 | 0.5 | 1.6 |
| | FR | 150 | 12 | 3.4 | 1.5 | 0.1 | 0.6 |
| Laundry greywaters | LD | N.A.[b] | 135 | 26 | 1.0 | 20 | 20 |
| | LF | N.A. | 126 | 55 | 1.3 | 40 | 41 |
| Shower greywaters | S | N.A. | N.M.[c] | 15 | 0.3 | 0.3 | 6.0 |
| | SS | N.A. | N.M. | 33 | 1.1 | 0.2 | 1.8 |
| | SB | N.A. | N.M. | 15 | 0.3 | 0.2 | 1.5 |
| Washbasin greywater | W | N.A. | N.M. | 78 | 0.7 | N.D.[d] | 1.1 |
| Kitchen greywaters | KD | N.A. | N.M. | 16 | 0.5 | N.D. | 0.6 |
| | KF | 100 | N.M. | 21 | 0.5 | N.D. | 3.3 |

[a]: U: raw urine sample collected before taking Zantac, diluted for 250 times in dechlorinated tap water; UR: raw urine sample collected after taking Zantac, diluted for 250 times; F: feces sample collected before taking Zantac, diluted for 150 times; FR: feces sample collected after taking Zantac, diluted for 150 times; LD: laundry greywater collected from washing machine discharge with the use of only laundry detergent; LF: laundry greywater containing detergent and fabric softener; S: shower greywater containing no personal care product; SS: shower greywater containing only shampoo; SB: shower greywater containing only body wash; W: bathroom washbasin greywater; KD: kitchen greywater containing only dishwashing detergent; KF: kitchen greywater containing only food ingredients, diluted for 100 times in dechlorinated tap water.

[b]: Not applicable because no dilution was made.

[c]: Not measured.

[d]: Not detected.

### *Analytical Methods*

Both *N*-nitrosamine UFC and FP tests were conducted with the blackwater and greywater samples. The UFC test used a practical chloramine condition (i.e., 5 mg $Cl_2$/L $NH_2Cl$, 3-d contact time), while the FP test used an excessive amount (i.e., 100 mg $Cl_2$/L) of pre-formed $NH_2Cl$ and prolonged contact time (i.e., 5 d). For laundry greywaters containing a high DOC, 10 mg $Cl_2$/L was used for the UFC test to obtain desired chloramine residuals (i.e., ~ 2 mg $Cl_2$/L) after 3-d contact time. Samples were buffered with 20-mM phosphate (pH 7.8±0.2), chloraminated under the UFC and FP tests, and incubated in dark at 23±2ºC. At the end of incubation, the residual chloramines were quenched by adding sodium thiosulfate ($Na_2S_2O_3$) powder. The quenched samples were then extracted and analyzed using a GC (Varian 3800, Agilent, US) equipped with MS/MS (Waters 4000, Agilent, US) according to US EPA Method 521 (US EPA, 2004). More details of *N*-nitrosamine analysis method are described elsewhere (Uzun, 2016; Beita-Sandi et al., 2019). In total, seven different *N*-nitrosamines were monitored from sewage components samples, including NDMA, NDEA, NPYR, NDPA, NMOR, NPIP and NDBA. These *N*-nitrosamines are among the most commonly analyzed and reported species in waters and wastewaters (Zeng and Mitch, 2015; Beita-Sandi, 2019). The MRL of *N*-nitrosamines is 3.0 ng/L.

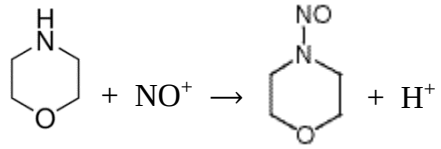
## Results and Discussion

### *N-nitrosamine UFC from Sewage Components*

Previous studies have investigated the occurrences and UFC of *N*-nitrosamines from selected sewage components (Zeng et al., 2015). The occurrence levels of *N*-nitrosamines were <10 ng/L in different sewage components, except for ~20 ng/L NMOR in laundry greywater (Zeng and Mitch, 2015). Similarly, <10 ng/L *N*-nitrosamines were found in different sewage components in this study, except for 29 ng/L NMOR in laundry greywater (**Table 5.2**). Morpholine, an NMOR precursor, is a known component of laundry detergent with high annual global production (Kuchowicz and Rydzyński 1998). Nitrosation of morpholine in the presence of nitrite, an impurity of synthesized nitrogen oxide surfactants used in laundry detergents (Miller et al., 1996), could form NMOR (see the following reactions; Masuda et al., 2006). Although the rate of nitrosation is slow under the near neutral pH condition, the presence of formaldehyde in some laundry detergent may catalyze nitrosation at circumneutral pH (Mitch et al., 2003).

$$2\ HNO_2 \longrightarrow N_2O_3 + H_2O$$

$$N_2O_3 \longrightarrow NO^+ + NO_2^-$$




Morpholine NMOR

**Table 5.2.** The occurrences and UFC of *N*-nitrosamines (ng/L) measured in sewage components.

| *N*-Nitrosamines | Samples | Sewage components | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | U | F | LD | SS | W | KD | KF |
| NDMA | Raw [a] | N.D.[c] | N.D. | N.D. | 3 | N.D. | 3 | N.D. |
| | UFC [b] | N.D. | N.D. | 11 | 9 | 9 | 64 | N.D. |
| NDEA | Raw | N.D. | N.D. | 8 | N.D. | N.D. | N.D. | N.D. |
| | UFC | N.D. | N.D. | 7 | N.D. | N.D. | N.D. | N.D. |
| NPYR | Raw | N.D. | N.D. | N.D. | N.D. | N.D. | 3 | N.D. |
| | UFC | N.D. | N.D. | 12 | N.D. | N.D. | 18 | N.D. |
| NMOR | Raw | N.D. | N.D. | 29 | N.D. | N.D. | N.D. | N.D. |
| | UFC | 4 | N.D. | 36 | N.D. | N.D. | N.D. | N.D. |
| NDPA | Raw | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | UFC | N.D. | N.D. | 19 | N.D. | N.D. | N.D. | N.D. |
| NPIP | Raw | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| | UFC | 5 | N.D. | 35 | N.D. | N.D. | N.D. | N.D. |
| NDBA | Raw | N.D. | N.D. | N.D. | N.D. | 4 | 8 | N.D. |
| | UFC | N.D. | N.D. | 22 | 28 | 33 | 12 | N.D. |

[a]: Measured in raw samples without chloramination. [b]: Measured under the UFC tests. [c]: Not detectable (i.e., <3 ng/L).

*N*-nitrosamine UFC values were reported to be <10 ng/L in urine and feces blackwaters (Zeng and Mitch, 2015), which are consistent with our observations (**Table 5.2**). Only NDMA (~ 100 ng/L) and NMOR (~ 20 ng/L) were reported in laundry greywater under UFC by Zeng and Mitch (2015). In this study, however, all seven *N*-nitrosamines (7-36 ng/L) in laundry greywater formed under UFC. The differences in *N*-nitrosamine UFC reported in these two studies are likely because of the different chloramination conditions adopted for the UFC tests. In the previous study conducted by Zeng and Mitch (2015), the laundry greywater was diluted in DDW approximately four times to achieve the target chloramine residuals (i.e., 1.0±0.4 mg $Cl_2$/L) after 3-d contact time in the UFC test. In the current study, however, laundry greywater was directly chloraminated without any dilutions, and a relatively high monochloramine dosage (i.e., 10 mg $Cl_2$/L, compared to 5

mg $Cl_2$/L used in the previous study) was used to yield the target chloramine residuals in the UFC test.

In shower greywater, Zeng and Mitch (2015) reported that 60-80 ng/L of NDMA formed, while only 9 ng/L of NDMA was found in the sample collected from Clemson. Higher NDBA UFC (i.e., 28 ng/L) was also detected in the current study. These discrepancies of *N*-nitrosamine UFC in shower greywater reported in the two studies are likely because of inconsistent sample collection and processing procedures that may cause different ingredients of shower greywaters (e.g., different brands of shampoo and body wash used during showering). In bathroom washbasin and kitchen greywaters, both studies found <10 ng/L NDMA UFC. In dishwashing greywater, NDMA, NPYR and NDBA UFC were measured to be 64, 18 and 12 ng/L, respectively, in this study. NDBA was detected at 28 and 33 ng/L in shower and washbasin greywaters, respectively. This suggests that laundry greywater contains a greater variety of *N*-nitrosamine precursors than the other sewage components, which may be released from laundry detergents and/or dyes of clothes. Except for KF, all greywaters were found to have NDMA and NDBA precursors

### *N-nitrosamine FP from Sewage Components*

*N*-nitrosamine FPs measured in different sewage components are summarized in **Table 5.3**. Relatively high NDMA FPs were detected in urine blackwaters collected before (U; 11338 ng/L) and after (UR; 7077 ng/L) taking Zantac, and kitchen greywater containing food leachates (KF; 11001 ng/L). Lower NDMA FP was found in kitchen greywater containing dishwashing detergent only (KD; 3001 ng/L), feces blackwaters

collected before (F; 664 ng/L) and after (FR; 1052 ng/L) taking Zantac., laundry greywaters (i.e., 470-501 ng/L), and shower greywaters (i.e., 353-449 ng/L).

**Table 5.3.** *N*-nitrosamine FP from sewage components (after dilution)[a].

| Sewage components | *N*-nitrosamine FP (ng/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | NDMA | NDEA | NPYR | NMOR | NDPA | NPIP | NDBA |
| U | 11338 (1958)[b] | N.D. | 110 (38) | 20 (14) | N.D. | 20 (7) | N.D. |
| UR | 7077 (1414) | N.D. | 141 (73) | 10 (1) | N.D. | 17 (3) | N.D. |
| F | 664 (331) | N.D. | N.D. | 15 (4) | N.D. | 10 (1) | N.D. |
| FR | 1052 (115) | N.D. | N.D. | 8 (2) | N.D. | 10 (2) | N.D. |
| LD | 470 (97) | 39 (16) | 108 (22) | 52 (8) | 20 (0) | 44 (0) | 61 (0) |
| LF | 501 (96) | 50 (1) | 98 (7) | 49 (1) | 12 (0) | 159 (0) | 56 (0) |
| S | 378 (8) | 16 (1) | 142 (13) | 4 (0) | N.D. | 14 (1) | 28 (2) |
| SS | 449 (0) | 10 (0) | 47 (0) | 6 (2) | N.D. | N.D. | 31 (0) |
| SB | 353 (5) | 8 (1) | 48 (1) | 4 (1) | N.D. | 6 (1) | 31 (2) |
| W | 175 (4) | 15 (1) | 135 (6) | N.D. | N.D. | N.D. | 44 (17) |
| KD | 3001 (289) | 4 (1) | 17 (2) | 8 (1) | N.D. | N.D. | 31 (11) |
| KF | 11001 (3696) | N.D. | 492 (16) | N.D. | N.D. | N.D. | 7 (2) |

[a]: Raw human urine (U and UR) and feces (F and FR) are diluted in tap water for 250 and 150 times, respectively, before *N*-nitrosamine FP tests. Kitchen greywater containing food leachates was diluted in tap water for 100 times, while other greywaters were not diluted before *N*-nitrosamine FP tests. [b]: Data in parenthesis represent the standard deviations of two or more measurements.

Other *N*-nitrosamine FPs were more than one magnitude lower than NDMA FPs regardless of sewage component types. In kitchen greywater containing food leachates (KF), NPYR FP was relatively high (i.e., 492 ng/L), followed by shower greywater not containing any personal care products (S; 142 ng/L), urine blackwaters (i.e., 110-141 ng/L), laundry greywaters (i.e., 103-108 ng/L), and shower greywaters containing shampoo (SS; 47 ng/L) and containing body wash (SB; 48 ng/L). In contrast, in feces blackwaters and the other greywaters, NPYR FP were <20 ng/L. Based on the volume fractions of blackwaters and greywaters in domestic sewage (Friedler et al., 2013; Zeng and Mitch, 2015), urine blackwater could be the predominant contributor (i.e., 65%) of NPYR FP in

domestic sewage, followed by KF contributing 25% of NPYR FP. According to Tricker et al. (1992), pyrrolidine, a typical NPYR precursor (Schreiber and Mitch, 2006; Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014), can be excreted via human urine (i.e., 19.4-23.8 mg per day). Pyrrolidine is also present in various food materials (e.g., bread, milk, cheese and vegetables) and drinks (e.g., coffee, cola and alcoholic beverages), presumably as the biodegradation product of proline, or as a flavoring agent (Neurath et al., 1977; Hamano et al., 1981). Human sweat also contains proline (i.e., 53.9 μM) that can be biodegraded to pyrrolidine via human skin microbiome (Mark and Harding, 2012; Dunstan et al., 2016). This may explain the presence of NPYR FP in laundry greywaters and shower greywaters. However, under UFC, the formation of NPYR was observed only in laundry (LD) and kitchen (KD) greywaters (**Table 5.2**).

NPIP FPs were 44 and 159 ng/L in LD and LF, respectively, 17-20 ng/L in urine blackwaters, 14 ng/L in S, and <10 ng/L in the other sewage components. It has been known that laundry detergents contain piperidine-based anionic surfactants (Wieczorek et al., 2017; Abdelmajeid et al., 2017) which may form NPIP under the FP chloramination condition (Schreiber and Mitch, 2006; Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014). Human urine excretes piperidine (i.e., 26.1-31.7 mg per day) (Tricker et al., 1992). Human sweat contains piperidine and phenylalanine (an amino acid) which can be biodegraded to piperidine (Gallagher et al., 2008; Dunstan et al., 2016). NDBA FPs were 56-61 ng/L in laundry greywaters, 31 ng/L in kitchen greywater containing dishwashing detergent (KD), 28-31 ng/L in shower greywaters, and <7 ng/L in the other sewage components. Dibutylamine, a typical NDBA precursor (Sacher et al., 2008; Bond

and Templeton, 2011; Zhou et al., 2014), is frequently used in the manufacture of emulsifiers as ingredients of detergents and personal care products (e.g., shampoo, body wash) (Lide, 1998). NDEA FPs and NMOR FPs were 39-50 and 40-46 ng/L in laundry greywaters, respectively, and <20 ng/L in the other sewage components. Diethylamine, an NDEA precursor (Schreiber and Mitch, 2006; Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014), is often used in the manufacture of surfactants contained in laundry detergents (Ozdil et al., 2016). NMOR can form via the reactions between morpholine and nitrite present in laundry detergents (Miller et al., 1996; Masuda et al., 2006; Glover et al., 2019). NDPA FPs were found only in laundry greywaters (i.e., 12-20 ng/L), and were undetectable (i.e., <3 ng/L) in the other sewage components. Among the blackwaters and greywaters tested, laundry greywater was the major source of NDEA (i.e., 70%), NPIP (i.e., 84%), NDPA (i.e., 100%) and NDBA precursors (i.e., 55%) in domestic sewage, also an important contributor of NMOR precursors (i.e., 30%).

In general, there were discrepancies between *N*-nitrosamine UFC and FP found in each component from sewage. In sewage components with both *N*-nitrosamine UFC and FP detected, the UFC of NDMA and NPYR were generally one or two orders of magnitudes lower than their FP. For other *N*-nitrosamines, their UFC were similar or less significantly lower than FP. However, a higher *N*-nitrosamine UFC does not necessarily indicate higher *N*-nitrosamine FP. For example, NDMA UFC were undetectable in urine blackwaters, but NDMA FP from urine blackwater were among the highest found in all tested sewage components. There were no clear patterns with *N*-nitrosamine UFC correlating with FP. More studies are still needed to evaluate the potential correlations

between *N*-nitrosamine UFC and FP, such as their responses to different impacting factors in selected water matrices.

## Conclusions

Among the tested sewage components, laundry greywater exhibited the highest *N*-nitrosamine formation under the UFC test, which is consistent with a previous study that used the same samples. Based on the FP tests with various sewage components, urine blackwater was the predominant source of NDMA and NPYR precursors present in domestic sewage, although laundry greywater was still the major sources of other *N*-nitrosamine precursors. To reduce *N*-nitrosamine precursors from wastewaters, it might be important to control the discharge of urine blackwater and laundry greywater, or to separate them from the main sewage drainage system. Applications of urine separation system and non-potable greywater reuse may thus help to reduce *N*-nitrosamine precursor loading in WWTP influents. Huge discrepancies were found between *N*-nitrosamine UFC and FP in each sewage component, and there were no clear patterns of *N*-nitrosamine UFC correlating with FP. Further studies are still needed to elucidate the potential relationships between *N*-nitrosamine UFC and FP as affected by different factors (e.g., water quality parameters).

# CHAPTER VI

# RE-EXAMINATION OF DIFFERENT FACTORS AFFECTING NDMA FORMATION FROM MODEL COMPOUNDS AND SURFACE WATERS

## Introduction and Objective

The disinfection process (e.g., chlorination, chloramination, ozonation, etc.) for drinking water is indispensable to deactivate waterborne pathogens and provide a safe potable product (Rook, 1974). However, chloramination of drinking waters may form NDMA, a probable carcinogenic DBP (Choi and Valentine, 2002). NDMA is formed via reactions between disinfectants (i.e., chloramines, ozone) and organic precursors (i.e., secondary, tertiary, and quaternary amines) (Mitch and Sedlak, 2004; Shen and Andrews, 2011a, b; Selbes et al., 2013). Many factors have been known to affect NDMA formation, such as pH, $Br^-$, $NH_2Cl$ dosage, and the presence of NOM in surface waters (Selbes et al., 2013; Shen and Andrews, 2011a, 2013a; Uzun et al., 2015; Zhang et al., 2015). So far, studies of different factors on NDMA formation have mostly focused on single-factor effects in a pure water (i.e., DDW) matrix. In natural surface waters, however, multiple factors co-exist and may simultaneously affect NDMA formation. No single factor was found to be fully attributable to NDMA formation (Uzun et al., 2015). Therefore, there is a need to elucidate the multi-factor effects on NDMA formation in surface waters.

Previous studies have reported inconsistent findings for pH effects on NDMA formation. Under different pH conditions, peak NDMA formations were found at pH 6 (Chen and Valentine, 2006), 8 (Mitch et al., 2003), or even 9 (Krasner et al., 2018). The

reasons for such inconsistent findings are unclear, likely because of the different water samples tested and distinct chloramination conditions (e.g., FP vs UFC tests) applied in these studies. Under the FP chloramination condition, RNTD, a reactive NDMA precursor, yielded its peak NDMA formation at pH 8 (Le Roux et al., 2011). Under the UFC chloramination condition, however, RNTD yielded peak NDMA formation at pH 7 (Shen and Andrews, 2013a). These results suggest that the effects of pH on NDMA formation may depend on chloramination conditions (i.e., UFC vs FP tests). So far, only a few compounds were investigated for their NDMA formation at different pH levels, including DMA, RNTD and SMTR (Mitch and Sedlak, 2002; Chen and Valentine, 2006; Le Roux et al., 2011; Shen and Andrews, 2013a). More studies are thus needed to investigate pH effects by testing more precursor compounds, and to evaluate the interactions between pH and other factors affecting NDMA formation.

The presence of NOM in surface waters was reported to decrease NDMA formation rates or yields (Shen and Andrews, 2011a; Selbes et al., 2014; Tan et al., 2018). NDMA formation rates of RNTD decreased with increasing DOC and $SUVA_{254}$ in water matrices (i.e., DDW, tap, river and lake waters; Shen and Andrews, 2011a). However, NDMA FP from RNTD was found to be higher in the presence of NOM than in DDW (Selbes et al., 2013). These contradictory findings are likely because of the different chloramination conditions (i.e., UFC vs FP tests) used in these studies. So far, NDMA formation from surface waters containing different amounts (i.e., DOC) and types (i.e., $SUVA_{254}$) of NOM are still not well evaluated, with their potential correlations largely unclear.

In natural surface waters, the mean concentration for $Br^-$ is approximately 100 µg/L (Kim et al., 2015). Elevated $Br^-$ concentrations (i.e., 250-1900 µg/L) may occur in desalinated seawaters or blending waters (Wajima, 2014; Kim et al., 2015). Less than 250 µg/L $Br^-$ was found to have a negligible effect on NDMA formation, while the presence of 500-1000 µg/L $Br^-$ enhanced NDMA formation in surface waters (Shah et al., 2012). So far, studies of $Br^-$ effects on NDMA formation have mainly focused on extremely high (i.e., 2-32 mg/L) $Br^-$ concentrations (Le Roux et al., 2012; Tan et al., 2018). The effects of $Br^-$ with concentrations (i.e., <2000 µg/L) typically found in natural surface waters and desalinated waters are still less understood, especially under different pH conditions. Previous studies found that $Br^-$ effects on NDMA formation from DMA depended on pH (Luh and Marinas, 2012). At relatively low pH (i.e., pH 6.0-6.6), NDMA formation from DMA was inhibited in the presence of a high level (i.e., 32 mg/L) of Br- in DDW. At a higher pH (i.e., pH 8-9), however, NDMA formation from DMA was enhanced in the presence of $Br^-$.

Increasing $NH_2Cl$ dosage typically increased NDMA formation (Choi and Valentine, 2002; Mitch et al., 2003; Hatt et al., 2013). However, there was a critical $NH_2Cl$ dosage above which NDMA formation reaches a plateau. So far, there is still no quantitative description of the effects of $NH_2Cl$ dosage on NDMA formation, and the critical $NH_2Cl$ dosages in different surface waters are still not well evaluated.

The major objective of this study is to investigate the multi-factor effects of selected factors (i.e., pH, DOC, $SUVA_{254}$, $Br^-$ and $NH_2Cl$ dosage) on NDMA formation from model compounds and surface waters, via measuring (i) NDMA UFC and NDMA FP from

different model compounds at pH 6.0, 6.8, 7.8 and 8.8 in DDW, (ii) NDMA UFC from model compounds at pH 6.8, 7.8 and 8.8 in Myrtle Beach raw and treated waters, (iii) NDMA UFC and NDMA FP in five raw surface waters and four treated waters, with the DOC of raw waters adjusted to 9-25, 5.0, 2.5 and 1.0 mg/L, respectively; (iv) NDMA UFC from model compounds in the presence of $Br^-$ (i.e., 1000 µg/L) in DDW at pH 6.8, 7.8 and 8.8; and (v) NDMA UFC and NDMA FP in surface waters with the DOC-normalized $NH_2Cl$ dosage (i.e., $NH_2Cl$/DOC) ranging 0.2-140 mg $Cl_2$/mg C.

## Materials and Methods

### *Model Precursor Compounds*

Sixteen model compounds were selected for the examination of their NDMA formation, including eight aliphatic amines (i.e., DMA, TMA, *N*,*N*-dimethylethylamine (DMEA), *N*,N-dimethylbutylamine (DMBA), *N*,*N*-dimethylisopropylamine (DMiPA), unsymmetrical dimethylhydrazine (UDMH), *N*,*N*-dimethylethylenediamine (DMEDA) and daminozide (DMNZD)) and eight aromatic amines (i.e., *N*,*N*-dimethylaniline (DMAN), *N*,*N*-dimethylbenzylamine (DMBzA), *N*,*N*-dimethylphenetylamine (DMPhA), RNTD, methylene blue (MB), minocycline (MNCL), SMTR and diuron (DRN)). All compounds were purchased from Sigma Aldrich (St. Louis, US) and dissolved in methanol (400 µM) to prepare primary standard solutions. The key physiochemical properties of these model compounds are summarized in **Table B-1**.

### *Surface Water Samples*

Five raw surface waters (RA, RB, RC, RD and RE) were collected from five watersheds in South Carolina, US. Water samples were grabbed from a river, lake or reservoir near to the intakes of water treatment plants. The five raw surface waters were selected to provide a variety of water source types (i.e., river, reservoir or lake), NOM concentrations (i.e., 4.9-25 mg/L DOC) and aromatic portions (i.e., 3.1-5.1 $L \cdot mg^{-1} \cdot m^{-1}$ $SUVA_{254}$). After conventional treatment, four treated waters (TA, TB, TCD and TE; RC and RD waters were mixed in a volume ratio of approximately 1:1 on the sampling date prior to treatment) were grabbed from the outlets of four water treatment plants prior to the disinfection process.

All collected water samples were filtered through 0.45-μm cellulose nitrate membrane (Whatman, Maidstone, UK) before storage at 4°C until used. Selected water quality parameters were measured according to Standard Methods (APHA et al., 2005) and shown in **Table B-2**.

### *Experimental Procedure*

The multi-factor effects of selected factors (i.e., pH, DOC, $SUVA_{254}$, $Br^-$ and $NH_2Cl$ dosage) were examined by monitoring NDMA formation from model compounds and surface waters, with the experimental matrix summarized in **Table 6.1**.

**Table 6.1.** Experimental matrix for the examination of multi-factor effects on NDMA formation.

| Factors | Ranges | NDMA precursors | Water matrices | NDMA formation tests |
|---|---|---|---|---|
| pH | 6.0, 6.8, 7.8 and 8.8 | Sixteen model compounds | DDW[a] | UFC & FP |
| | 6.8, 7.8 and 8.8 | Four model compounds | Myrtle Beach raw (DOC = 1.0 and 2.5 mg/L) and treated (DOC = 1.0 mg/L) waters | UFC |
| | 6.8, 7.8 and 8.8 | Raw surface waters (RA-RE)[c] | N.A.[b] | UFC & FP |
| | 6.8 and 7.8 | Treated surface waters (TA-TE)[d] | N.A. | UFC |
| DOC | 1.0 and 2.5 mg/L | Four model compounds | Myrtle Beach raw and treated waters | UFC |
| | 1.0, 2.5, 5.0 and 9-25 mg/L | Raw surface waters (RA-RE) | N.A. | UFC & FP |
| $SUVA_{254}$ | 1.7-5.1 $L \cdot mg^{-1} \cdot m^{-1}$ | Raw and treated surface waters | N.A. | UFC & FP |
| $Br^-$ | 0 and 1000 µg/L | Seven model compounds | DDW | UFC |
| $NH_2Cl/DOC$[e] | 0.2-100 mg $Cl_2$/mg C | Raw and treated surface waters | N.A. | UFC & FP |

[a]: Distilled and deionized water. [b]: Not applicable. [c]: Five raw surface water samples (RA, RB, RC, RD and RE) collected from five watersheds in southeast US. [d]: Four treated surface waters (TA, TB, TCD and TE) collected from water utilities after conventional treatment of RA-RE prior to disinfection process (RC and RD were mixed before treatment in a water utility). [e]: DOC-normalized $NH_2Cl$ dosage.

In brief, the effects of pH were examined by measuring NDMA UFC and NDMA FP from sixteen model compounds (i.e., 1000 nM) in DDW at pH 6.0, 6.8, 7.8 (all buffered with 20-mM phosphate) and 8.8 (buffered with 20-mM borate), respectively. In addition, NDMA UFC from four selected model compounds (i.e., TMA, DMiPA, DMBzA and DMPhA) were measured at pH 6.8, 7.8 and 8.8 in DDW, Myrtle Beach raw waters (1.0 and 2.5 mg/L DOC), and Myrtle Beach treated water (1.0 mg/L DOC). Further, NDMA UFC and NDMA FP from the five raw surface waters (RA-RE) and four treated waters (TA-TE) were monitored at pH 6.8, 7.8 and 8.8, respectively.

The effects of NOM on NDMA formation were examined by measuring NDMA UFC from seven selected compounds (i.e., DMA, TMA, DMBA, DMiPA, DMAN, DMBzA and DMPhA) in DDW and in a Suwannee River (SR) NOM solution (i.e., 10 mg/L), respectively. NDMA UFC from four selected compounds (i.e., TMA, DMiPA, DMBzA and DMPhA) were further measured in DDW, Myrtle Beach raw (1.0 and 2.5 mg/L DOC) and treated (1.0 mg/L DOC) waters, respectively. Besides, NDMA UFC and NDMA FP were measured in the five raw surface waters (RA-RE; 9-25 mg/L DOC) with their DOC adjusted to 1.0, 2.5, 5.0 mg/L, respectively. The effects of $SUVA_{254}$ were examined by evaluating potential correlations between NDMA UFC (or NDMA FP) and $SUVA_{254}$ measured in different surface waters, with the effects of DOC on NDMA formation accounted (i.e., NDMA UFC/DOC, NDMA FP/DOC).

The effects of $Br^-$ were evaluated by monitoring NDMA UFC from seven model compounds (i.e., DMA, TMA, DMBA, DMiPA, DMAN, DMBzA and DMPhA) in DDW in the presence of 1000 µg/L $Br^-$ at pH 6.8, 7.8 and 8.8, respectively. The effects of $NH_2Cl$ dosage on NDMA formation were evaluated by evaluating potential correlations between NDMA UFC/DOC (or NDMA FP/DOC) measured in each surface water with $NH_2Cl$/DOC.

The molar yields (%) of NDMA from model compounds were calculated based on the following equation:

$$NDMA\ molar\ yields\ (\%) = \frac{NDMA\ UFC\ or\ FP(ng/L)}{MW \times C} \times 100\% \quad \textbf{Equation 6.1}$$

where MW is the molecular weight of NDMA (74.5 g/mol), and *C* is the initial concentration of model compounds (i.e., 1000 nM).

### *NDMA Formation Tests*

The formation of NDMA from model compounds and surface waters were measured under both the UFC and FP conditions. Specifically, a monochloramine stock solution (~2000 mg $Cl_2$/L) was freshly prepared by adding a sodium hypochlorite solution (NaClO, ~4000 mg $Cl_2$/L) into an ammonium chloride solution ($NH_4Cl$, ~1000 mg N/L) drop by drop at pH 9 with a Cl:N mass ratio of 4:1. During the UFC and FP tests, a predetermined amount of monochloramine stock solution was added to water samples to achieve target chloramine dosages (i.e., 5 mg $Cl_2$/L for UFC test, 100 mg $Cl_2$/L for FP test). The chloraminated samples were then incubated at 23±2° in dark for 3 d (UFC test) or 5 d (FP test). At the end of incubation, excess amounts of sodium thiosulfate ($Na_2S_2O_3$) powder were added to quench residual chloramines.

### *Analytical Methods*

NDMA concentrations were analyzed according to US EPA Method 521 (US EPA, 2004). For the sample analysis, 250 mL of chloraminated model compounds' solutions or surface water samples were added with a predetermined amount of a standard solution of NDMA-d6 before extraction. The samples were then passed through cartridges packed with 2-g coconut charcoal (UTC, Farmington, US) and pre-conditioned with DCM, methanol and DDW driven by a vacuum system. After extraction, cartridges were dried under a mild vacuum and eluted with DCM. Eluates were collected, passed through a drying column packed with 6-g anhydrous sodium sulfate ($Na_2SO_4$) powder, and

concentrated to ~1 mL under a gentle nitrogen gas flow. Concentrated extracts were spiked with NDPA-$d_{14}$ as internal standard and transferred to 2-mL amber GC vials for GC/MS/MS (7000 QQQ, Agilent, US) analysis. The minimum reporting level of NDMA in DDW and surface water samples was 0.5 ng/L.

$NH_2Cl$ and dichloramine ($NHCl_2$) concentrations were monitored according to US EPA Method 330.5 (US EPA, 1978). The detection limits of $NH_2Cl$ and $NHCl_2$ were 0.025 mg $Cl_2$/L. All samples and blank controls were prepared and analyzed in duplicates.

## Results and Discussion

### *Effects of pH on NDMA UFC and FP*

NDMA UFC yields from all tested compounds consistently increased with pH increasing from 6.0 to 6.8, while decreased with pH further increasing from 7.8 to 8.8 (**Figure 6.1**). With pH increasing from 6.0 to 6.8, precursor compounds would become more deprotonated, which may facilitate the electrophilic reactions between compounds and chloramines to form NDMA (Shen and Andrews, 2013a; Selbes et al., 2013). With pH further increasing from 7.8 to 8.8, however, $NHCl_2$ concentrations were more limited (**Figure B-1**; Vikesland et al., 2001), and NDMA formation was inhibited because $NHCl_2$ was found to be mainly attributable to NDMA formation in chloraminated waters (Shen and Andrews, 2013a; Selbes et al., 2013).

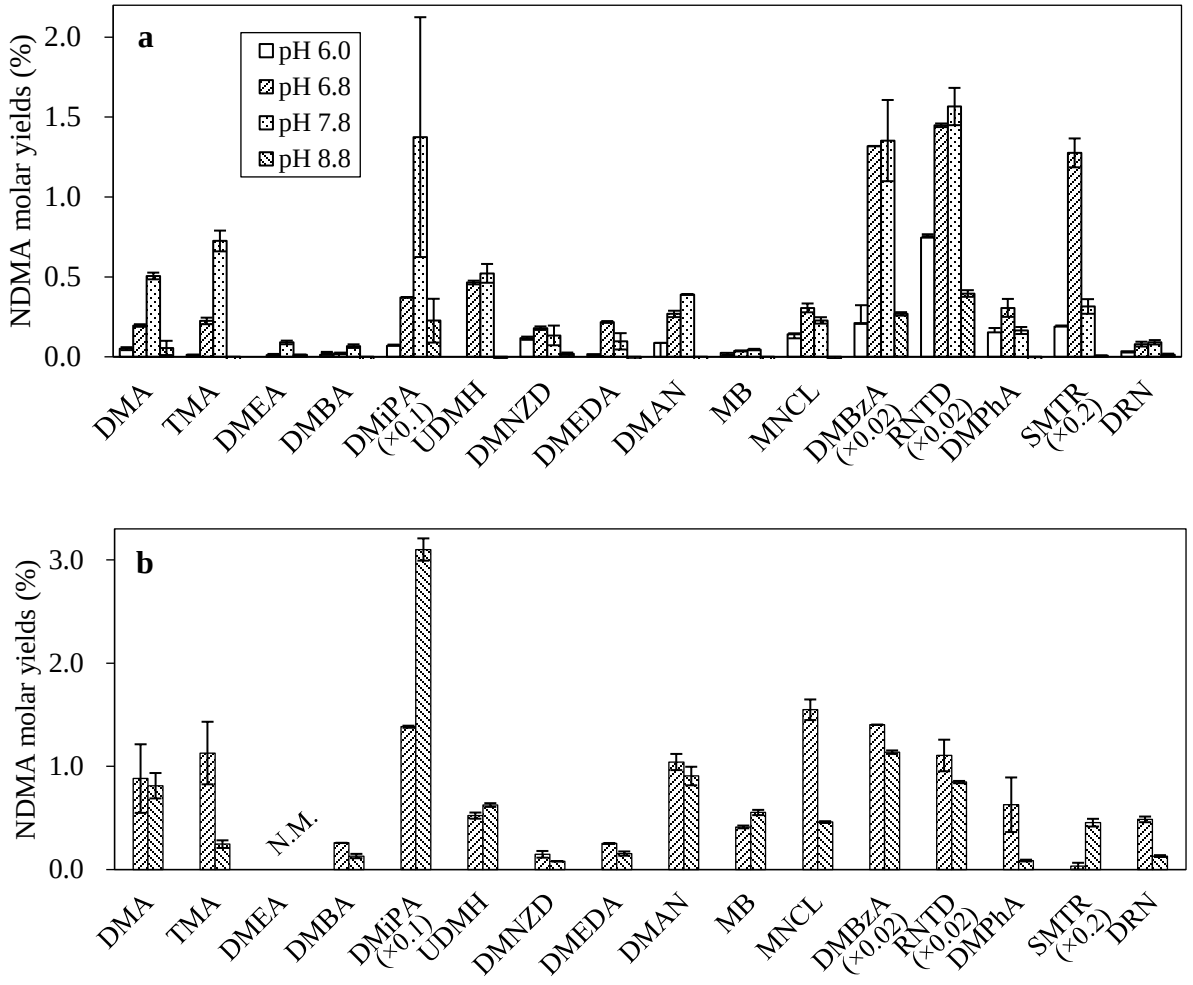



**Figure 6.1.** NDMA molar yields from model compounds measured in DDW under (a) UFC and (b) FP tests (FP only measured at pH 6.8 and 8.8; DMEA was not measured for NDMA FP). DMA: dimethylamine, TMA: trimethylamine, DMEA: *N*,*N*-dimethylethylamine, DMBA: *N*,*N*-dimethylbutylamine, DMiPA: *N*,*N*-dimethylisopropylamine, UDMH: unsymmetrical dimethylhydrazine, DMNZD: daminozide, DMEDA: *N*,*N*-dimethylethylenediamine, DMAN: *N*,*N*-dimethylaniline, MB: methylene blue, MNCL: minocycline, DMBzA: *N*,*N*-dimethylbenzylamin, DMPhA: *N*,*N*-dimethylphenetylamine (DMPhA), SMTR: sumatriptan, DRN: diuron. NDMA yields from DMiPA were multiplied by 0.1 (i.e., ×0.1), yields from DMBzA and RNTD were multiplied by 0.02 (i.e., ×0.02), and yields from SMTR were multiplied by 0.2 (i.e., ×0.2). The bar graphs hereafter represent the average values of duplicate measurements, and error bars hereafter represent the standard deviations.

All sixteen compounds yielded their peak NDMA UFC at pH 6.8 or 7.8. Previous studies proposed that increasing pH could enhance the deprotonation of NDMA precursors, and decrease $NHCl_2$ concentrations, thus the peak NDMA UFC were achieved at pH 7-8 where the deprotonated compounds and $NHCl_2$ concentrations were both abundant (Shen and Andrews, 2013a). However, compounds such as MB, DMNZD and DRN have pKa of 3.8, 4.7 and 13.6, respectively, and increasing pH (i.e., from 6.0 to 8.8 in this study) may not affect their deprotonation. The results that MB, DMNZD and DRN yielded their peak NDMA UFC at pH 6.8-7.8 suggest that other factors may affect NDMA formation from these precursors. At acidic pH (i.e., pH 6.0), free chlorine (i.e., HClO) concentrations can be relatively high due to acid catalyzed decays of $NH_2Cl$ and $NHCl_2$ which can form HClO (Jafvert and Valentine, 1992). HClO is known to deactivate NDMA precursors and inhibit NDMA formation (Shen and Andrews, 2013a; Selbes et al., 2014). Therefore, NDMA UFC at pH 6.0 were lower than that at pH 6.8 or 7.8, regardless of compounds' pKa.

NDMA UFC values from all tested compounds were higher at pH 6.8 than pH 8.8. In contrast, NDMA FPs from most tested compounds were comparable or even lower at pH 6.8 than at pH 8.8 (**Figure 6.1**). Under the FP condition, $NHCl_2$ concentration was more abundant than under the UFC condition (**Figure B-2**), due to the excessive $NH_2Cl$ dosage used for the FP test. $NH_2Cl$ decays can lead to the formation of $NHCl_2$ (Vikesland et al., 2001). With pH increasing from 6.8 to 8.8, $NHCl_2$ was less limited under the FP condition because of the extremely high dosage applied, and thus NDMA FP would be able to increase with pH increasing from 6.8 to 8.8.

NDMA UFC from four selected compounds (i.e., TMA, DMiPA, DMBzA and DMPhA; each 1000 nM) were monitored at pH 6.8, 7.8 and 8.8 in Myrtle Beach raw (i.e., 1.0 and 2.5 mg/L DOC) and treated (i.e., 1.0 mg/L DOC) waters. In Myrtle Beach treated water, NDMA UFC from the four compounds consistently increased with pH increasing from 6.8 to 7.8, while decreased with pH further increasing from 7.8 to 8.8 (**Figure 6.2**). This finding was consistent with that observed in DDW. In Myrtle Beach raw water containing 1.0 mg/L DOC, however, NDMA UFC values from DMiPA, DMBzA and DMPhA were constant or decreased with pH increasing from 6.8 to 7.8. In Myrtle Beach raw water containing 2.5 mg/L DOC, NDMA UFC from all four compounds decreased with pH increasing from 6.8 to 7.8. These results suggest that in surface waters with higher DOC or $SUVA_{254}$, NDMA UFC from model compounds more tended to decrease with pH increasing from 6.8 to 7.8.

At pH 6.8, $NHCl_2$ concentration was relatively abundant, which can compete with NOM to react with NDMA precursors. At pH 7.8, however, $NHCl_2$ concentrations were relatively limited (**Figure B-3**), and NOM complexations can inhibit NDMA formation. NOM in raw waters were considered more hydrophobic than that in treated waters, likely exhibiting stronger complexations with model NDMA precursors. Previous studies have indicated that hydrophobic NOM exhibited stronger complexations with cationic ions than hydrophilic NOM (Gu et al., 1995; Meier et al., 1999; Inam et al., 2019). Under the near neutral pH condition, NOM in raw waters may exhibit stronger complexations with NDMA precursors than that in treated waters. Because of the stronger NOM complexations with NDMA precursors in raw waters, NDMA formation was more inhibited at pH 7.8 in raw

waters, and thus NDMA UFC tended to decrease more with pH increasing from 6.8 to 7.8. Similarly, with the DOC of raw waters increasing from 1.0 to 2.5 mg/L, NOM complexations with NDMA precursors were stronger, and NDMA UFC thus tended to decrease more with pH increasing from 6.8 to 7.8.

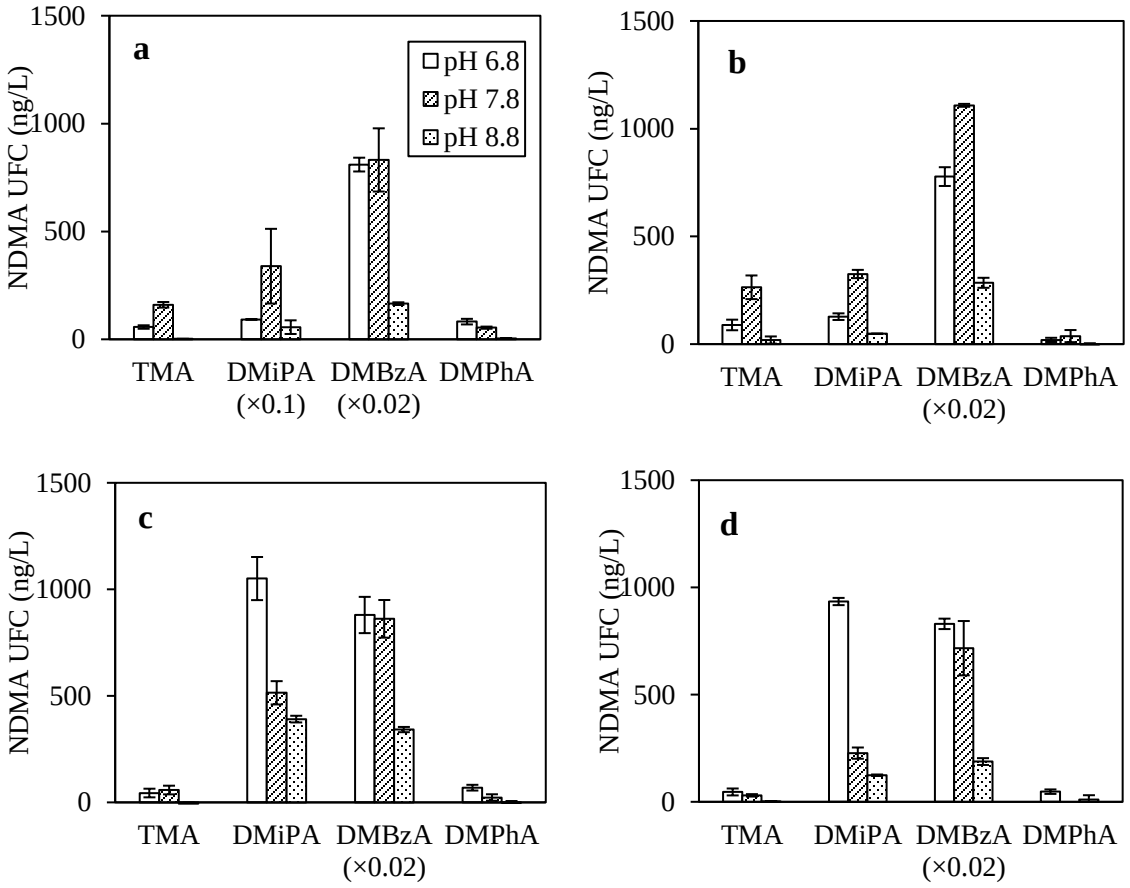



**Figure 6.2.** NDMA UFC from model compounds measured in (a) DDW, (b) Myrtle Beach treated water with 1.0 mg/L DOC, (c) Myrtle Beach raw water with 1.0 mg/L DOC, and (d) Myrtle Beach raw water with 2.5 mg/L DOC.

Without any model compounds added, NDMA UFC and NDMA FP were measured in five raw surface waters (RA, RB, RC, RD and RE, DOC = 9-25 mg/L) and four treated waters (TA, TB, TCD, TE, DOC = 2.4-5.9 mg/L) at pH 6.8, 7.8 and 8.8, respectively. With pH increasing from 6.8 to 8.8, NDMA UFC from all five raw waters consistently decreased (**Figure 6.3**). This observation is consistent with that (i.e., model compounds) in Myrtle Beach raw waters. In contrast, NDMA FP from raw waters increased with pH increasing from 6.8 to 7.8, though decreased with pH further increasing from 7.8 to 8.8. Under the FP condition, $NHCl_2$ concentration was more abundant than that under the UFC condition **(Figure B-4**). With pH increasing from 6.8 to 7.8, $NHCl_2$ concentration was less limited under the FP condition, and thus NDMA FP more likely increased with pH increasing from 6.8 to 7.8.

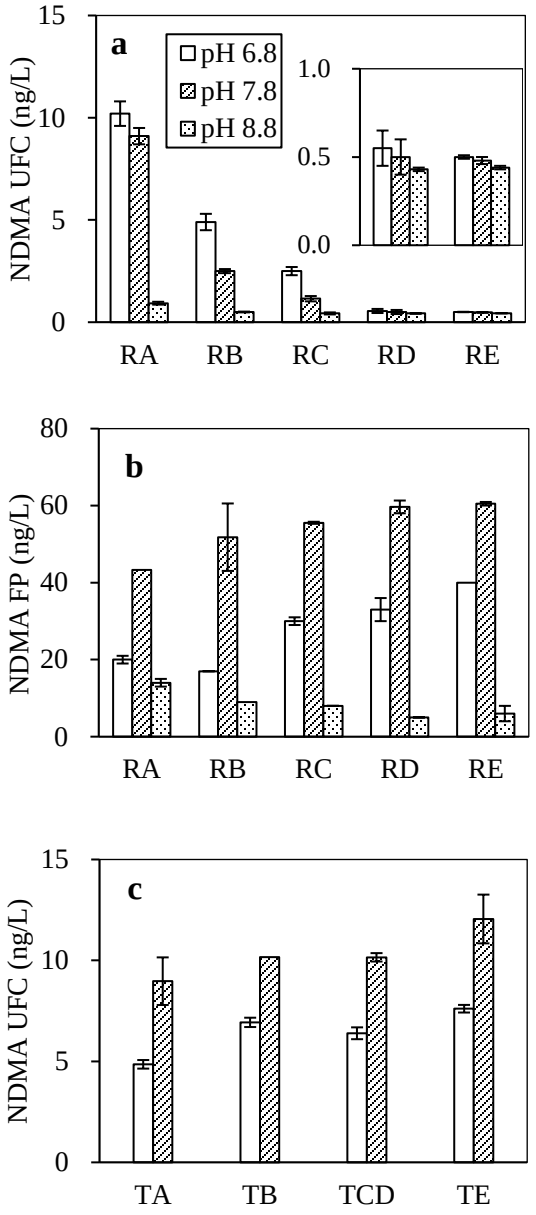



**Figure 6.3.** NDMA (a) UFC from raw surface waters, (b) FP from raw surface waters, and (c) UFC from treated surface waters (not measured at pH 8.8).

NDMA UFC from the four treated waters (TA, TB, TCD, TE, DOC = 2.4-5.9 mg/L) consistently increased with pH increasing from 6.8 to 7.8, contrary to that found in raw waters. This was likely because treated waters contained lower DOC, and $NHCl_2$ was less consumed by NOM at pH 7.8 (**Figure B-5**). With pH increasing from 6.8 to 7.8, NDMA UFC from treated waters thus increased more than that in raw waters. With the DOC of five raw waters decreasing from 9-25 mg/L to 5.0, 2.5 and 1.0 mg/L, NDMA UFC increased with pH increasing from 6.8 to 7.8. In raw waters with 5.0 mg/L DOC, NDMA UFC consistently decreased with pH increasing from 6.8 to 7.8; in raw waters with 2.5 mg/L DOC, NDMA UFC values were generally constant with pH increasing from 6.8 to 7.8, while in raw waters with 1.0 mg/L DOC, NDMA UFC increased with pH increasing from 6.8 to 7.8, except that NDMA UFC from RE water still slightly decreased (**Figure 6.4**). At lower DOC, NOM exhibited weaker complexations with NDMA precursors, and less consumptions of $NHCl_2$ (**Figure B-6**). With pH increasing from 6.8 to 7.8, the concentrations of $NHCl_2$ and NDMA precursors were both less limited in waters with lower DOC, and thus NDMA UFC increased with pH increasing from 6.8 to 7.8 more than in waters with higher DOC.

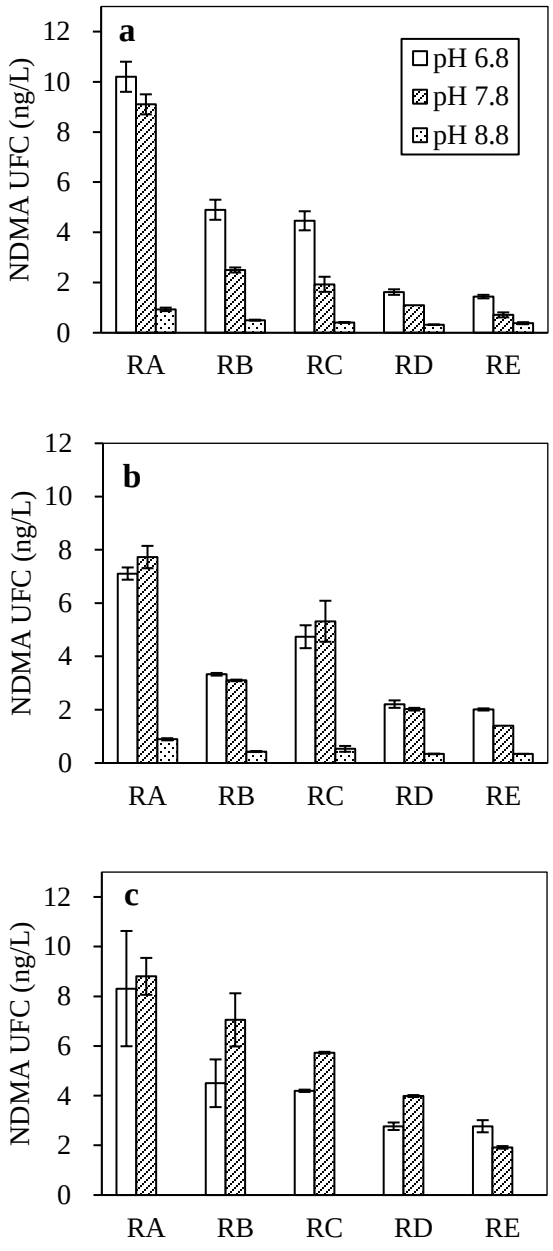



**Figure 6.4.** NDMA UFC from raw surface waters containing (a) 5 mg/L DOC, (b) 2.5 mg/L DOC, and (c) 1.0 mg/L DOC (not measured at pH 8.8).

These results suggest that the effects of pH on NDMA formation depended on the amounts (i.e., DOC) and types (e.g., raw vs treated, or $SUVA_{254}$) of NOM present in water samples. In water utilities, DOC and $SUVA_{254}$ of surface waters could both decrease after conventional treatment, and NDMA UFC in finished waters may thus be higher at pH 7.8 than at pH 6.8. To minimize NDMA formation, water utilities may need to adjust pH of finished waters to 6.8 or 8.8. Because utilities need to maintain water pH above 7.6 for corrosion control purposes, pH 8.8 might be more proper for NDMA formation controls.

### *Effects of NOM on NDMA UFC and FP*

To evaluate the effects of NOM on NDMA formation, NDMA UFC from seven selected compounds (i.e., DMA, TMA, DMEA, DMiPA, DMAN, DMBzA, DMPhA) were monitored in SR NOM solution (10 mg/L) and in DDW at pH 6.8, 7.8 and 8.8, respectively. In SR NOM solution, NDMA UFC values from all seven compounds were <substantially lower than in DDW, regardless of pH (**Figure 6.5**). NOM can affect NDMA formation in two different ways. First, NOM enhances the consumption of $NHCl_2$ (Vikesland et al., 2001), and reduces NDMA formation rates. Second, NOM complexes with NDMA precursors and inhibits the reaction rates between NDMA precursors and $NHCl_2$ to form NDMA (Shen and Andrews, 2011a). $NHCl_2$ concentrations were comparable in SR NOM solution and in DDW (**Figure B-7**) suggesting that $NHCl_2$ consumption by SR NOM may not be an important factor affecting NDMA formation. Rather, NOM complexation with NDMA precursors may play a more important role. At near neutral pH, NOM complexes with NDMA precursors via electrostatic attractions between negatively charged carbonyl

or hydroxyl groups of NOM and positively charged amine groups of NDMA precursors. Aromatic NDMA precursors can complex with NOM via covalent bindings between precursors' amine groups and carbonyl (or quinone) groups of NOM (Shen and Andrews, 2011a).

At pH 7.8, NDMA UFC values from four selected compounds (i.e., TMA, DMiPA, DMBzA and DMPhA) were lower in Myrtle Beach raw waters than in DDW (**Figure 6.6**), likely because NOM complexations with NDMA precursors inhibited NDMA formation at pH 7.8. At pH 6.8, however, NDMA UFC from the four compounds were generally comparable in Myrtle Beach raw waters and in DDW. At pH 6.8, $NHCl_2$ concentration was relatively abundant (Vikesland et al., 2001; Chen and Valentine, 2006; **Figure B-3**), and may exhibit stronger competition with NOM to react with NDMA precursors. Therefore, NOM complexations less affected NDMA UFC at pH 6.8. This suggests that NOM effects on NDMA formation depend on pH.

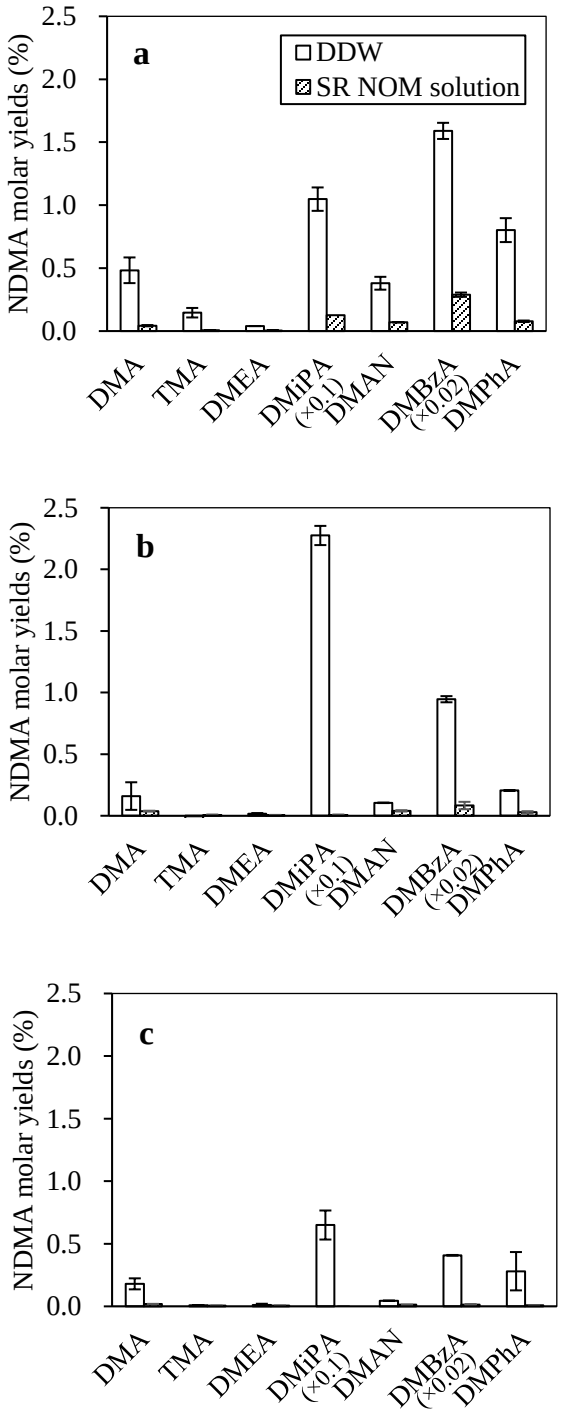



**Figure 6.5.** NDMA UFC yields from model compounds measured in DDW and in Suwannee River (SR) NOM solution (i.e., 10 mg/L) at (a) pH 6.8, (b) pH 7.8, and (c) pH 8.8.

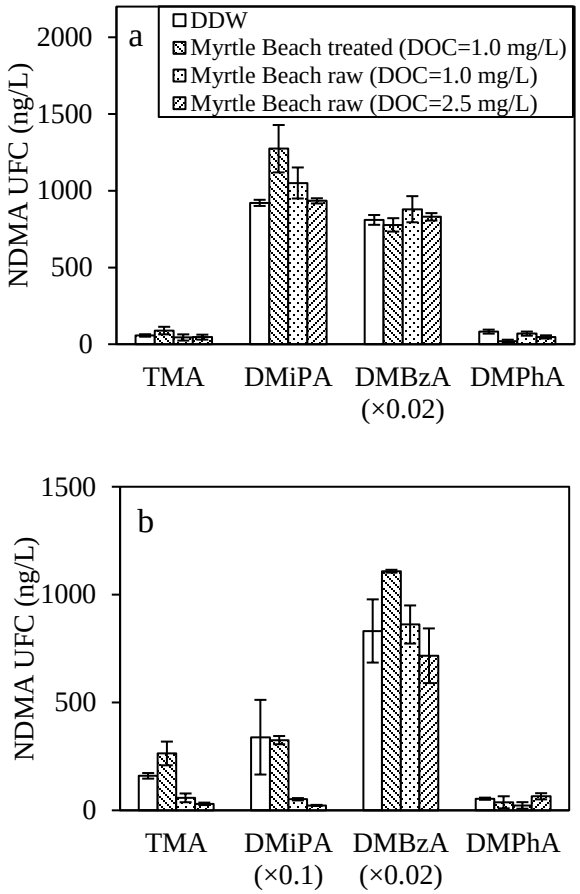



**Figure 6.6.** NDMA UFC from model compounds measured in different water matrices at (a) pH 6.8, and (b) pH 7.8.

With the DOC of Myrtle Beach raw waters increasing from 1.0 to 2.5 mg/L, NDMA UFC from the four compounds decreased, likely because of the higher NOM complexations with NDMA precursors that inhibited NDMA formation. At the same DOC (i.e., 1.0 mg/L), NDMA UFC values from the four compounds were comparable or higher in Myrtle Beach treated water than in Myrtle Beach raw water, except NDMA UFC from DMPhA being relatively lower. This was probably because NOM in Myrtle Beach treated water were more hydrophilic (e.g., lower $SUVA_{254}$), and thus exhibited weaker

complexations with NDMA precursors. Therefore, NDMA UFC from model compounds were less inhibited in Myrtle Beach treated water than Myrtle Beach raw water. These results suggest that NDMA UFC from model compounds were dependent on DOC and $SUVA_{254}$ of water samples.

Without any model compounds added, NDMA UFC and NDMA FP from the five raw waters were monitored at pH 6.8, 7.8 and 8.8, with their DOC adjusted to 5.0, 2.5 and 1.0 mg/L, respectively. NDMA UFC from most (i.e., three at pH 6.8, and four at pH 7.8) of the five raw waters increased with DOC decreasing from 5.0 to 1.0 mg/L (**Figure 6.7**). With DOC decreasing from 5.0 to 1.0 mg/L, NOM would exhibit less complexations with NDMA precursors, and thus NDMA UFC increased. In contrast, NDMA FP consistently decreased with DOC decreasing from 5.0 to 1.0 mg/L. Under the FP condition, $NHCl_2$ concentrations were more abundant than under the UFC condition (**Figures B-6** and **B-8**), and $NHCl_2$ exhibited stronger competition with NOM to react with NDMA precursors, thus NDMA FPs were less affected by NOM complexations. With DOC decreasing from 5.0 to 1.0 mg/L, the amounts of NDMA precursors decreased, and thus NDMA FP decreased. These results suggest that the effects of NOM on NDMA formation depend on the distinct $NHCl_2$ concentrations in the UFC and FP tests.

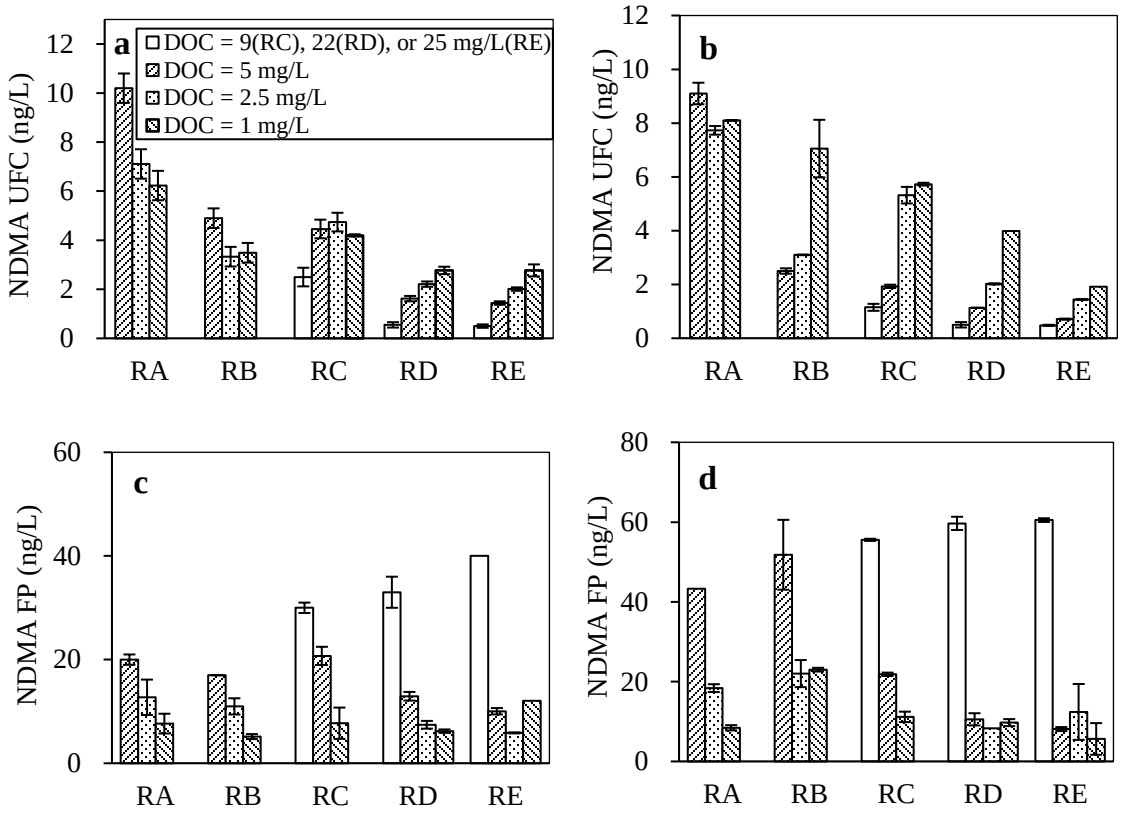



**Figure 6.7.** NDMA formation from surface waters measured under (a) UFC tests at pH 6.8, (b) UFC tests at pH 7.8, (c) FP tests at pH 6.8, and (d) FP tests at pH 7.8.

In four treated waters (TA-TE), NDMA UFC values (i.e., 6-12 ng/L at pH 6.8 or 7.8) were higher than those (i.e., 1-5 ng/L) in raw waters, except that NDMA UFC from TA (i.e., 5-9 ng/L) was lower than RA (i.e., 9-10 ng/L). Even with the DOC of raw waters adjusted to 5.0 or 2.5 mg/L which is comparable to that (i.e., 2.5-3.6 mg/L DOC) in treated waters, NDMA UFC values in raw waters (i.e., 2-5 ng/L) were still lower than those in treated waters. This suggests a potential effect of NOM types (i.e., raw vs treated, or $SUVA_{254}$) on NDMA UFC. With the effects of DOC accounted, both NDMA UFC/DOC and NDMA FP/DOC were significantly ($p$ <0.05, $R^2$ = 0.58-0.85, n = 9-22) correlated with

$SUVA_{254}$ (**Figure 6.8** and **Figure B-9**). Previous studies have indicated that hydrophilic NOM fractions with lower $SUVA_{254}$ (e.g., proteins, peptides, amino sugars, polysaccharides and small hydrophilic acids) yielded higher NDMA formation than hydrophobic NOM fractions (i.e., humic-like substances) (Chen and Valentine, 2007; Kristiana et al., 2013; Wang et al., 2013; Yang et al., 2015). Similarly, in surface waters collected from nine different watersheds in southeastern US, NDMA FP/DOC were significantly ($p$ <0.05; $R^2$ = 0.83, n = 9; **Figure B-10**) correlated with $SUVA_{254}$ (Uzun, 2016). These results further demonstrated the potential effects of $SUVA_{254}$ on NDMA formation in surface waters.

Although bulk organic matters (i.e., DOC) cannot represent the amounts of NDMA precursors, these results suggest that DOC could still play an important role in NDMA formation, mainly through influencing the concentrations of $NHCl_2$ and availability of NDMA precursors to react and form NDMA.

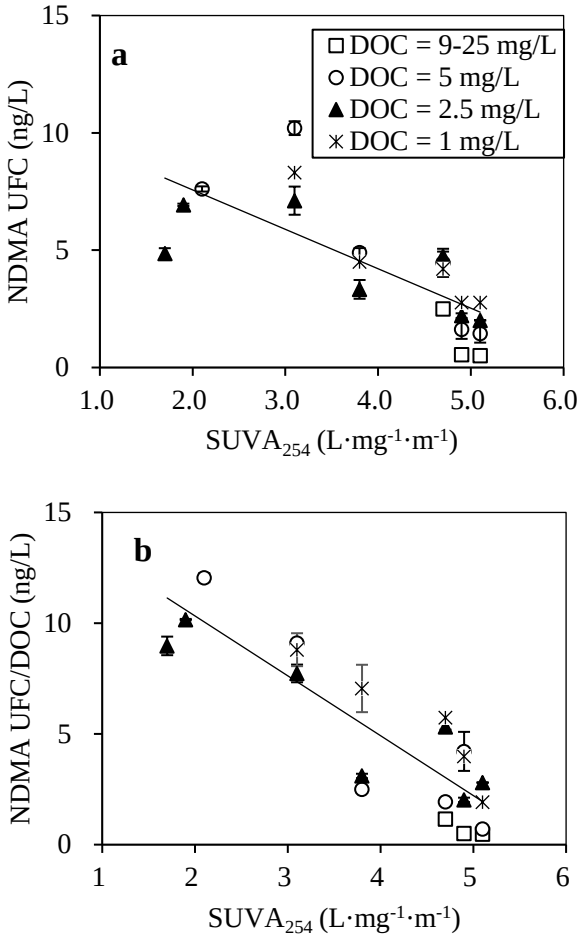



**Figure 6.8.** Correlations between DOC-normalized NDMA UFC (i.e., NDMA UFC/DOC) and SUVA254 in surface waters at (a) pH 6.8, and (b) pH 7.8 (n = 20).

NDMA UFC/DOC and NDMA FP/DOC were significantly ($p$ <0.05) correlated at pH 6.8 ($R^2$ = 0.82, n = 17) and 7.8 ($R^2$ = 0.52, n = 17) (**Figure B-11**), although NDMA UFC was poorly correlated with NDMA FP ($R^2$ = 0.04-0.06, n = 17; **Figure B-12**). Previous studies also indicated that NDMA UFC was poorly ($R^2$ = 0.27, n = 25) correlated with NDMA FP (Zeng et al., 2016). Because DOC exhibits opposite effects on NDMA UFC and NDMA FP, its effects need to be accounted for evaluating potential correlations between NDMA UFC and NDMA FP.

In water utilities, DOC and $SUVA_{254}$ can both decrease after conventional treatment. As a result, NDMA UFC in finished waters may increase. To effectively control NDMA formation, water utilities may need to strike a balance between removal of NOM and reduction of NDMA formation.

### *Effects of Bromide on NDMA UFC from Model Compounds*

In the presence of 1000 µg/L $Br^-$, NDMA UFC values from seven selected compounds (i.e., DMA, TMA, DMEA, DMiPA, DMAN, DMBzA and DMPhA) were generally comparable at pH 6.8 or higher at pH 7.8, while lower at pH 8.8 than in DDW (i.e., no $Br^-$ present) (**Figure 6.9**). The only exception was DMA, which showed higher NDMA UFC at pH 8.8 in the presence of $Br^-$ than in DDW. So far, the mechanisms of $Br^-$ effects on NDMA formation at different pH are still unclear, because of the different bromamine species formed at different pH. At pH 6.8 or 7.8, bromochloramine (NHBrCl) was the predominant bromamine species, and its concentration was over one order of magnitude higher than other bromamine species (i.e., monobromamine ($NH_2Br$) and dibromamine ($NHBr_2$); Luh and Marinas, 2014). NHBrCl was found to be more reactive with NDMA precursors than other bromamine species and even $NH_2Cl$ (Luh and Marinas, 2012; Liu and Zhong, 2017), and NDMA UFC thus increased in the presence of $Br^-$ at pH 6.8 or 7.8. At pH 8.8, however, $NH_2Br$ was the dominant bromamine species (Luh and Marinas, 2014), which was less reactive with NDMA precursors than other bromamine and chloramine species (Luh and Marinas, 2012; Liu and Zhong, 2017). Therefore, NDMA UFC was suppressed in the presence of $Br^-$ at pH 8.8.

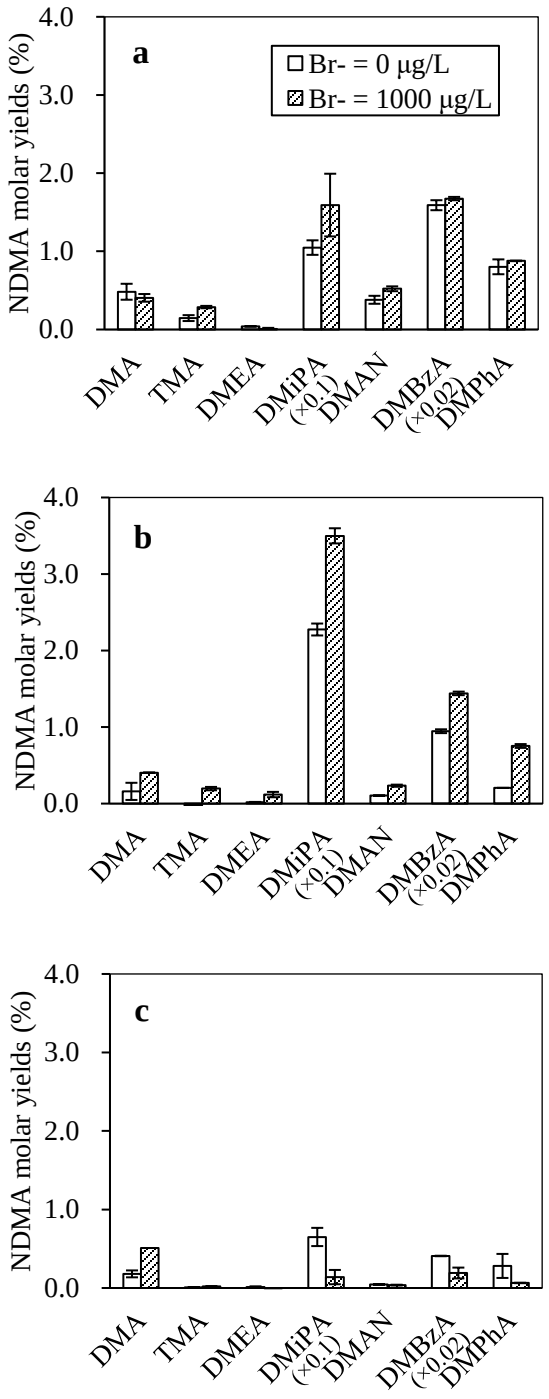



**Figure 6.9.** NDMA UFC yields from model compounds measured in the presence and absence of Br- (1000 µg/L) in DDW at (a) pH 6.8, (b) pH 7.8, and (c) pH 8.8.

Previous studies reported that DMA yielded a higher NDMA UFC in the presence of a high level (i.e., 32 mg/L) of $Br^-$ at pH 9 than in DDW (Luh and Marinas, 2012). This finding was consistent with our observations. Among the seven compounds tested, DMA was the only secondary amine, while other compounds were tertiary amines. It has been shown that NDMA formation pathway from bromamines reacting with secondary amines (e.g., DMA) may be different from that with tertiary amines (Chen et al., 2010), which may help explain the opposite findings of $Br^-$ effects on NDMA UFC from DMA than other compounds at pH 8.8.

### *Effects of $NH_2Cl$/DOC on NDMA UFC*

NDMA UFC/DOC showed linear correlations ($R^2$ = 0.86-0.99, n = 5-8) with DOC-normalized $NH_2Cl$ dosage (i.e., $NH_2Cl$/DOC) in each surface water (**Figure 6.10** and **Figure B-13**), within the range of 0-5.0 mg $Cl_2$/mg C $NH_2Cl$/DOC at pH 6.8 and 7.8, or 0-20 mg $Cl_2$/mg C at pH 8.8. Further increasing $NH_2Cl$/DOC up to 140 mg $Cl_2$/mg C slightly increased or negligibly affected NDMA UFC/DOC, probably because all NDMA precursors were oxidized to NDMA under such high $NH_2Cl$/DOC.

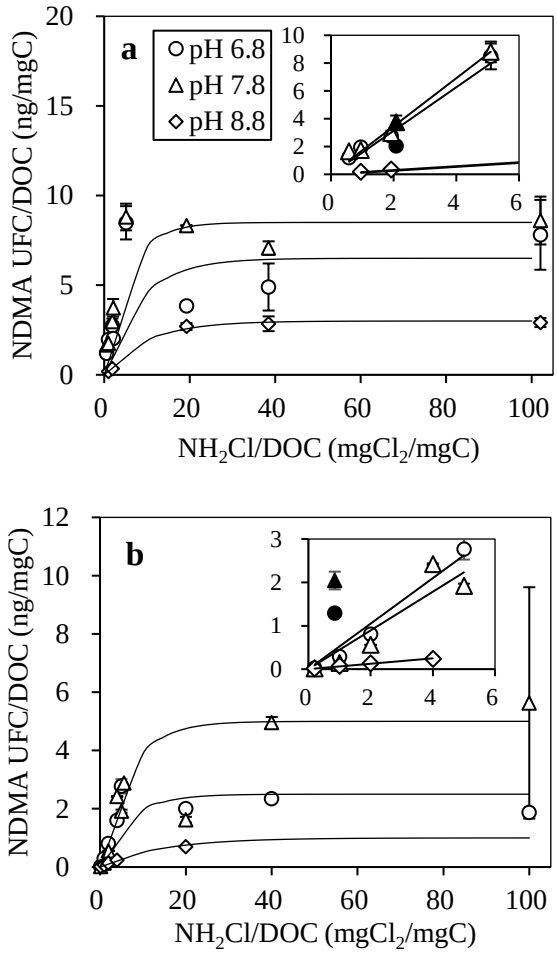



**Figure 6.10.** Effects of NH2Cl/DOC on NDMA UFC/DOC from (a) RA water, and (b) RE water (n = 5-8). Solid points represent NDMA UFC/DOC measured in (a) TA water, and (b) TE water.

Krasner et al. (2004) has proposed a DOC-normalized free chlorine dosage for the FP tests of halogenated DBPs (**Equation 6.2**). Here, we further identified a DOC-normalized $NH_2Cl$ dosage for NDMA FP tests. The minimum $NH_2Cl$ dosage required to oxidize all NDMA precursors was found to be 5-fold of DOC at pH 6.8 and 7.8, and 20-fold of DOC at pH 8.8. Our results further indicate that the minimum $NH_2Cl$ dosage may depend on water types. In surface waters (e.g., TA-TE, RA, RB) with lower $SUVA_{254}$, the

minimum $NH_2Cl$ dosage could be lower, because NDMA UFC/DOC increased more readily with increasing $NH_2Cl$/DOC in these waters. This was understandable because NOM in waters with lower $SUVA_{254}$ were more hydrophilic, exhibiting less complexations with NDMA precursors. Therefore, more NDMA precursors were available to react with $NHCl_2$ in these waters, and NDMA formation was favored under the same $NH_2Cl$ dosage.

$$\text{NaClO dosage (mg } Cl_2\text{/L)} = 3 \times \text{DOC} + 8 \times NH_3\text{-N} + 10 \qquad \textbf{Equation 6.2}$$

In water utilities, $NH_2Cl$ dosage is typically below 5-fold of DOC, and NDMA UFC may linearly correlate with $NH_2Cl$/DOC in drinking waters. To reduce NDMA formation, water utilities may need to minimize $NH_2Cl$ dosage without compromising disinfection effectiveness. Further, water utilities may predict NDMA formation levels based on the DOC and $NH_2Cl$ dosage applied in drinking waters. For lab researchers, the DOC of water samples may need to be considered during NDMA FP tests, to reach the minimum $NH_2Cl$ dosage required for converting NDMA precursors.

## Conclusions

Though many factors have been known to affect NDMA formation, most current findings of their effects on NDMA formation have focused on single-factor effects in pure water (i.e., DDW) matrix. In natural surface waters, however, multiple factors can simultaneously affect NDMA formation. In the current study, the co-effects of selected factors (i.e., pH, DOC, $SUVA_{254}$, $Br^-$ and $NH_2Cl$ dosage) on NDMA formation were comprehensively investigated by monitoring NDMA UFC and NDMA FP from model compounds and surface waters under various conditions of multiple factors. Results

showed that model compounds consistently yielded peak NDMA UFC at pH 6.8 or 7.8 in DDW, despite their distinct pKa (i.e., 3.8-13.6). NDMA UFC from model compounds increased with pH increasing from 6.8 to 7.8 in Myrtle Beach treated water, while decreased with pH increasing from 6.8 to 7.8 in Myrtle Beach raw waters. In surface waters with lower DOC or $SUVA_{254}$, NDMA UFC tended to increase more with pH increasing from 6.8 to 7.8. However, NDMA FP consistently increased with pH increasing from 6.8 to 7.8 regardless of DOC or $SUVA_{254}$ in surface waters.

In treated surface waters, NDMA UFC values from model compounds were comparable or higher than those in raw waters. In surface waters with lower DOC and $SUVA_{254}$, NDMA UFC tended to be higher. However, NDMA FPs in waters with lower DOC were relatively lower. Because bulk organic matters (i.e., DOC) could affect NDMA UFC and NDMA FP differently, the effects of DOC need to be accounted for when evaluating potential correlations between NDMA UFC and NDMA FP. NDMA UFC/DOC was significantly correlated ($p$ <0.05; $R^2$ = 0.52-0.82, n = 17) with NDMA FP/DOC in tested surface waters, although NDMA UFC and FP were poorly correlated ($R^2$ = 0.04-0.06, n = 17). NDMA UFC/DOC and NDMA FP/DOC were both negatively correlated ($p$ <0.05; $R^2$ = 0.58-0.85, n = 9-22) with $SUVA_{254}$ in surface waters.

The effects of $Br^-$ with concentrations (i.e., 1000 µg/L) typically found in desalinated or blending waters on NDMA formation strongly depended on pH. The presence of 1000 µg/L $Br^-$ negligibly affected NDMA UFC at pH 6.8 and enhanced NDMA UFC at pH 7.8, while decreased NDMA UFC at pH 8.8. For utilities treating waters

containing high $Br^-$ concentrations (e.g., desalinated or blending waters), adjusting the pH of finished waters to a higher level (i.e., pH 8.5) could benefit control of NDMA formation.

Under practical chloramination conditions (i.e., $NH_2Cl$/DOC <5 mg $Cl_2$/mg C), NDMA UFC/DOC was linearly correlated ($R^2$ = 0.86-0.99, n = 5-8) with $NH_2Cl$/DOC. To reduce NDMA formation, therefore, water utilities may need to minimize $NH_2Cl$ dosage in finished waters. For lab researchers, the DOC of water samples may need to be considered during NDMA FP tests, to reach the minimum $NH_2Cl$ dosage required for converting NDMA precursors.

# CHAPTER VII

# THE FATES OF MODEL NDMA PRECURSORS DURING BIOLOGICAL WASTEWATER TREATMENT

## Introduction and Objective

Municipal wastewater discharges are considered one of the major sources of NDMA precursors that could impact downstream drinking water qualities (Krasner et al., 2013). NDMA precursors can be deactivated to various degrees during secondary biological treatment (e.g., the AS process) at WWTPs. So far, however, few studies have investigated biological removal of specific NDMA precursors during AS treatment. The factors that potentially impact NDMA precursor removal efficiencies are still poorly understood. Many known NDMA precursors such as PPCPs are xenobiotics with unfamiliar chemical structures to microorganisms and their biodegradability could be recalcitrant or persistent (Radjenovic et al., 2007; Sgroi et al., 2018). The characteristics of the microbial community in AS (i.e., bacterial speciation and population) and treatment conditions in the AS process (i.e., HRT, SRT) may affect biodegradation rates for such persistent NDMA precursors, as reported for other trace-level emerging organic contaminants (Grady, 1993; Ahmed et al., 2007; Zhang et al., 2009; Gros et al., 2010; Jin et al., 2010; Li and Zhang, 2010; Sarma and Joshi, 2015; Liu et al., 2017).

NDMA precursors are considered to be deactivated via biotic (i.e., biodegradation) and abiotic (i.e., biosorption, volatilization) processes during the AS process at typical WWTPs (Byrns, 2001). At neutral pH, tertiary amine NDMA precursors are positively

charged, and their biosorption onto negatively charged AS solid particles could be significant (Shen and Andrews, 2013a). RNTD and tetracycline tended to be adsorbed on AS particles rather than being biodegraded (Li and Zhang, 2010; Jelic et al., 2011). Precursors such as DMA and TMA, however, are more readily biodegradable with their concentrations frequently measured at WWTP influents and secondary effluents (Mitch and Sedlak, 2004; Sedlak et al., 2005; Wang et al., 2014). Due to the significant spatial and temporal variations in wastewater influents and WWTP operational parameters leading to varying AS characteristics (Zhao et al., 2006; Grady, 2011), roles of biotic and abiotic processes deactivating NDMA precursors may be accordingly impacted.

Conventional wastewater treatment processes are designed to remove contaminants such as chemical oxygen demand (COD) and nutrients (i.e., nitrogen, phosphorus), while exhibiting less effectiveness in removing xenobiotic compounds such as NDMA precursors (Grady et al., 2011). Potential strategies to enhance the bio-deactivation of NDMA precursors during the AS process include biostimulation (i.e., adding readily biodegradable substrates) and bioaugmentation (i.e., enhance the populations of potential degraders), which have been applied in WWTPs to facilitate the biodegradation of bio-refractory organic contaminants (Jittawattanarat et al., 2007a; Chong and Huang, 2012; Lee et al., 2015; Leu and Stenstrom, 2010). So far, however, the mechanisms of bio-deactivation of NDMA precursors are still little understood, which impedes the selection and application of potential biostimulation or bioaugmentation strategies. A few studies indicated that some NDMA precursors (such as RNTD), if at high concentrations, could inhibit microbial activities (Carucci et al., 2006), and their deactivation during the AS process is likely via

co-metabolic biodegradation with non-specific oxygenase. It is thus hypothesized that biodegradation of NDMA precursors is closely associated with the activities of non-specific oxygenase. So far, however, the efficiencies of such non-specific oxygenase in deactivating NDMA precursors are still little investigated.

The major objectives of this study are to (i) investigate the deactivation efficiencies of selected model NDMA precursors during biological wastewater treatment (i.e., the AS process), (ii) examine the main deactivation pathways (i.e., biodegradation, biosorption, and volatilization processes) of model NDMA precursors, (iii) evaluate the effects of AS types (i.e., domestic rural, domestic urban, textile and lab-grown AS) and selected treatment conditions (i.e., HRT or incubation time of the AS treatment test, SRT, seasonal variations in AS activities) on the deactivation efficiencies of NDMA precursors, and (iv) investigate the roles of biostimulation and non-specific oxygenases affecting the removal of NDMA precursors (i.e., RNTD).

## Materials and Methods

### *Model NDMA Precursors*

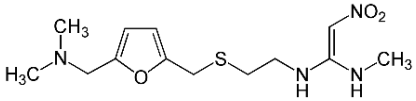
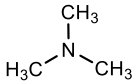
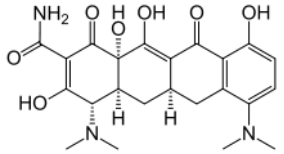
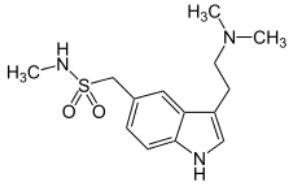
Three amine-based pharmaceuticals (i.e., RNTD, MNCL and SMTR) and TMA were selected as model NDMA precursors, with ~2%-90% of their NDMA FP yields on a molar basis (Le Roux et al., 2011; Selbes et al., 2013; Shen and Andrews, 2013b). RNTD, MNCL, and SMTR are the main active ingredients of the most widely sold and prescribed amine-based pharmaceuticals in the US in recent years (Shen and Andrews, 2013b; Zeng and Mitch, 2016). TMA is a tertiary amine commonly present (i.e., 0-2.7 mmol/mol

creatinine) in human urine and feces (Svensson et al., 1994; Mitch and Sedlak, 2004; Lee et al., 2010). RNTD, MNCL, and SMTR were purchased from TCI (Duncan, US) in solid forms, which were then dissolved in methanol to 0.4 mM and further diluted in DDW to 20 μM (RNTD) or 200 μM (MNCL and SMTR). An aqueous solution of TMA (4% mass concentration) was purchased from Sigma-Aldrich (St. Louis, US) which was further diluted in DDW to 200 μM. The chemical structures and key physiochemical properties of selected model precursor compounds are shown in **Table 7.1**.

### *Experimental Procedure*

A modified version of EPA standard test method (OPPTS 835.3280 -314B) (US EPA, 2008) was employed to assess i) overall removal of NDMA FP (via biotic plus abiotic processes), ii) removal of NDMA FP via abiotic biosorption process only, and iii) removal of NDMA FP via abiotic volatilization process only, during AS treatment tests. A modified version of a respirometry test method was also employed to assess the removal of NDMA FP via biodegradation processes (OECD, 2003). NDMA FPs were monitored before and after batch tests to evaluate the removal (or deactivation) of NDMA precursors.

**Table 7.1.** Physicochemical properties of selected model precursors.

| Model precursors | Ranitidine (RNTD) | Trimethylamine (TMA) | Minocycline (MNCL) | Sumatriptan (SMTR) |
|---|---|---|---|---|
| Chemical structure |  |  |  |  |
| Molecular formula | $C_{13}H_{22}N_4O_3S$ | $C_3H_9N$ | $C_{23}H_{27}N_3O_7$ | $C_{14}H_{21}N_3O_2S$ |
| Molecular weight (g/mol) | 314.4 | 59.1 | 457.5 | 295.4 |
| Molar volume ($cm^3$)[a] | 265.4 | 85.3 | 289.6 | 237.6 |
| $LogK_{ow}$ | 0.27[1] | 0.16[2] | 0.05[1] | 0.93[3] |
| pKa | 8.2[4] | 9.8[5] | 2.8, 5.0, 7.8, 9.5[6] | 9.5, 11.2[b] |
| Solubility (mg/L) | 24.7[c] | 410000[7] | 52000[c] | 21400[c] |
| H (mm Hg, 25ºC)[a] | 0.0 (±1.0) | 1717 (±0.0) | 0.0 (±2.6) | 0.0 (±1.3) |
| Reported NDMA molar yields in FP test (%) | 80~90[8-10] | 1.9[8] | ~8[11] | 4~6[9,10] |

[a]: Data was predicted by Advanced Chemistry Development, Inc. (ACD/Labs). [b]: Data were predicted by ChemAxon software company. [c]: Data were cited from DrugBank database. [1]: Sangster, 1997. [2]: Hansch, et al., 1995. [3]: Adlard, et al., 1995. [4]: Kortejarvi, et al., 2005. [5]: Dewick, 2013. [6]: Food and Drug Administration (FDA), 2000. [7]: Mackay, et al., 2006.

**Table 7.2.** Key information of selected wastewater treatment facilities.

| AS sample | Facilities | Influent type | Treatment capacity (mgd)[a] | HRT[b] (h) | SRT[c] (d) | Treatment Process | Nutrients Removal | Industrial impact |
|---|---|---|---|---|---|---|---|---|
| AS 1 | WWTP 1 | Predominantly domestic wastewater | 2.0 | 22-24 | 20 | Extended aeration | Nitrification | 1% |
| AS 2 | WWTP 2 | Domestic wastewater and industrial discharge | 70 | 13.5 | 12 | Anaerobic-anoxic-oxic | Biological nutrient removal | 25% |
| AS 3 | WWTP 3 | Textile wastewater | 1.7 | 5 | 26 | Extended aeration | None (N is added) | 100% |
| AS 4 | Lab-scale SBR[d] | Synthetic wastewater[e] | 12 (L/d) | 6 | 8[f] | Anoxic-oxic | Partial nitrification | None |

[a]: Million gallons per day. [b]: Hydraulic retention time. [c]: Solids retention time. [d]: Sequencing batch reactor. [e]: Consisting of glucose and yeast extract as growth substrates.
[f]: Nominal SRT without considering the loss of suspended solids from discharging effluents

Four types of AS were collected to treat NDMA precursors during batch tests. AS liquor samples were grabbed from aeration basins of a rural municipal WWTP (AS 1), an urban municipal WWTP (AS 2), a textile WWTP (AS 3), and a laboratory SBR (AS 4) fed with synthetic wastewater. The AS 4 was acclimated to synthetic wastewater for at least three SRTs (i.e., 24 d for 8-d SRT) in the lab-scale SBR before being collected. Key information for the WWTPs and lab-scale SBR is summarized in **Table 7.2**. The recipe of synthetic wastewater (consisting of glucose and yeast extract as growth substrates) is provided in **Table C-1**. Detailed operating conditions for the SBR are provided in **Appendix C (Table C-2)**.

The collected AS liquor samples were transported to the lab within 1 h upon collection with adequate ventilation to maintain an aerobic condition, and then aerated for 4-12 h at 23±2 ºC for preconditioning. Prior to testing, AS liquor was centrifuged (2000×g, 5 min) with the solid portion harvested and resuspended in the mineral solution (150 mL/g of mixed liquor suspended solids (MLSS)). The recipe of the mineral solution is shown in **Table C-3** (OECD, 2003). The resuspended AS was then centrifuged with the solid portion harvested and then resuspended in the mineral solution. The same procedures were repeated three times to remove background NDMA precursors from the AS liquor. The washed AS biomass was then resuspended in the mineral solution with MLSS adjusted to ~3000 mg/L. AS liquor (500 mL, washed and resuspended) was transferred to a 1-L incubation bottle, dosed with a predetermined amount of precursor compound solution (i.e., with a target NDMA FP of ~1000 ng/L from each compound) under vigorous mixing condition, and then aerated for 6 or 24 h using compressed air at 25±2 ºC. The air flow rate

(i.e., 150 L/$m^3$·min) was set as close as possible to aeration rates used in practice (20-90 L/$m^3$·min) in aeration basins at WWTPs (Grady et al., 2011). At the end of test (i.e., after 6 or 24 h), a 125-mL sample was harvested and filtered through a 0.45-μm membrane. Then the filtrate was used for the NDMA FP tests.

To monitor removal of NDMA FP via biosorption, sodium azide ($NaN_3$, 5 g/g MLSS) was added to the resuspended AS liquor under gentle mixing conditions. After 24-h contact time, the residual $NaN_3$ was removed by washing the AS biomass four times with the mineral solution (150 mL/g MLSS). The washed AS biomass was then resuspended in the mineral solution and the MLSS was adjusted to ~3000 mg/L. To confirm that $NaN_3$ deactivation was effective, oxygen ($O_2$) uptake was monitored throughout the 6-h (occasionally 10-h) biosorption tests using a respirometer (Oxymax ER-10, Columbus Instruments, US). The measured $O_2$ uptake curves are shown in **Figure C-1**. A negligible $O_2$ uptake was presumed to be an adequate metric for a lack of microbial activity.

Removal of NDMA FP via biodegradation was examined by employing a low concentration of MLSS (~200 mg/L) with prior exposure for pre-biosorption (i.e., 30-min pre-contact of AS biomass with NDMA precursors) to minimize the interference from the biosorption process. The incubation duration of the biodegradation tests was extended up to 10 d to obtain measurable changes of NDMA FPs. Removal of NDMA FP via volatilization process only was evaluated by dosing NDMA precursors into the mineral solution and aerating for 6 h in the absence of any AS biomass. NDMA FPs were measured before and after 6-h incubation.

### *Roles of Biostimulation and Non-specific Oxygenase*

To examine the role of biostimulation, selected biostimulants were added to AS 1 liquor (MLSS = 200 mg/L) before batch treatment tests with RNTD (100 nM), including benzoate, ammonia, glucose and ammonia, acetate and ammonia, and ethanol and ammonia. The concentrations added were 60-100 mg/L of glucose, acetate, benzoate, 600 mg/L of ethanol, and 25 mg N/L of ammonia. The incubation time was up to 10 d.

*P. putida* was selected as a model microbe to produce non-specific oxygenase, because *P. putida* has versatile metabolic pathways in biodegrading aromatic compounds. Strain *P. putida* (Trevisan) Migula was purchased from American Type Culture Collection (ATCC #49128) and inoculated in nutrient broth (NB; pH 6.8±0.2) to grow at 23±2°C under aerobic conditions. After 48-h incubation, the cells reached the late exponential phase of growth, at an optical density of ~1.0, measured at 600 nm ($OD_{600}$). The *P. putida* cells were then inoculated into a phenol medium by transferring ~1 mL NB liquor with actively growing cells to 500 mL phenol medium. All these operations were conducted in a biosafety cabinet (Labconco Corporation, Kansas City, US). The NB medium was freshly prepared by dissolving peptone (5 g/L) and beef extract (3 g/L) (Becton Dickinson & Company, Franklin Lakes, US) in a mineral solution consisting of phosphate buffer (pH 7.4±0.1), ethylenediaminetetraacetic acid (EDTA), and mineral salts (**Table C-4**). The phenol medium was prepared by dissolving ~100 mg/L phenol in a mineral solution (**Table C-3**). The prepared NB and phenol medium were filter sterilized by passing through 0.2-μm nylon membrane filter (Whatman, Maidstone, UK) before used to maintain aseptic conditions.

The *P. putida* cells were incubated in phenol medium for 24 h and the residual phenol concentration was monitored (e.g., via measuring the ultraviolet absorbance at 270 nm ($UV_{270}$) on spectrophotometer). Then, a determined volume of phenol PDS (i.e., 80 g/L) was added to supplement consumed phenol and maintain phenol concentration at ~100 mg/L, until the total phenol consumption exceeded 1000 mg/L. The cells were then harvested, washed, and resuspended in the mineral solution (**Table C-3**). The purity of *P. putida* strain grown in NB and phenol medium was monitored and confirmed periodically using a microscope (Zeiss Group, Oberkochen, DE).

To verify the activity of a non-specific enzyme (i.e., phenol 2-monooxygenase), the removal of trichloroethylene (TCE), a reference compound, was monitored during incubation with *P. putida*. The removal of TCE served as an indicator of the activity of ring-cleaving oxygenase (i.e., phenol 2-monooxygenase from *P. putida*), because enzymes that can cleave aromatic rings are associated with TCE degradation (Nelson et al., 1987). To measure the removal of TCE, 70 mL washed and resuspended *P. putida* cells were transferred to a 160-mL serum bottle, closed with a Teflon-faced septum and crimp cap, and injected with TCE-saturated water to achieve TCE concentrations of 1, 2 or 5 mg/L in the aqueous phase. The mixed liquor was then incubated on a shaker (200 rpm) at 23±2°C for 1, 2, 3, 5, 7 and 10 d (until TCE degradation ceased). The oxygen levels in the headspace of serum bottle were monitored on a Hewlett Packard 5890 Series II GC equipped with a TCD in conjunction with a MS-5A 60/80 Mesh Molecular Sieve column (Alltech, Nicholasville, US). The response from the GC was calibrated using the room air (i.e., 21% volume fraction). During incubation, pure oxygen was supplemented periodically to

maintain aerobic conditions in the serum bottle. The TCE concentration in 0.5-mL headspace sample was analyzed using a Hewlett-Packard 5890 Series II GC equipped with an FID and a column packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco Inc., Bellefonte, US). The transformation capacity of TCE was then calculated based on the total TCE consumed divided by and the initial cell concentration. In parallel, the removal of NDMA FP from RNTD was tested by dosing 1000 nM RNTD to the washed and resuspended *P. putida* cells under aerobic conditions. The incubation time of *P. putida* cells treating RNTD was set at 2 h, 5, 10, 15 and 20 d.

In addition to aerobic conditions, the biodegradation tests for RNTD with *P. putida* were also conducted under unfavorable conditions as negative controls, including (i) under aerobic conditions in the presence of a high concentration of a selected oxygenase inhibitor (i.e., 10%-20% volume ratio of acetylene in the serum headspace), and (ii) under anaerobic conditions, i.e., in the absence of oxygen prepared in a glove box atmosphere containing to a mixture of ~96% nitrogen and ~4% hydrogen gas. During biodegradation tests in the presence of acetylene, the serum bottles were sealed with Teflon-faced septa and crimp caps; acetylene was injected via a syringe to reach a 10%-20% volume ratio of acetylene in headspace. During biodegradation tests under anaerobic conditions, the serum bottle containing washed and resuspended *P. putida* cells were transferred to the glove box, sealed with Teflon-faced septa and crimp caps after at least 2 h, and then placed on a shaker (200 rpm) for incubation. At the end of the incubation period, 15-mL sample was harvested with a syringe, filtered through 0.45-μm membrane filter (Whatman, GE Healthcare Life Sciences, US), and the filtrate was used for the NDMA FP test.

### *NDMA Formation Tests*

For the FP test, samples were buffered with 20-mM phosphate (pH 7.8±0.2) and chloraminated by adding pre-determined volume of monochloramine stock solution (~2000 mg $Cl_2$/L) to achieve the target monochloramine dosage (i.e., 100 mg $Cl_2$/L). The monochloramine stock solution was freshly prepared by adding a sodium hypochlorite solution (NaClO, ~4000 mg $Cl_2$/L) to an ammonium chloride solution ($NH_4Cl$, ~1000 mg N/L) drop by drop at pH 9 with a Cl:N mass ratio of 4:1. The chloraminated samples were then incubated in dark at 23±2ºC for 5 d, with the residual chloramines after 5-d incubation quenched by adding sodium thiosulfate ($Na_2S_2O_3$) powder.

### *Analytical Methods*

The quenched samples were then extracted and analyzed using GC (Varian 3800, Agilent, US) coupled with MS/MS (Waters 4000, Agilent, US) for NDMA FPs according to US EPA Method 521 (US EPA, 2004). More details of NDMA analysis method were described elsewhere (Selbes et al., 2013; Beita-Sandi et al., 2019). The MRL of NDMA is 3 ng/L.

## Results and Discussion

### *Deactivation Efficiencies of Model NDMA Precursors*

NDMA FPs of RNTD were reduced by 34%-87% after 24-h incubation with four types of AS (**Figure 7.1**). NDMA FPs of TMA and MNCL were reduced by 92%-98% and 77%-97%, respectively, after 24-h incubation. The lowest level of removal occurred with SMTR, with its NDMA FPs reduced by only 29a%-46% depending on the types of AS. These overall deactivation efficiencies were achieved via both biotic (i.e., biodegradation) and abiotic (i.e., biosorption, volatilization) processes. TMA is known to be readily biodegradable, with >71% removals achieved during various biological treatment processes reported in previous studies (Mitch and Sedlak, 2004; Wang et al., 2014). MNCL contains hydroxyl functional groups attached to ring structures, which can enhance its biodegradability (Okey and Stensel, 1996). Unlike TMA or MNCL, RNTD contains a nitryl ($-NO_2$) functional group, and SMTR contains a sulfonamide ($-NH-SO_2-$) structure, both of which were considered to impede biodegradation (Okey and Stensel, 1996).

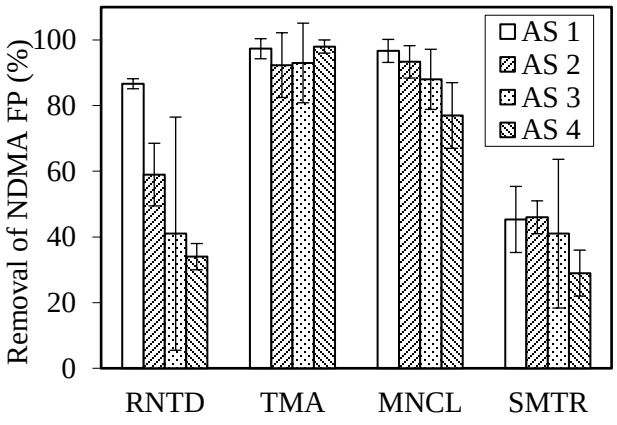


**Figure 7.1.** Removal of NDMA FPs from model precursor compounds during 24-h treatment with four types of AS. Bar graph represents an average removal of NDMA FP during three different batches of treatment with AS collected in winter, spring, and summer, respectively; error bar represents standard deviation. AS 4 was collected only in spring and the error bar represents standard deviation of duplicate tests. Initial NDMA FP ~1000 ng/L from each compound.

In addition to NDMA precursors' chemical structures, the sources of AS biomass can also affect the overall removal of NDMA FPs. AS 1 which was collected from a rural municipal WWTP with an extended aeration process (i.e., 22-24 h HRT) and an excellent nitrifying performance (i.e., undetectable ammonia nitrogen at secondary effluent) showed exceptionally high deactivation of RNTD with 87% of NDMA FP removal after 24-h incubation. AS 2 which was collected from an urban municipal WWTP with biological nutrient removal process (14 h HRT) reduced 59% NDMA FP of RNTD. Both AS 1 and AS 2 exhibited relatively efficient deactivations of the three amine-based pharmaceuticals (i.e., RNTD, MNCL and SMTR), while the lab-grown AS (i.e., AS 4) showed the least deactivation efficiencies. Previous studies found that municipal AS has more diverse microbial communities due to the complexity of organic contaminants in municipal wastewater influents, while AS fed with simple nutrients (such as AS 4 fed with synthetic wastewater and AS 3 fed with textile wastewater) has less diverse microbial communities (Zhao et al., 2006). The diversity and richness of microbial species could favor the removal of xenobiotic compounds (Jelic et al., 2011).

### *Factors that Influence the Deactivation of NDMA Precursors*

Selected factors including seasonal variations in AS activity, incubation time or HRT, and SRT were examined for their impacts on the deactivation efficiencies of model NDMA precursors. Among the four types of AS tested, AS 3 collected from a textile WWTP exhibited seasonal variations in the deactivation efficiencies of some NDMA precursor compounds (**Figure 7.1**). NDMA FP removal of RNTD and SMTR varied by 36% and 23%, respectively, after 24-h incubation with AS 3 collected in three seasons, while removal of NDMA FP from TMA and MNCL varied by 9%-12%. In contrast, only <10% variations in NDMA FP removals were observed after 24-h treatment with municipal WWTP AS (i.e., AS 1 or AS 2) collected during three seasons. In the textile WWTP, seasonal adjustments would be applied to textile manufacturing processes and wastewater treatment conditions, leading to substantial fluctuations in textile influent components and AS 3 characteristics (e.g., color, MLSS, settleability), which may thus cause the instabilities of treatment efficiencies of NDMA precursors. In contrast, there were no adjustments of operation conditions at the two municipal WWTPs (i.e., WWTP 1 and WWTP 2) during our sampling campaigns. Occasionally, WWTP 1 adopted rain mode to cope with heavy influent flows during rain events (i.e., with precipitation >0.5 inch per day), for which the oxygen supply to the aeration tanks was shut off and the MLSS of AS 1 dropped to ~1000 mg/L in aeration basin. AS 1 collected during rain mode (~1000 mg/L MLSS) showed low deactivation efficiencies for all tested model precursor compounds, with removals of NDMA FP 12%-34% less than those measured under routine operational

mode with ~3000 mg/L MLSS (**Figure C-2**), suggesting a potential impact of MLSS on the deactivations of NDMA precursors.

The effects of operational parameters (i.e., incubation time or HRT, SRT) on the deactivations of model precursor compounds were also examined. With incubation time increasing from 6 to 24 h, NDMA FP removal from RNTD increased from 81% to 88% during the treatment with AS 1, from 34% to 54% with AS 2, and from 32% to 62% with AS 3 (**Figure 7.2**). These observations are consistent with previous studies showing a significant ($p$ <0.05) positive correlation between removals of RNTD and the HRTs of AS process at different WWTPs (**Figure C-3**). On the contrary, NDMA FP removal from the other three compounds were less affected by incubation time. TMA and MNCL were readily removable and nearly 100% of NDMA FP were eliminated within 6 h incubation time. Further increasing incubation time up to 24 h negligibly increased their NDMA FP removal. SMTR is relatively bio-refractory and thus increasing incubation time would not enhance its NDMA FP removal. Differently, RNTD has a moderate biodegradability (i.e., with average removal efficiencies between 40% and 70% after 24-h treatment with the AS process at WWTPs) and the removal of its NDMA FP were thus more affected by HRT (Byrns, 2001).

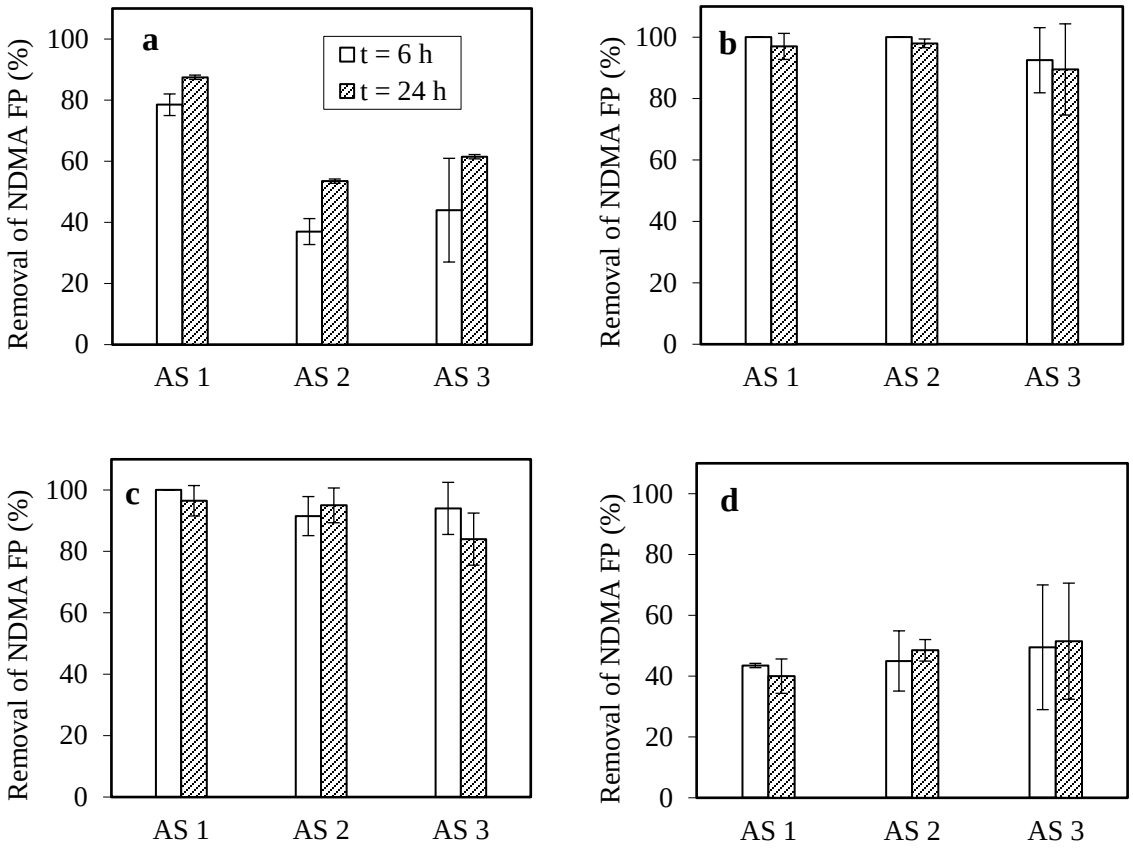



**Figure 7.2.** Effects of incubation time on the removal of NDMA FP from (a) RNTD, (b) TMA, (c) MNCL, and (d) SMTR. Bar graph represents an average removal from two batches of AS treatment tests with AS collected in spring and summer, respectively.

The effect of SRT on AS deactivations of RNTD was examined using lab-grown AS 4 acclimated to a synthetic wastewater. As SRT increased from 4 d to 8 d or 12 d, NDMA FP removal increased from 26% (±2%) to 30% (±5%) or 38% (±6%). Usually, a longer SRT favored the diversity of microbial communities of AS by helping retain microorganisms with relatively long generation times (e.g., nitrifiers), which may thus enhance the deactivation of compounds with a moderate biodegradability such as RNTD (Jelic et al., 2011). On the other hand, a longer SRT may also favor the biosorption of some

polar compounds (such as RNTD) onto AS particles (Petrie et al., 2014). The enhanced removal of NDMA FP under a longer SRT may have resulted from biodegradation and/or biosorption processes.

### *Deactivation of Model NDMA Precursors via Biosorption*

The deactivation pathways (i.e., biodegradation, biosorption and volatilization) of precursor compounds were evaluated by subtracting NDMA FP removal achieved via each deactivation mechanism from the overall removal of their NDMA FPs. For instance, the removal achieved via biodegradation were defined as the differences between the overall NDMA FP removal and the removal via biosorption plus the removal via volatilization. NDMA FPs of RNTD were removed mainly via biosorption (i.e., >75%), while biodegradation contributed less than<25% to overall removal during 6-h treatment with the four types of AS (**Figure 7.3**). Similarly, biodegradation accounted for only <1% and 2%-11% of NDMA FP removal for MNCL and SMTR, respectively, when treated with the three WWTP AS (i.e., AS 1, AS 2 and AS 3). In contrast, TMA was mainly deactivated via biodegradation, with 74%-98% of NDMA FPs removed via biodegradation during treatment with AS 2, AS 3 and AS 4, although nearly 100% of NDMA FP was removed via biosorption during treatment with AS 1, probably because of the strong biosorption capability of AS 1. The lower deactivation tendency of TMA via biosorption compared to the other model precursors is probably because TMA tends to remain in the aqueous phase rather than on the AS solids, as indicated by its relatively higher water solubility (i.e., 410 g/L, compared to 0.025-0.0052 g/L of the other three compounds). For all tested precursor

compounds, NDMA FPs were negligibly altered via volatilization during 6-h incubation in the absence of any AS biomass (**Figure 7.4**). These results suggest that biosorption is an important deactivation pathway of NDMA precursors, especially for high-molecular-weight compounds, while volatilization contributes the least to removal of NDMA precursors.

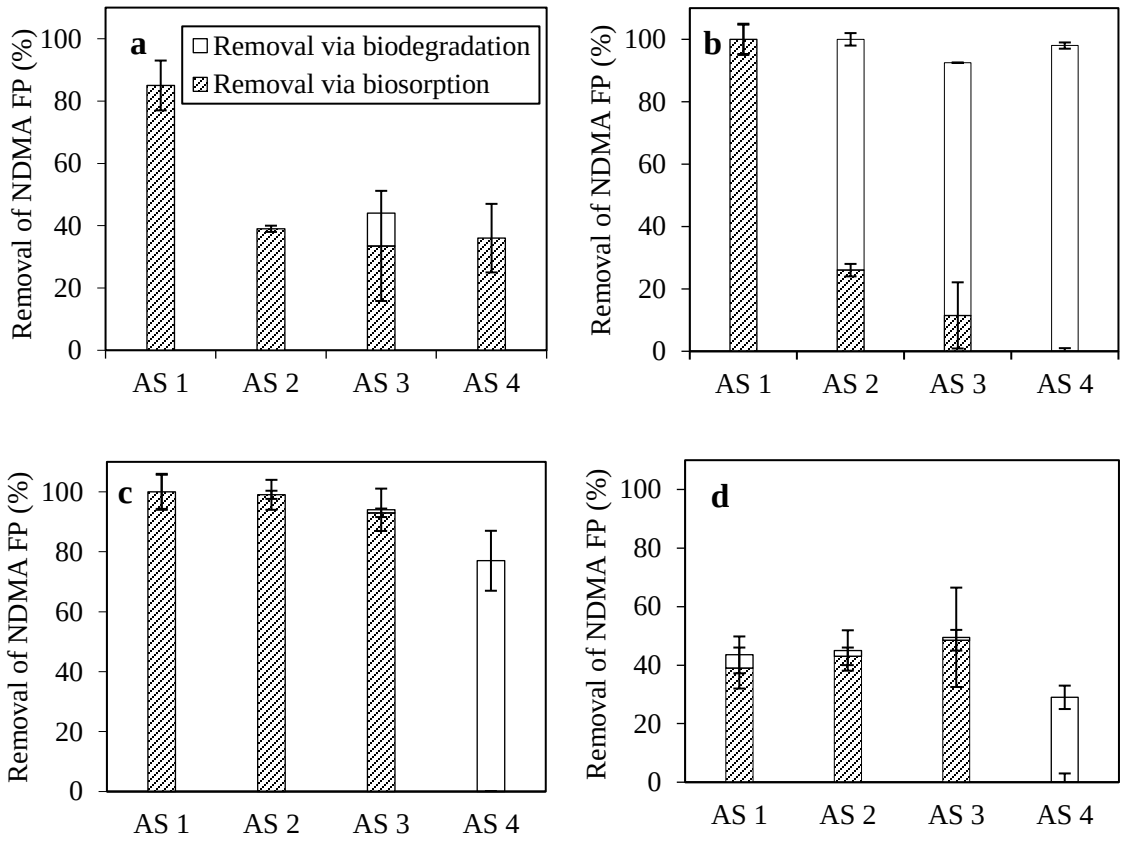



**Figure 7.3.** Contributions of biosorption and biodegradation in deactivating model precursor compounds during AS treatment. (a) RNTD, (b) TMA, (c) MNCL, (d) SMTR. Incubation time = 6 h.

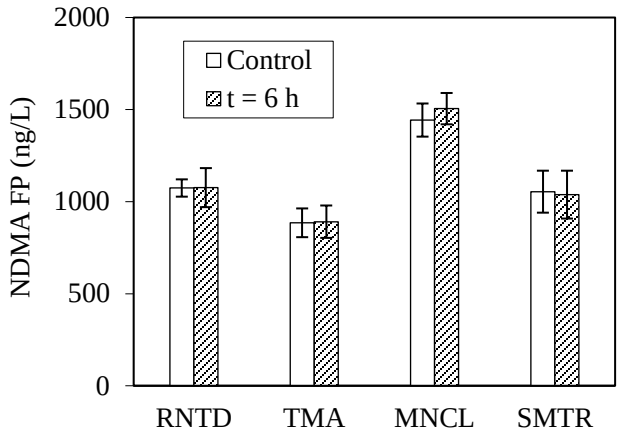



**Figure 7.4.** NDMA FP from model precursor compounds during 6-h volatilization test (i.e., in the absence of AS).

Deactivation of model NDMA precursors via biosorption depended on the sources of AS biomass. AS 1 exhibited a relatively strong biosorption tendency to all four model compounds, while AS 4 exhibited the least biosorption deactivation of TMA, MNCL and SMTR, with their NDMA FPs removed predominantly via biodegradation. Previous studies indicated that the settling properties of AS (such as sludge volume index) were directly related to the surface charges of AS particles, and thus impact AS adsorption of cations (Steiner et al., 1976). In this study, AS 1 exhibited the highest settleability among the four tested AS, while AS 4 had the lowest settleability due to its relatively shorter SRT (i.e., 8 d) and larger aeration rates (i.e., 150 $L/m^3 \cdot min$, compared to 20-90 $L/m^3 \cdot min$ typically applied at aeration basins of WWTPs) (Ahn et al., 2013; Zhang et al., 2015; Li and Stenstrom, 2018). Increasing SRT can enhance AS settleability and thus promote biosorption of cations onto AS biomass (Ahn et al., 2013). In our study, AS 4 with a longer SRT (i.e., 12 d) was found to exhibit NDMA FP higher removal (i.e., 38±6%) of RNTD

than that (i.e., 26±2%) with a shorter SRT (i.e., 4 d), presumably because of its stronger biosorption of RNTD at a longer SRT. This assumption was verified in the following biodegradation tests indicating that NDMA FP of RNTD was not readily removed via biodegradation during the treatment with AS 4 (i.e., <13% after 24-h incubation).

The solid-water distribution coefficients ($K_d$) of precursor compounds biosorbed onto different AS were calculated based on NDMA FPs measured in both aqueous and solid phases (**Table 7.3**). The log $K_d$ values of all tested model precursors biosorbed onto different AS biomasses were at least twofold higher than log $K_{ow}$. This may suggest that adsorption of precursor compounds onto AS biomass was governed more by factors other than hydrophobicity, such as electrostatic interaction (Golet et al., 2013). At near neutral pH, dimethylamine functional groups (i.e., $-N(CH_3)_2$) of precursor compounds are positively charged and tend to bind to negatively charged carboxyl and/or hydroxyl groups on AS surfaces (Shen and Andrews, 2013b).

**Table 7.3.** Calculated $K_d$ of model NDMA precursors during 6-h biosorption tests.

| Precursor compounds | Log$K_{ow}$ | Calculated log$K_d$ | | | | Reported log$K_d$ |
|---|---|---|---|---|---|---|
| | | AS 1 | AS 2 | AS 3 | AS 4 | |
| RNTD | 0.27 | 3.3 | 2.3 | 2.2 | 2.3 | 0.5-2.8[a], 2.7±1.9[b], 2.2±1.5[c] |
| TMA | 0.16 | >3.5 | 2.0 | 1.7 | ~1.0 | N.A.[d] |
| MNCL | 0.05 | >3.5 | 3.4 | 3.6 | ~1.0 | 3.6[e] |
| SMTR | 0.93 | 2.3 | 2.4 | 2.5 | ~1.0 | N.A. |

[a]: Jelic et al., 2011. [b]: Vasiliadou et al., 2013. [c]: Vasiliadou et al., 2014. [d]: Not available. [e]: Guerra et al., 2014.

Biosorption of xenobiotic compounds tends to be a rapid process which could be completed within hours or even minutes (Savci, 2013; Vasiliadou et al., 2013; Martinez-Hernandez et al., 2016). To further examine the biosorption kinetics, NDMA FPs of model precursor compounds were monitored up to 10 h during the biosorption tests with $NaN_3$-deactivated AS liquor (~3000 mg/L MLSS). The two municipal AS (AS 1 and AS 2), and an additional AS 3' were employed for the biosorption kinetics tests. AS 3' was collected from the textile WWTP after a new textile manufacturing process was launched, with characteristics that were substantially altered (e.g., distinct colors, MLSS concentrations, and enhanced settleability). AS 3' exhibited 2%-56% higher removals of NDMA FP via biosorption than AS 3 after 6-h incubation (**Figure 7.5**). With the incubation duration increasing from 2 to 10 h during the biosorption tests, relatively consistent NDMA FP removal (i.e., <18% differences) was observed for RNTD, MNCL and SMTR despite the different types of AS. For TMA, however, NDMA FP removal was 33%- 41% higher after 6 or 10-h incubation than 2-h incubation with AS 2 or AS 3', likely because of the gradually recovered microbial activities during the biosorption tests, as indicated by the slight increase in oxygen uptake rates with increasing incubation duration (**Figure C-1**) which contributed to TMA deactivations via biodegradation. These results suggest that deactivation of model NDMA precursors via biosorption could be completed within a shorter time frame (i.e., 2 h) than the lower end (i.e., 4 h) of typical HRTs utilized at WWTPs.

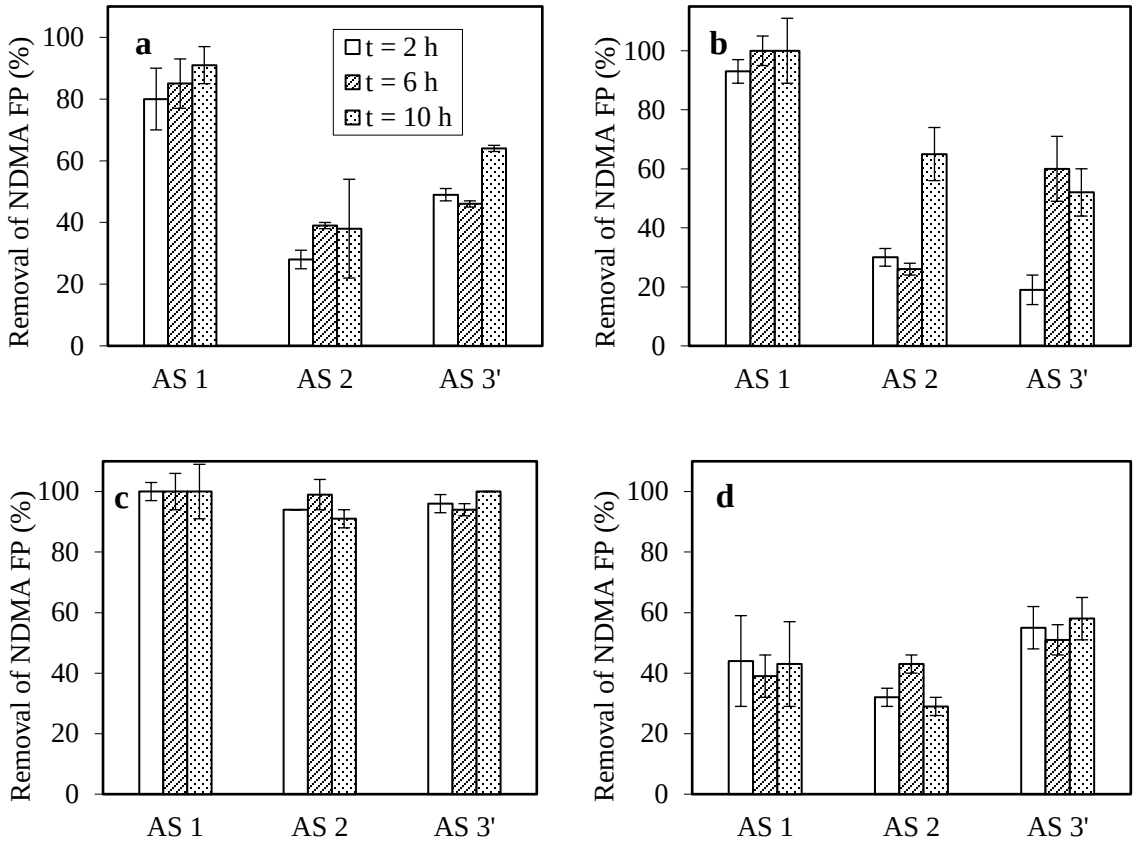



**Figure 7.5.** Biosorption of NDMA FP from model precursor compounds during 2, 6 and 10-h biosorption tests. (a) RNTD, (b) TMA, (c) MNCL, (d) SMTR. AS 3' was collected from the textile WWTP after a new textile manufacturing process was launched and AS characteristics were substantially altered.

### *Deactivation of Model NDMA Precursors via Biodegradation*

The biodegradation of xenobiotics can be affected by microbial communities and other factors such as pH and temperature (Grady, 1993; Sarma and Joshi, 2015). Therefore, the deactivation of RNTD (100 nM) and SMTR (200 nM) via biodegradation was further investigated with different AS biomasses including AS 1 collected during spring and summer, and AS 4 with 8 and 24-d SRT at constant pH (7.4±0.2, buffered with 4 mM phosphate) and temperature (23±2°C). The effects of biosorption on deactivation were

minimized by using a low MLSS (~200 mg/L) and pre-biosorption (i.e., 30 min pre-contact of AS biomass with precursor compounds). After 24-h incubation of RNTD, 4% and 45% of NDMA FPs were reduced by AS 1 collected from spring and summer, respectively, while only 13% and 2% of NDMA FP reductions were achieved with AS 4 with 8 and 24-d SRT, respectively (**Figure 7.6**). AS 1, in general, showed higher biodegradation rates of RNTD than AS 4. These results suggest that deactivation of RNTD via biodegradation depends on AS sources (i.e., domestic vs lab-grown) and AS seasonal reactivity. AS 1 collected in summer exhibited higher reactivity than AS 1 collected in spring, as indicated by a higher removal of organic carbon and ammonia during summer at WWTP 1 than in spring (**Table C-5**). Similarly, AS 4 with a shorter SRT could be more reactive than that with a longer SRT, and thus exhibited a higher deactivation of RNTD via biodegradation. It has been studied that AS biomass acclimated exhibited higher microbial activities biodegrading organic contaminants as SRT decreased (Ouyang and Liu, 2009). Negligible reductions of NDMA FP, however, were observed for SMTR after 24-h incubation with all AS. Extending incubation up to 10 d increased the NDMA FP removal of RNTD up to 71% and 44% with AS 1 and AS 4, respectively, while up to 29% NDMA FP removal was achieved for SMTR with AS 1 collected in summer.

The pseudo-first-order equation fits well the biodegradation kinetics of RNTD ($R^2$ >0.86, **Figure C-4**) during 10-d incubation with AS 4 (8 and 24-d SRT) and AS 1 collected in spring, and their pseudo-first-order biodegradation rate constants were 0.0667, 0.0428 and 0.0828 $d^{-1}$, respectively. During treatment with AS 1 collected in summer, however, the results of biodegradation kinetics were less correlated ($R^2$ <0.69) with the

pseudo-first-order model. Instead, NDMA FP of RNTD was reduced substantially (i.e., 45%) during 1-d incubation, and less (i.e., 26%) during 2-10 d incubation (**Figure 7.6**).

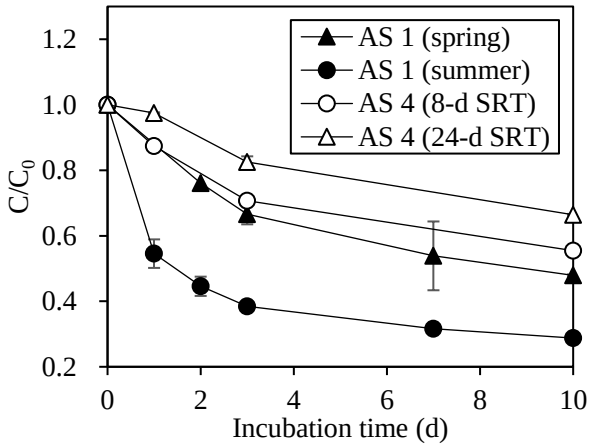



**Figure 7.6.** Biodegradation curves of NDMA FP from RNTD during 10-d incubation with AS 1 collected in spring and summer, AS 4 with 8-d and 24-d SRT. Initial concentration of RNTD = 100 nM. MLSS ≈ 200 mg/L.

### *Roles of Biostimulation and Non-specific Oxygenase*

The removals of NDMA FP from RNTD during treatment with AS 1 with and without the addition of biostimulants, are shown in **Figure 7.7**. Adding selected biostimulants did not promote the removal of NDMA FP from RNTD. To contrary, adding ammonia may slightly decrease NDMA FP removal, likely because of the pH drop due to nitrification process. The pH dropped from 7.5 before incubation, to 6.9, 6.3 and 5.0 after 2, 5 and 7-d incubation, respectively. These results suggest that adding the selected biostimulants could not significantly increase the deactivation efficiencies of RNTD during incubation with AS 1. The reason was likely because the growth of microbes, or the

activities of target enzymes attributable to RNTD biodegradation, were not significantly promoted by adding the selected biostimulants.

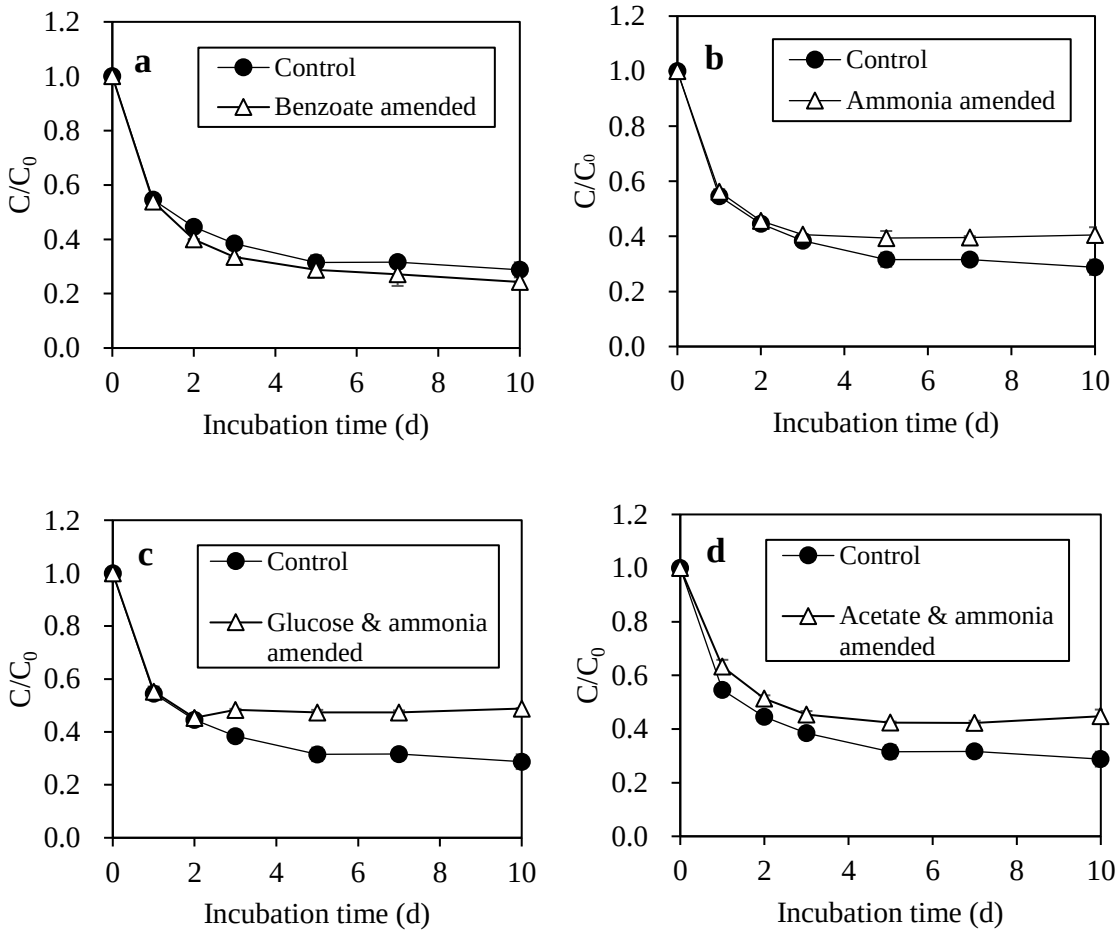



**Figure 7.7.** Removal of NDMA FP from RNTD during biodegradation tests with AS 1 in the presence of (a) benzoate, (b) ammonia, (c) glucose and ammonia, (d) acetate and ammonia as biostimulants. RNTD concentration = 100 nM, MLSS = 200 mg/L. *C* represents NDMA FP from RNTD measured after incubation with AS 1, while $C_0$ represents the initial NDMA FP from RNTD.

Removal of NDMA FP from RNTD by *P. putida* grown on phenol is shown in **Figure 7.8**. Under aerobic condition, NDMA FP from RNTD dropped from 18163 ng/L before incubation, to 9969 ng/L after 2-h incubation with *P. putida*. After 5-d incubation, the NDMA FP from RNTD further decreased to 1586 ng/L. With incubation time further increasing from 5 d to 20 d, NDMA FPs from RNTD remained at 1302-1731 ng/L.

In the presence of acetylene, NDMA FP from RNTD slightly increased from 18163 ng/L before incubation, to 22592 ng/L after 2-h incubation with *P. putida*. After 5-d incubation, NDMA FP from RNTD decreased to 3848 ng/L, which was higher than that (i.e., 1586 ng/L) measured without any acetylene added. During 10-20 d of incubation, NDMA FP from RNTD remained relatively constant at 3564-5914 ng/L. Removals of NDMA FP from RNTD were generally lower in the presence of acetylene than those in the absence of acetylene. This result suggests that the inhibition of oxygenase activity by acetylene could decrease the removal of NDMA FP from RNTD. Oxygenases (i.e., phenol 2-monooxygenase) may contribute to removal of NDMA FP from RNTD.

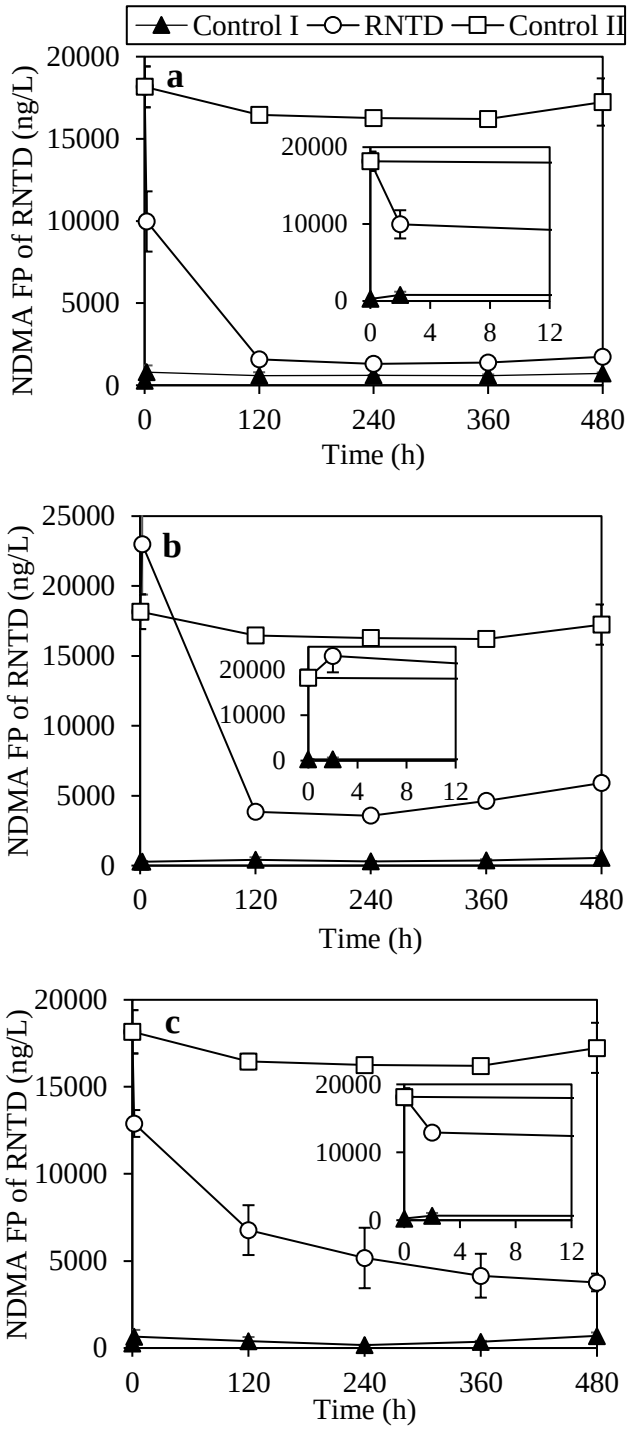



**Figure 7.8.** Removal of NDMA FP from RNTD during incubation with P. putida under (a) aerobic condition in the absence of acetylene, (b) aerobic condition in the presence of acetylene, and (c) anaerobic condition. RNTD concentration = 1000 nM, SS = 700 mg/L. Control I and II: NDMA FP in the absence of RNTD and *P. putida* cells, respectively.

Under anaerobic conditions, the NDMA FP from RNTD dropped from 18163 ng/L before incubation, to 12897 ng/L after 2-h incubation with *P. putida* because of biosorption. After 5-d incubation, NDMA FP from RNTD further decreased to 6772 ng/L, which was still higher than that (i.e., 1586 ng/L) measured under aerobic condition. With further incubation to 10, 15 and 20 d, NDMA FP from RNTD further decreased to 5172, 4153 and 3764 ng/L, respectively. These results suggest that anaerobic biodegradation processes can partially remove NDMA FP from RNTD. However, the removal efficiencies under anaerobic condition were lower than those under aerobic condition. This may suggest that activity of oxygenases favors the removal of NDMA FP from RNTD.

### *Deactivation of NDMA Precursors under Practical Chloramination Condition*

NDMA formations from selected model compounds were monitored under practical chloramination condition (i.e., UFC tests) in the AS-treated mineral solutions (i.e., model compounds were dosed into the mineral solutions which had been treated by AS). The NDMA UFC yields of RNTD measured in the mineral solutions after treatment with AS 1 and AS 2 (i.e., Effluent 1 and Effluent 2, respectively) were 82.5% and 80.9%, respectively, while its NDMA FP yields were 82.5% and 73.1%, respectively (**Table 7.4**). These results are generally consistent with those (i.e., 62.8-97% NDMA UFC yields and 42.2-94.2% NDMA FP yields) reported in previous studies measured in different water matrices (i.e., DDW, tap water and surface waters) (Shen and Andrews, 2011a; Shen and Andrews, 2011b; Selbes et al, 2013; Shen and Andrews, 2013a; Schmidt et al., 2006). RNTD has been found to be reactive with both monochloramine and dichloramine to form

NDMA (Selbes et al., 2013). Because the monochloramine concertation is generally higher than dichloramine under the UFC or FP tests, or in different water matrices (Selbes et al., 2013), NDMA yields of RNTD may be less affected by changes of mono-/dichloramine ratios.

NDMA UFC yields of TMA dosed in Effluent 1 and Effluent 2 were 0.6% and 0.2%, respectively, consistent with those (i.e., 0.4%) measured in DDW (Selbes et al., 2013). Its FP yields in Effluent 1 and Effluent 2 were 1.7% and 1.4%, respectively, slightly lower than those measured in DDW (i.e., 1.9%) and surface waters (i.e., ~ 2%). TMA has been known to be more reactive with dichloramine to form NDMA (Selbes et al., 2013), and thus its NDMA UFC yields were less than FP yields due to the lower amounts of dichloramine present in UFC than FP tests. MNCL yielded negligible NDMA UFC, while its NDMA FP yields were 1.3% and 0.7% in Effluent 1 and Effluent 2, respectively. SMTR showed 0.7% NDMA UFC yields in Effluent 1 and Effluent 2, which are lower than its NDMA FP yields (i.e., 1.5% and 1.8%, respectively). MNCL and SMTR are probably more reactive with dichloramine than monochloramine to form NDMA.

**Table 7.4.** Summary of NDMA formation from model compounds measured in different water matrices.

| Model compounds | Measured NDMA UFC yields in this study (%) | | Measured NDMA FP yields in this study (%) | | Reported NDMA UFC yields (%) | | | Reported NDMA FP yields (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WWTP 1 effluents[a] | WWTP 2 effluents[a] | WWTP 1 effluents | WWTP 2 effluents | DDW | Tap water | Surface water | DDW | Tap water | Surface water |
| RNTD | 82.5 (0.8) | 80.9 (2.6) | 82.5 (7.9) | 73.1 (7.1) | 82.7-85.2[b]; ~97[d]; 62.8-90.6[e] | 83.4-88.4[b]; >77[c] | 72.9.76.9[b] (lake); 82.2-84.1[b] (river) | 89.9 (0.3)[c], 80.5 (2.9)[d]; 42.2-59.6[g] | 94.2 (4.4)[c]; 62.9[f] | ~89-93[d] |
| TMA | 0.6 (0.1) | 0.2 (0.1) | 1.7 (0.1) | 1.4 (0.7) | 0.4[d] | | | 1.9 (0.2)[d] | | ~2[d] |
| MNCL | N.D. | N.D. | 1.3 (0.1) | 0.7 (0.6) | | | | 8.2 (0.7)[g] | | |
| SMTR | 0.7 (0.0) | 0.7 (0.1) | 1.5 (0.2) | 1.8 (0.4) | 2.8-4.2[e] | ~2-5[c] | | 6.1[c] | 6.1[c] | |

[a]: Effluents after 6 and 24-h incubation with AS 1 and AS 2 in the mineral salts solution without any NDMA precursors added before the tests. [b]: Shen and Andrews, 2011a. [c]: Shen and Andrews, 2011b. [d]: Selbes et al, 2013a. [e]: Shen and Andrews, 2013a. [f]: Schmidt et al., 2006. [g]: Le Roux et al., 2011.

In addition, NDMA UFC (i.e., 0.7%) and FP (i.e., 1.5%-1.8%) yields from SMTR measured in Effluent 1 and Effluent 2 were lower than those (i.e., 2-5% UFC yields, 6.1% FP yields) measured in DDW and tap waters (Shen and Andrews, 2011b; Shen and Andrews, 2013a). This was likely because the higher amounts of bulk organic matters present in Effluent 1 and 2 may exhibit a stronger complexation with model compounds and decreased their NDMA UFC and FP (Shen and Andrews, 2011b; Selbes et al., 2013).

NDMA UFC of RNTD, TMA and SMTR all decreased after treatment with AS 1 or AS 2 (**Figure 7.9**). Removals of NDMA UFC from RNTD were 69% and 83% after 6 and 24-h treatment with AS collected from WWTP 1, respectively, while removals of NDMA UFC from TMA were nearly 100%, and removals of NDMA UFC from SMTR were 32% and 47%, respectively. After 6 and 24-h treatment with AS collected from WWTP 2, removals of NDMA UFC were 48% and 76% for RNTD, respectively, 100% for TMA, and 59% and 55% for SMTR, respectively. MNCL yielded negligible NDMA UFC before and after treatment with AS 1 or AS 2. In general, there is no significant ($p$ >0.05) differences between the removal of NDMA UFC after treatment with AS 1 and AS 2 (except for a higher NDMA UFC removal from SMTR after 6-h treatment with AS 2 than AS 1), or between the removal of NDMA UFC and removal of NDMA FP from each model compound.

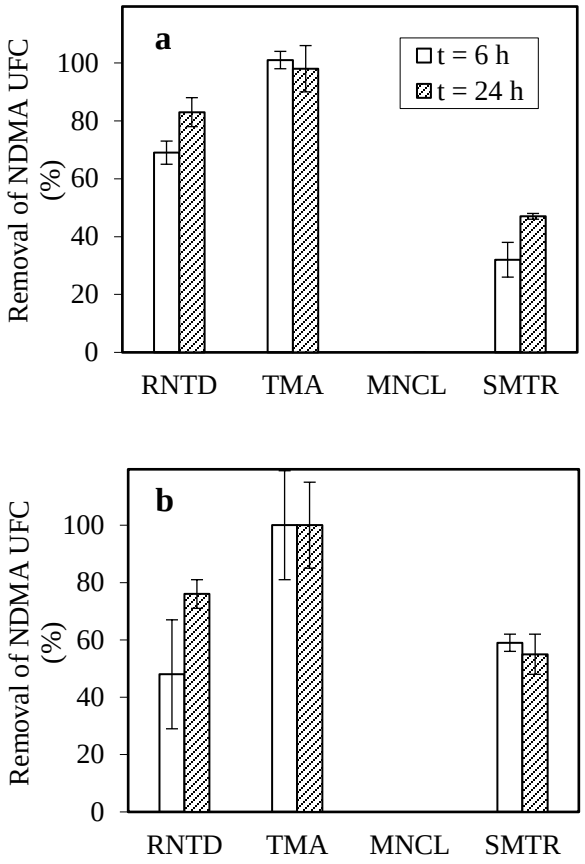



**Figure 7.9.** Removal of NDMA UFC from model compounds after treatment with (a) AS 1, and (b) AS 2.

## Conclusions

The deactivation efficiencies of selected model NDMA precursors were investigated via monitoring their NDMA FPs during batch AS treatment tests. The effects of treatment conditions (i.e., incubation time or HRT, SRT) were carefully evaluated. AS treatment deactivated NDMA precursors to different degrees depending on the functional groups of precursor compounds and AS sources. After 24-h incubation with four types of AS (i.e., domestic rural, domestic urban, textile and lab-grown AS), TMA and MNCL were readily removed, RNTD was moderately removed, and SMTR was the least removable. NDMA FP removal from RNTD depended on AS types and incubation time. Among the four types of AS tested (i.e., domestic rural, domestic urban, textile and lab-grown AS), AS 1 exhibited the highest deactivation efficiencies of RNTD. Increasing incubation time from 6 to 24 h increased NDMA FP removal from RNTD, but negligibly affected NDMA FP removal from other compounds. The lab SBR AS with a longer SRT (i.e., 12 d) exhibited higher removal of NDMA FP from RNTD.

Among the tested deactivation pathways (i.e., biosorption, biodegradation, volatilization), biosorption was predominant for RNTD, MNCL and SMTR, while biodegradation was the major deactivation pathway of TMA during AS treatment. The biodegradation rates for RNTD (i.e., with minimized biosorption) were affected by both seasonal AS reactivity and SRT. Higher biodegradation rates were observed when RNTD was treated by AS 1 collected in summer than in spring, or by the lab-grown AS with a shorter SRT (i.e., 8 d) than longer SRT (i.e., 24 d). Biostimulation may not be an effective method to enhance the deactivation of NDMA precursors during AS treatment. Although

the biodegradation of NDMA precursors (e.g., RNTD) was partially attributed to non-specific oxygenase, the roles of oxygenase may not be significant in promoting the removal of NDMA precursors. Future studies are still needed to investigate other types of oxygenase and precursor compounds to better elucidate the mechanisms involved in the biodegradation of NDMA precursors.

Under the UFC chloramination condition, NDMA formation of model compounds was generally less than that under the FP condition, except for RNTD which showed similar NDMA UFC and FP results. NDMA UFC from RNTD, TMA and SMTR decreased after 6 or 24-h treatment with AS 1 and AS 2, which is generally consistent with their NDMA FP removals. However, MNCL yielded negligible NDMA UFC before and after AS treatment.

# CHAPTER VIII

# DEACTIVATION OF *N*-NITROSAMINE PRECURSORS FROM SEWAGE COMPONENTS AND WASTEWATER INFLUENTS DURING THE AS PROCESS

## Introduction and Objective

Wastewater influents contain human urine and feces, laundry, shower, washbasin and kitchen greywaters as major constituents (Friedler et al., 2013; Zeng and Mitch, 2015). Among all these sewage components (i.e., blackwaters and greywaters), laundry greywater was found to be the most significant contributor of *N*-nitrosamines UFC, followed by shower greywater and urine blackwater (Zeng and Mitch, 2015). So far, however, the deactivation efficiencies of *N*-nitrosamines UFC from different sewage components during the AS process are still largely unknown. Human urine is the major source of organic nitrogen present in domestic wastewater (Hanson and Lee, 1971), and also an important contributor to *N*-nitrosamine precursors (Zeng and Mitch, 2015). It has been found that the dilution factors for human urine in domestic wastewater vary diurnally, depending on the urine production (i.e., 100-300 mL per void) and wastewater flow rates (Friedler et al., 2013). The peak concentration of human urine, estimated based on ammonia concentrations in WWTP influents, was found to occur during the morning hours, three-fold of that typically found in the evening (Henze et al., 2008). The impacts of human urine dilution factors (i.e., the degrees of human urine diluted in tap water; 250 or 125 times in this study) on the removal efficiencies of its *N*-nitrosamines UFC during the AS process

are still largely unclear, as are the potential effects of other factors (e.g., influent types, incubation time or HRT) on the *N*-nitrosamines UFC removal from different sewage components.

For potential improvements in biodegradation efficiencies, adding exogenous electron donors (EEDs; or biostimulation) has been applied for in-situ bioremediation at contaminated sites with chlorinated solvents (Adams et al., 2015). The application of adding EEDs, however, is still limited for AS when applied to removing emerging trace contaminants from wastewaters. Lab studies showed that addition of readily biodegradable growth substrates (e.g., ammonia, glucose, acetate) enhanced the removal of persistent organic compounds (such as PPCPs) during biodegradation with different bacterial strains (Yi and Harper, 2007; Tran et al., 2009; Fernandez-Fontaina et al., 2012; Lee et al., 2015). The addition of primary growth substrates stimulates the production of reductive co-factors (e.g., NADH) that are required for the oxidation of target contaminants (Arcier et al., 1989; Chen et al., 2008). On the other hand, however, the primary growth substrates may compete with target contaminants for binding sites on enzymes and thus reduce the removal rates of contaminants (Drillia et al., 2005; Saratale et al., 2009; Muller et al., 2013). So far, the roles of adding EEDs in the removal efficiencies of *N*-nitrosamine precursors from wastewaters during the AS process are still unclear.

In this study, the deactivation efficiencies of *N*-nitrosamine precursors, which were determined by their FP changes before and after treatment, in different sewage components (i.e., urine and feces blackwaters, laundry, shower and kitchen greywaters) and wastewater influents (i.e., with <1%-100% contributions of industrial discharges) were monitored

during batch AS treatment tests. The effects of precursor types, AS sources (i.e., domestic rural, domestic urban, and textile AS), and incubation time (i.e., 6 and 24 h) on the deactivation efficiencies of *N*-nitrosamine precursors were evaluated. Further, *N*-nitrosamine UFC from different sewage components were also measured during the AS process under selected treatment conditions (i.e., incubation time or HRT, addition of EEDs, dilution factors of urine in wastewater). Finally, the relative contributions of each sewage component to the total *N*-nitrosamine FP and UFC found in raw sewage and secondary effluents were estimated.

## Materials and Methods

### *Sewage Components*

Four blackwaters (i.e., two urine and two feces blackwaters) and seven greywaters (i.e., two laundry greywaters, three shower greywaters and two kitchen greywaters) were collected for batch AS treatment tests. Raw human urine (U) and feces (F) were collected from a volunteer before (U and F, respectively) and after (UR and FR, respectively) taking a Zantac tablet containing 150 mg RNTD as an active ingredient. The collected urine and feces samples were then filtered through 0.7-µm glass fiber filter followed by 0.45-µm cellulose nitrate membrane filter (Whatman, GE Healthcare Life Sciences, US). Laundry greywater samples were collected from washing machine discharges after a batch of old, mixed white and colored clothes were washed with a single brand of detergent (LD), and with both detergent and fabric softener (LF), respectively. The urine, feces and laundry

greywater samples were collected by Dr. William Mitch's group at Stanford University, which were then shipped to Clemson University for AS treatment tests. Shower greywater samples were collected from a bathtub after a volunteer took a hot shower without the use of any personal care products (S), with the use of a single brand of shampoo only (SS), and with the use of a single brand of body wash only (SB). Kitchen greywater samples were collected from a stoppered kitchen sink after a volunteer manually washed a pile of clean dishes using a single brand of dishwashing detergent only (KD), and after soaking (for 30 min) and boiling (for 2 min) mixed food materials (i.e., rice, noodle, vegetables, meats and seafood, each ~50 g/L), respectively. The soaking water and boiled soup were then mixed in a 1:4 volume ratio (KF) to mimic leachates from food residuals. All collected blackwaters and greywaters were filtered through 0.45-μm cellulose nitrate membrane filter (Whatman, GE Healthcare Life Sciences, US) before being stored. Urine, feces and KF samples were stored at -20ºC to minimize microbial activity, while other samples were stored at 4ºC until used. Selected water quality parameters of filtered blackwaters and greywaters were measured based on Standard Methods (APHA et al., 2005), as shown in **Table 5.1**.

### *Wastewater Influents*

Wastewater influents (WW1-WW4) were collected from four WWTPs with industrial impacts (i.e., contributions of industrial discharge in influents) ranging <1%-100%. Key operational parameters of the four WWTPs are available in **Table 8.1**. The four wastewater influents covered a fairly wide range of water qualities, with COD ranging from 26-63 mg/L, and $NH_3$-N from 7-26 mg/L (**Table 8.2**). Approximately 10 L of wastewater were grabbed from the inlet of an aeration basin (i.e., outlet of grid screen) at each WWTP, transported to the lab within two hours upon collection, and then filtered through 0.45-μm membrane filter (Whatman, GE Healthcare Life Sciences, US) for storage at 4°C until used.

**Table 8.1.** Key information of wastewater utilities for the collection of wastewater influents.

| Wastewater influents | Influent type | Treatment capacity (mgd)[a] | HRT (h) | SRT (d) | Treatment Process | Industrial impact |
|---|---|---|---|---|---|---|
| WW 1 | Domestic wastewater | 2.0 | 22-24 | 20 | Extended aeration | <1% |
| WW 2 | Domestic wastewater with partial industrial discharge | 70 | 13.5 | 12 | Anaerobic-anoxic-oxic | 25% |
| WW 3 | Predominantly domestic wastewater | 3.0 | N.A.[b] | N.A. | N.A. | 8-15% |
| WW 4 | Predominantly domestic wastewater | 4.0 | N.A. | N.A. | Membrane bioreactor | 8-15% |

[a]: Million gallons per day. [b]: Not available.

**Table 8.2.** Selected water quality parameters measured in wastewater influents.

| Wastewater influents | COD (mg/L) | $NH_3$-N (mg/L) | DOC (mg/L) | $SUVA_{254}$ |
|---|---|---|---|---|
| WW 1 | 63 | 26 | 13 | 2.0 |
| WW 2 | 51 | 24 | 15 | 2.0 |
| WW 3 | 39 | 19 | 11 | 2.1 |
| WW 4 | 26 | 7 | 13 | 1.8 |

### *AS Samples*

Three types of AS samples were collected to evaluate treatment of *N*-nitrosamine precursors, including AS from a rural domestic WWTP (AS 1), an urban domestic WWTP (AS 2) and a textile WWTP (AS 3), respectively. The three WWTPs were selected to cover a variety of treatment capacities (i.e., 1.7-70 million gallons per day), HRT (i.e., 5-24 h), SRT (i.e., 12-26 d), and nutrient removal levels (i.e., nitrification, biological N and P removal, and nitrogen deficient condition, respectively). Key operational parameters of the three WWTPs are summarized in **Table 7.2**. During AS collection, ~1 L liquor was grabbed from the aeration basins of the WWTPs, transported to the lab within 1 h upon collection with adequate ventilation to maintain an aerobic condition, and then aerated for 4-12 h at 23±2 ºC for preconditioning. Prior to testing, the AS solids were washed three times to remove residual NDMA precursors from wastewaters. The procedures used are as follows: (i) AS liquor was centrifuged at 2000×g for 5 min, (ii) the pellet portion was harvested and resuspended in a mineral salts solution (recipe shown in **Appendix C**; 6000 mg/L MLSS). The same procedures were repeated for three times, with the final MLSS adjusted to 3000 mg/L.

### *Batch AS Treatment Tests*

*N*-nitrosamine precursors were treated with AS using a simulated EPA batch test method (OPPTS 835.3280 -314B) (US EPA, 2008). The washed AS liquor was transferred to 1-L incubation bottle, dosed with a predetermined volume of human urine, feces or KF sample to achieve the target dilution factors (i.e., 250, 150 and 100 times, respectively). For treatment of laundry, shower and kitchen greywaters, the washed AS solids were resuspended in greywaters with the MLSS adjusted to ~3000 mg/L. The mixed liquor was then incubated at 25±2 ºC for 6 and 24 h with constant aeration (i.e., 150 L/m$^3$·min air flowrate). At the end of incubation, 125-mL liquor was harvested and filtered through 0.45-μm membrane filter (Whatman, GE Healthcare Life Sciences, US). The filtered samples were used in the *N*-nitrosamine FP tests.

All tests were run in duplicates. Controls were prepared whenever necessary, run in parallel with tested samples. The deactivation efficiencies of *N*-nitrosamine precursors (i.e., removal of *N*-nitrosamine FP) were evaluated using the following equation:

$$R\ (\%) = (1 - \frac{C_e - C_0}{C_{in}}) \times 100\% \qquad \textbf{Equation 8.1}$$

where $R$ is the removal (%) of *N*-nitrosamine FP, $C_{in}$ is *N*-nitrosamine FP (ng/L) measured in blackwaters and greywaters before AS treatment, $C_e$ is *N*-nitrosamine FP measured in blackwaters and greywaters after AS treatment, and $C_0$ is *N*-nitrosamine FP measured in the mineral salts solutions after AS treatment without any external *N*-nitrosamine precursors dosed.

### *N-Nitrosamine Formation Tests*

Seven of the most detected *N*-nitrosamines, including NDMA, NDEA, NPYR, NDPA, NMOR, NPIP and NDBA were evaluated during the AS treatment tests. During *N*-nitrosamine FP testes, a monochloramine stock solution was fresh prepared by adding a sodium hypochlorite solution (NaClO, ~4000 mg $Cl_2$/L) to an ammonium chloride solution ($NH_4Cl$, ~1000 mg N/L) drop by drop at pH 9 with a Cl:N mass ratio of 4:1. A predetermined volume of monochloramine stock solution was dosed into filtered samples to achieve the target monochloramine dosage (i.e., 100 mg $Cl_2$/L). The chloraminated samples were then incubated at 23±2ºC in the dark for 5 d. During *N*-nitrosamine UFC tests, a predetermined volume of monochloramine stock solution was dosed into filtered samples to achieve the target monochloramine dosage (i.e., 5 mg $Cl_2$/L). A higher monochloramine dosage (i.e., 10 mg $Cl_2$/L) was used for the UFC tests of laundry greywaters, because of the high DOC present. The chloraminated samples were then incubated at 23±2ºC in dark for 3 d. At the end of incubation, the residual chloramines were quenched by adding excess amounts of sodium thiosulfate ($Na_2S_2O_3$) powder. *N*-nitrosamines in samples were analyzed according to US EPA Method 521 (US EPA, 2004), with more details described elsewhere (Uzun, 2016). The MRLs of the tested *N*-nitrosamines were determined to be 2 (for NDMA and NDEA) or 3 ng/L (for the other *N*-nitrosamines).

## Results and Discussion

### *Deactivation of N-Nitrosamine Precursors from Urine and Feces Blackwaters*

Before the treatment with AS 1, AS 2 and AS 3, NDMA FPs from raw urine collected before taking Zantac (U) were 9105, 12760 and 12150 ng/L, respectively. After 6-h treatment with the three types of AS, NDMA FP from U decreased to 5621, 8491 and 4241 ng/L, respectively, and further to 3150, 6222 and 2894 ng/L after 24-h treatment respectively (**Figure 8.1**). NDMA FPs from UR were 5493, 8213 and 7524 ng/L before treatment with AS 1, AS 2 and AS 3, respectively. After 6-h treatment with the three types of AS, NDMA FP decreased to 2881, 5879 and 4966 ng/L, respectively, and further to 1157, 3689 and 3747 ng/L after 24-h treatment, respectively. AS 1 exhibited higher removal (i.e., 65%-79% after 24-h treatment) of NDMA FP from urine blackwaters than either AS 3 (i.e., 50%-76% after 24-h treatment) or AS 2 (i.e., 51%-55% after 24-h treatment). AS 1 has excellent nitrification performance with the $NH_3$-N concentrations undetectable at secondary effluents. Nitrification was found to enhance the removal of persistent contaminants (e.g., pharmaceuticals) via co-metabolic biodegradation process (Yi and Harper, 2007; Quintana et al., 2005; Haiss and Kummerer, 2006).

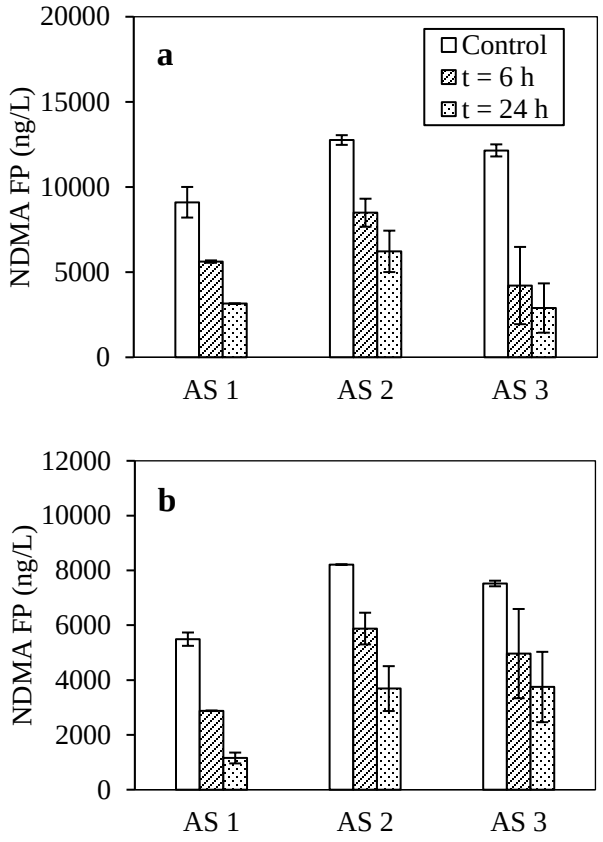



**Figure 8.1.** Removal of NDMA FP from urine blackwaters collected (a) before taking Zantac (U), and (b) after taking Zantac (UR) during 6 and 24-h treatment with three types of AS (i.e., AS 1, AS 2 and AS 3).

NDMA FPs from feces blackwater collected before taking Zantac (F) were 767, 910 and 314 ng/L before treatment with AS 1, AS 2 and AS 3, respectively. After 6-h treatment with the three types of AS, NDMA FP from F decreased to 245, 508 and 207 ng/L, respectively, and further to 75, 317 and 162 ng/L after 24-h treatment, respectively (**Figure 8.2**). Before treatment with AS 1, AS 2 and AS 3, NDMA FPs from feces blackwater collected after taking Zantac (FR) were 1183, 1006 and 967 ng/L, respectively. After 6-h treatment with the three types of AS, NDMA FP from FR decreased to 118, 411

and 318 ng/L, respectively, and further to 77, 282 and 99 ng/L after 24-h treatment, respectively. Still, AS 1 showed higher removal of NDMA FP (i.e., 90%-93% after 24-h treatment) from feces blackwaters than AS 3 (i.e., 48%-90% after 24-h treatment) and AS 2 (i.e., 65%-72% after 24-h treatment).

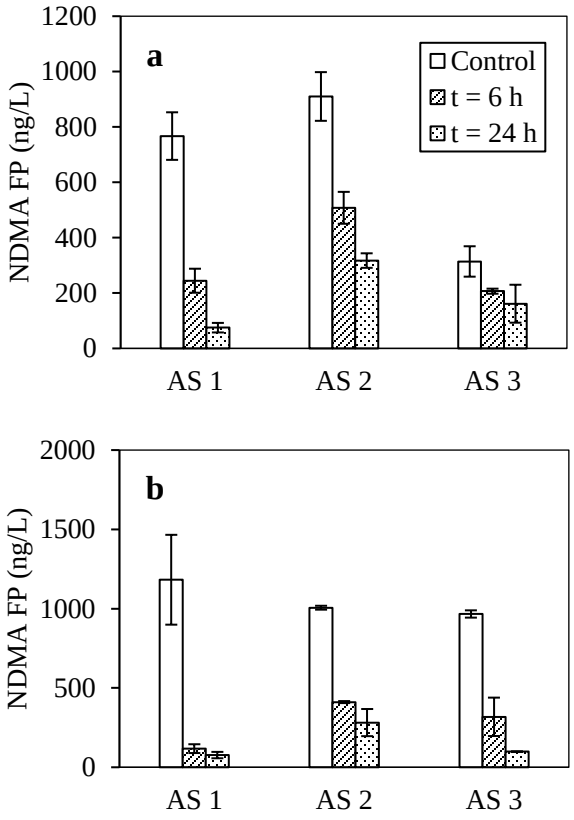



**Figure 8.2.** Removal of NDMA FP from feces blackwaters collected (a) before taking Zantac (F), and (b) after taking Zantac (FR) during 6 and 24-h treatment with three types of AS.

NPYR precursors estimated by its FPs were readily removable during AS treatment of urine blackwaters. Removals of NPYR FP from urine blackwaters were >85% and >94% after 6 and 24-h treatment with the three types of AS, respectively (**Figure D-1**).

Pyrrolidine, a known NPYR precursor (Schreiber and Mitch, 2006; Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014), has been found to be readily biodegradable, with its removal >75% during 24-h treatment with different bacterial strains (Emtiazi and Knapp, 1994; Poupin et al., 1999; Schrader et al., 2000; Trigui et al., 2003). After 24-h treatment with the three types of AS, NPYR FP from urine blackwaters were <10 ng/L. Because the precursors of *N*-nitrosamines other than NDMA and NPYR were relatively low (i.e., FP <20 ng/L), they were not examined for removal during AS treatment.

### *Deactivation of N-Nitrosamine Precursors from Shower Greywater*

NDMA FPs from shower greywaters were removed differently among the three types of AS. NDMA FP from shower greywater not containing any personal care products (S) were removed by 100%, 81%-100%, and <2% after treatment (for 6 and 24 h) with AS 1, AS 2 and AS 3, respectively (**Figure 8.3**). Among the three types of AS, AS 3 exhibited the lowest removal of NDMA FP from S. NDMA precursors in S could be mostly from the ingredients of human sweat (e.g., trimethylamine *N*-oxide, betaine, choline) (Ayesh et al., 1993; Mitch and Sedlak, 2004; Craig et al., 2010; Subramaniam and Fletcher, 2018). Such biologically originated substances likely present in textile wastewater at lower levels than in municipal wastewaters, because their specific sources (i.e., laundry and shower greywaters) are collected mainly via domestic sewage systems. Because of the lack of such compounds in textile wastewater, AS 3 may not have developed a microbial community that can actively biodegrade these NDMA precursor sources, including those from S. NDMA FPs from the shower greywater containing shampoo only (SS) were removed by

<8% after 6-h treatment, and 77%, 84% and 58% after 24-h treatment with AS 1, AS 2 and AS 3, respectively. There was a slight increase in NDMA FP from SS after 6-h incubation with AS 1, which was likely because the biodegradation of NDMA precursors (i.e., surfactants, dyes) contained in shampoo formed some intermediate products containing DMA functional group (**Figure D-5**), and these products could exhibit higher NDMA FP yields than the parent compounds.

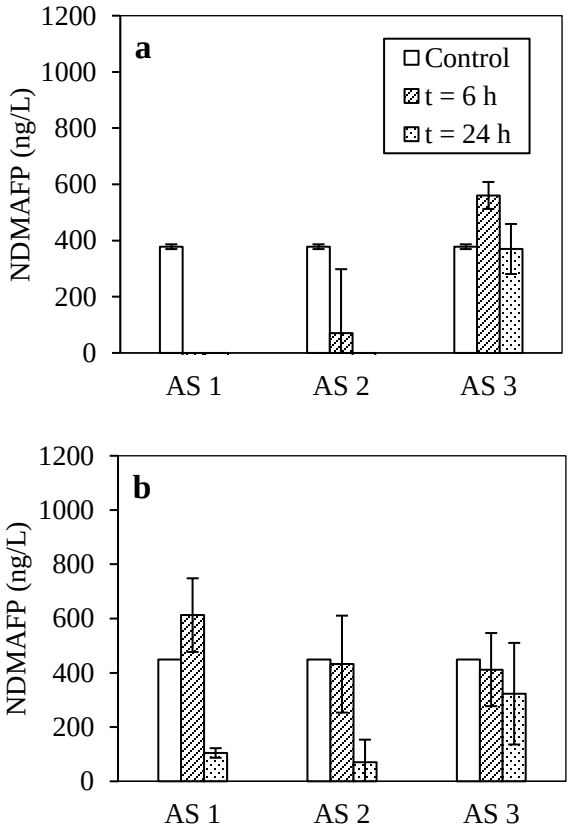



**Figure 8.3.** Removal of NDMA FP from shower greywaters (a) containing no personal care products (S), and (b) containing shampoo only (SS) during 6 and 24-h treatment with three types of AS.

Different from S, NDMA precursors from SS were presumably from shampoo ingredients (i.e., surfactants, emulsifiers, forming agents). It has been reported that textile wastewaters contained abundant (i.e., tens to hundreds of mg/L) surfactants (Yaseen and Scholz, 2019). Therefore, AS 3 may have a microbial community that can actively biodegrade these ingredients (Meerbergen et al., 2017). AS 3 showed higher removal of NDMA FP from SS than S.

NPYR precursors from shower greywaters were readily removable. NPYR FPs from S were removed by >77% and >94% after 6 and 24-h treatment with the three types of AS, respectively, >66% and >87% from SS, respectively, and >66% and >94% from SB, respectively (**Figure D-2**). After 24-h treatment with the three types of AS, NPYR FPs from shower greywaters were <8 ng/L. NDBA FPs from shower greywaters were removed differently after treatment with the three types of AS. After 6-h treatment with AS 1, AS 2 and AS 3, NDBA FPs from S were removed by 14%, 58% and 87%, respectively, 61%, 58% and 87% from SS, respectively, and 39%, 55% and 90% from SB, respectively (**Figure D-3**). AS 3 showed higher removal of NDBA FP from shower greywaters than the AS 1 and AS 2. Textile wastewater has been known to contain dibutylamine, a typical NDBA precursor used as an intermediate in the manufacture of textile and dye auxiliaries (Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014; NPCS, 2009). Dibutylamine and its derivatives present in textile wastewater may facilitate the growth of microorganisms in the textile AS (AS 3) that can actively biodegrade NDBA precursors from shower greywaters. Increasing the incubation time from 6 to 24 h enhanced removal of NDBA FP from shower greywaters. After 24-h treatment with the three types of AS,

NDBA FP from S, SS and SB were >89%, >74% and >94%, respectively. Because the FP of other *N*-nitrosamines (i.e., NDEA, NPIP, NMOR and NDBA) from shower greywaters are relatively low (i.e., <20 ng/L), they were not examined for biological removal during the AS treatment tests.

### *Deactivation of N-Nitrosamine Precursors from Kitchen Greywater*

Kitchen greywater containing food leachates (KF) was an important contributor of NPYR FP (i.e., 25%) and NDMA FP (i.e., 9%) in domestic sewage. Before the treatment with AS 1, AS 2 and AS 3, NDMA FPs from KF were 10723, 7452 and 14828 ng/L, respectively. After 6-h treatment with the three types of AS, NDMA FP from KF decreased to 32, 464 and 6003 ng/L, respectively, and further to 32, 73 and 55 ng/L after 24-h treatment, respectively (**Figure 8.4**). NDMA precursors from KF were mainly from the ingredients of food leachates (e.g., DMA, trimethylamine, etc.) which are readily biodegradable (Mitch and Sedlak, 2004; Wang et al., 2014; Radosevic et al., 2015). AS 1 and AS 2 showed higher removal of NDMA FP from KF than AS 3, likely because food ingredients are typically present in the municipal wastewaters, but less in textile wastewater. Before the treatment with AS 1, AS 2 and AS 3, NPYR FPs from KF were 492, 463 and 606 ng/L, respectively. After 6 and 24-h treatment with the three types of AS, NPYR FPs from KF were <6 and <3 ng/L, respectively (**Figure D-4**), suggesting that NPYR precursors from KF were readily removable during AS treatment.

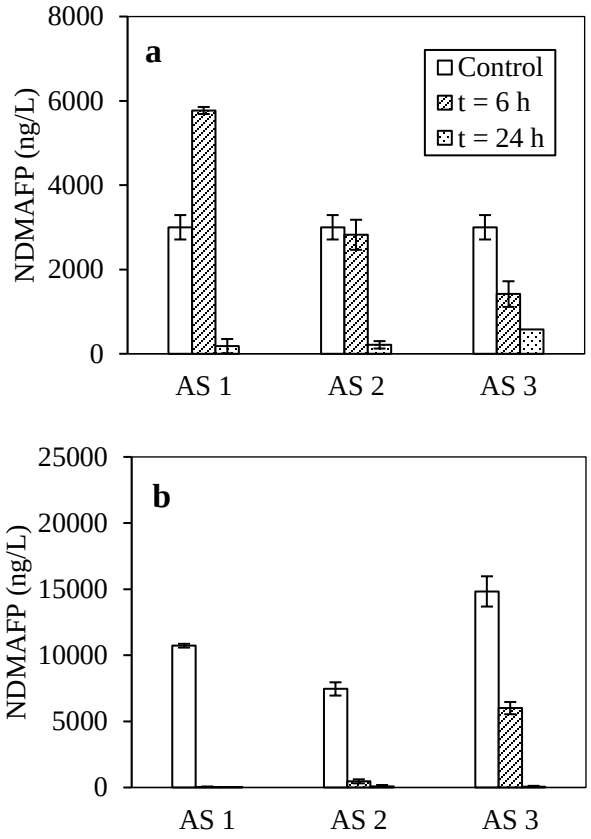



**Figure 8.4.** Removal of NDMA FP from kitchen greywaters containing (a) dishwashing detergent only (KD), and (b) food leachates only (KF) during 6 and 24-h treatment with three types of AS.

NDMA FP from the kitchen greywater containing dishwashing detergent (KD) was 3001 ng/L before AS treatment. After 6-h treatment with AS 1, NDMA FP from KD increased to 5771 ng/L, while it decreased to 2822 and 1416 ng/L after 6-h treatment with AS 2 and AS 3, respectively. The increase in NDMA FP after 6-h treatment with AS 1 was presumably because biodegradation of dishwashing detergent ingredients (e.g., quaternary ammonium surfactants) formed secondary and tertiary amines exhibiting higher NDMA FP yields than their parent compounds (**Figure D-5**; Verschueren, 2009; Kemper et al.,

2010; Selbes et al., 2013). After 24-h treatment with the three types of AS, NDMA FP from KD decreased to 180, 212 and 575 ng/L, respectively. In general, NDMA precursors from KD were less removable than those from KF, likely because dishwashing detergent ingredients (e.g., cationic surfactants) were relatively persistent during AS treatment (Mitch and Sedlak, 2004; Verschueren, 2009; Wang et al., 2014). NDBA FP from KD decreased from 31 ng/L to 10, 13 and 3 ng/L after 6-h treatment with AS 1, AS 2 and AS 3, respectively, and further to <3 ng/L after 24-h treatment (**Figure D-6**). AS 3 exhibited higher removal (i.e., 90%) of NDBA FP from KD than the AS 1 (i.e., 68%) and AS 2 (i.e., 58%) during 6-h treatment. NDBA precursors from KD were mainly from dishwashing detergent ingredients (e.g., surfactants) which could be abundantly present in textile wastewaters. Following exposure to these ingredients, AS 3 may thus develop a microbial community that can actively biodegrade them.

*N*-nitrosamines (i.e., NDEA, NPIP, NMOR and NDPA) that exhibited low FP (i.e., <20 ng/L) from kitchen greywaters were not examined for their precursor removal during the AS treatment tests.

### *Deactivation of N-Nitrosamine Precursors from Laundry Greywater*

Although NDMA FPs from laundry greywaters were relatively low (i.e., <501 ng/L), they increased after AS treatment. NDMA FP from the laundry greywater containing detergent only (LD) increased from 470 ng/L before AS treatment, to 2152, 1634 and 2077 ng/L after 6-h treatment with AS 1, AS 2 and AS 3, respectively. FPs then decreased to 186, 361 and 255 ng/L after 24-h treatment, respectively (**Figure 8.5**). Similarly, NDEA FP from LD increased from 39 ng/L before AS treatment, to 208, 85 and 48 ng/L after 6-h treatment with AS 1, AS 2 and AS 3, respectively, then decreased to 46, 8 and 7 ng/L after 24-h treatment, respectively. The increases in NDMA and NDEA FP from LD after 6-h AS treatment was presumably attributable to biodegradation of NDMA and NDEA precursors (i.e., cationic surfactants and synthetic dyes) from laundry detergents, leading to formation of DMA and diethylamine (DEA)-based biodegradation products (**Figure D-5**). These products may exhibit higher NDMA and NDEA FP yields than their parent compounds.

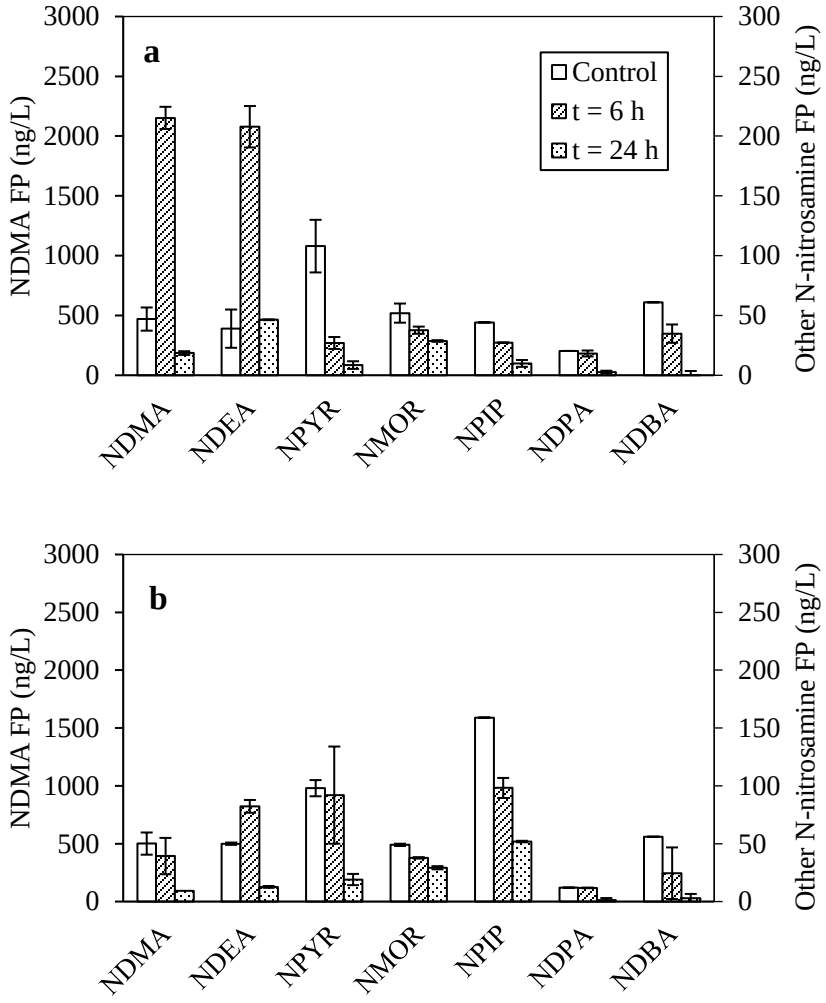



**Figure 8.5.** Removal of *N*-nitrosamine FP from laundry greywaters containing (a) detergent only (LD), and (b) detergent and fabric softener (LF) during 6 and 24-h treatment with AS 1.

FPs for the other *N*-nitrosamines (i.e., NPYR, NMOR, NPIP, NDPA and NDBA) decreased after treatment with the three types of AS (**Figure 8.5, Figures D-7** and **D-8**). NPYR FP, NDBA FP and NDPA FP from laundry greywaters were removed by >76%, >77%, and >85% after 24-h AS treatment, respectively. Less NPIP FP was removed (60%-90%) after 24-h AS treatment. However, NMOR FP was relatively persistent, with removals ranging from 48%-58% after 24-h AS treatment of laundry greywaters. Typical NDPA and NDBA precursors, dipropylamine and dibutylamine (Sacher et al., 2008; Bond and Templeton, 2011; Zhou et al., 2014), are aliphatic amines known to be easily biodegradable (Verschueren, 2009). Within 14-d incubation with an activated sludge inoculum, dibutylamine was found to be completely degraded (Calamari et al., 1980). Dipropylamine was determined to be biodegradable using batch biodegradation test protocol (Howard, 1997). NPYR and NPIP precursors (e.g., pyrrolidine and piperidine) were found also to be biodegradable, with essentially complete removal after 4-h treatment with *Mycobacterium sp.* (Rothkopf and Bartha, 1984; Poupin et al., 1999; Schrader et al., 2000). However, NMOR has been found to be bio-refractory, with <50% removal during biological treatment (Krauss et al., 2009; Wijekoon et al., 2013; Glover et al., 2019). Because of their relatively low removal, NMOR and NPIP were the predominant *N*-nitrosamine species following NDMA after 24-h AS treatment of laundry greywaters, with their FP ranging from 22-29 and 4-64 ng/L, respectively. NPYR FP, NDPA FP, and NDBA FP were all <10 ng/L after 24-h AS treatment.

Among the three types of AS tested, AS 1 exhibited the lowest removal of *N*-nitrosamine FP from laundry greywaters. Removals of NPYR FP, NMOR FP, NPIP FP,

NDPA FP, and NDBA FP from LD after 6-h treatment with AS 1 were 70%, 30%, 37%, 19% and 39%, respectively, which are lower than those with AS 2 (i.e., 89%, 53%, 54%, 52% and 73%)or AS 3 (i.e., 93%, 58%, 57%, 88% and 73%) (**Figure 8.6**). Similar trends were found with *N*-nitrosamine FPs from LF. *N*-nitrosamine precursors in laundry greywaters were presumably from the ingredients of detergent and fabric softener (e.g., surfactants, foaming agents), and synthetic dyes shed from clothes (Zeng and Mitch, 2015). The abundant presence (i.e., tens to hundreds of ng/L) of surfactants and dyes from industrial (e.g., textile) discharges in the urban municipal and textile wastewaters may favor the growth of microorganisms in AS that can actively biodegrade them. AS 2 and AS 3 thus showed higher levels of removal of *N*-nitrosamine FP from laundry greywaters than AS 1. Increasing the incubation time from 6 to 24 h increased the removal of *N*-nitrosamine FP by 18%-100%, 1%-48%, and 0%-21%, respectively, during treatment with AS 1, AS 2 and AS 3 (**Figure D-9**). Among the blackwaters and greywaters tested, laundry greywater was still the dominant (i.e., 56%-100%) contributor of NDEA FP, NPIP FP, NMOR FP, NDPA FP, and NDBA FP after AS treatment. Shower greywaters contributed 45%-92% of NDBA FP after treatment with AS 1 and AS 2.

Before AS treatment, laundry greywater was found to be the major contributor of most *N*-nitrosamine FP in domestic sewage, as shown in Chapter 5. After treatment with different types of AS, laundry greywater was still the most important contributor to *N*-nitrosamine FP except for NDMA and NPYR FP which were mainly contributed by urine blackwater. These findings suggest that AS treatment generally exhibited little impact on the relative importance of sewage components as potential sources of *N*-nitrosamine

precursors. Controlling the discharges of urine blackwater and laundry greywater may favor the reduction of *N*-nitrosamine precursors from secondary wastewater effluents.

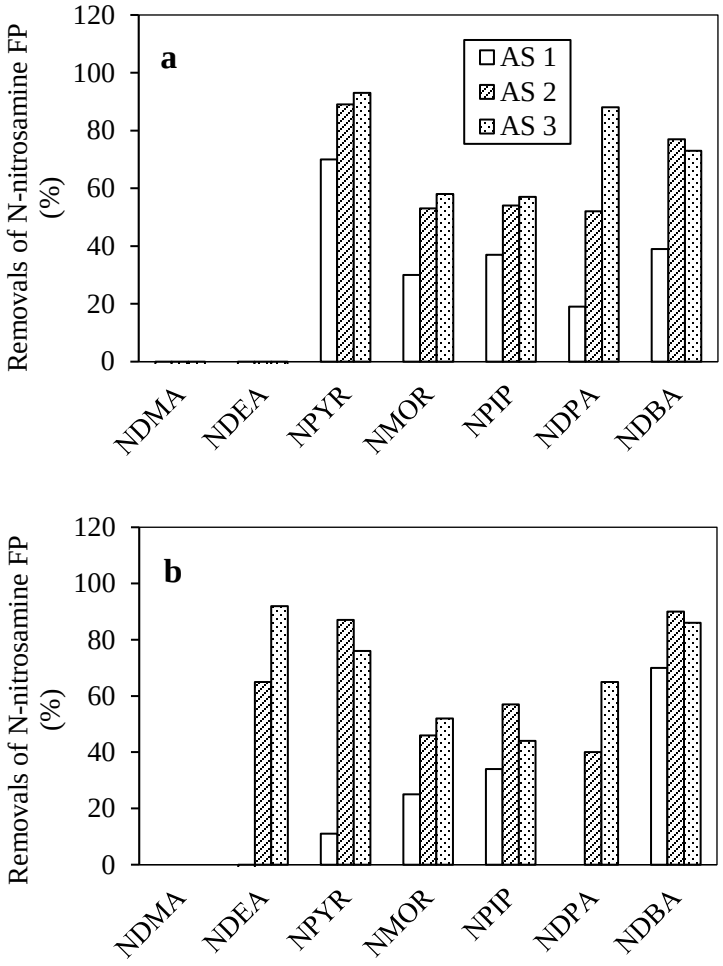



**Figure 8.6.** Removal of *N*-nitrosamine FP from laundry greywaters containing (a) detergent only (LD), and (b) detergent and fabric softener (LF) during 6-h treatment with three types of AS.

### *Deactivation of N-Nitrosamine Precursors from Wastewater Influents*

To investigate the roles of wastewater influent types and seasonal changes in AS activity toward removal of *N*-nitrosamine FP from wastewaters, four wastewater influents (WW1-WW4) were collected and treated with AS 1 (collected in summer) and AS 2 (collected in spring and summer), respectively. Before AS treatment, the FPs of seven *N*-nitrosamine species in WW1-WW4 were measured and are summarized in **Table 8.3**. NDMA FP from WW1-WW4 ranged 1529-3650 ng/L, NPYR FP 99-424 ng/L, NMOR FP 12-37 ng/L, NDBA FP 14-30 ng/L and NDEA FP 4-27 ng/L, while NDPA FP was <3 ng/L in WW1-WW4. The abundance of *N*-nitrosamine FP from WW1-WW4 was generally consistent with what was found in blackwaters and greywaters. The exception is that NPIP FP, which was not measurable in wastewater influents due to the interferences from a neighboring peak (114 m/z) to the target NPIP peak (115 m/z) on the GC spectrum.

**Table 8.3.** *N*-nitrosamine FP measured in different wastewater influents.

| Wastewater influent source | Season[a] | NDMA | NDEA | NPYR | NMOR | NDPA | NPIP | NDBA |
|---|---|---|---|---|---|---|---|---|
| WW1 | Spring | 3650 | 4 | 138 | 21 | N.D.[b] | N.M.[c] | 27 |
| | Summer | 1529 | 5 | 99 | 20 | N.D. | 29 | 14 |
| WW2 | Spring | 2440 | 10 | 424 | 24 | N.D. | 113 | 24 |
| | Summer | 3078 | 27 | 304 | 37 | N.D. | N.M. | 16 |
| WW4 | Summer | 1849 | 10 | 131 | 12 | N.D. | N.M. | 30 |
| WW5 | Summer | 2561 | 13 | 108 | 21 | N.D. | N.M. | 14 |

[a]: Seasons during which the wastewater influents were collected. [b]: Not detectable (i.e., <3 ng/L). [c]: NPIP FP were not measurable in WW1 collected during spring, WW2 collected during summer, WW3 and WW4, because the target NPIP peak (115 m/z) was interfered with a neighbor peak (114 m/z) on GC spectrum.

NDMA FP from WW1-WW4 decreased by 73%-80% and 69%-77% after 6-h treatment with AS 1 and AS 2, respectively (**Table 8.4**). Increasing the incubation time to 24 h increased the removal of NDMA FP to 79%-93% and 82%-87% after treatment with

AS 1 and AS 2, respectively. Differences among the removal of NDMA FP from the four wastewater influents were insignificant (i.e., <8%). AS 2 collected in summer showed higher removal of NDMA FP (i.e., 85%-87%) from WW1 and WW2 after 24-h incubation than that collected in spring (i.e., 69%-79%). These results suggest that removal of NDMA FP might be less dependent on wastewater influent types, but more on the seasonal changes in AS activities.

**Table 8.4.** Removal of NDMA FP from wastewater influents during batch AS treatment tests.

| Samples | Season[a] | AS 1 (summer)[b] | | AS 2 (spring)[c] | | AS 2 (summer)[d] | | Removal at WWTP[e] (%) |
|---|---|---|---|---|---|---|---|---|
| | | 6-h removal (%) | 24-h removal (%) | 6-h removal (%) | 24-h removal (%) | 6-h removal (%) | 24-h removal (%) | |
| WW1 | Spring | 80 | 93 | 63 | 69 | 73 | 85 | 92 |
| | Summer | 74 | 78 | N.M.[f] | N.M. | N.M. | N.M. | 71 |
| WW2 | Spring | 73 | 86 | 74 | 79 | 69 | 87 | 77 |
| | Summer | 72 | 83 | N.M. | N.M. | N.M. | N.M. | 62 |
| WW3 | Summer | 74 | 79 | N.M. | N.M. | 77 | 83 | 67 |
| WW4 | | 78 | 87 | N.M. | N.M. | 76 | 82 | 51 |

[a]: Seasons during which the wastewater influents were collected. [b]: AS 1 collected in summer. [c]: AS 2 collected in spring. [d]: AS 2 collected in summer. [e]: Estimated by measuring NDMA FP in primary effluents and secondary effluents at WWTPs collected at WWTPs. [f]: Not measured.

Potential effects of AS types on removal of NDMA FP from wastewater influents were evaluated by comparing NDMA FP removal during treatment with AS 1, AS 2, and AS from the two WWTPs where WW3 and WW4 were collected. Removals of NDMA FP from WW3 and WW4 monitored at WWTPs (i.e., via measuring NDMA FP from primary and secondary effluents) were 67% and 51%, respectively, which are lower than those treated with AS 1 (i.e., 74%-83%) or AS 2 (i.e., 76%-87%) during batch tests. This suggests the removal of NDMA FP from wastewaters depends on the type of AS. At the two

WWTPs (i.e., the domestic WWTPs) where WW1 and WW2 were collected, NDMA FPs decreased by 92% and 77%, respectively. These removals were similar to those (i.e., 93% and 79%) found in batch tests using AS 1 and AS 2 collected from these two WWTPs, respectively.

Among the seven *N*-nitrosamines evaluated, NPYR FP and NDBA FP were readily removable from WW1-WW4 after 24-h AS treatment (i.e., 90%-99% and 100% removal, respectively). NDEA FP was removed variably (i.e., 27%-100% removal), while NMOR FP was relatively persistent (i.e., 0%-81% removal; **Table D-1**). Though readily biodegradable, NPYR FP was still relatively high (i.e., tens of ng/L) in secondary effluents, mainly because of its abundant occurrences in influents (i.e., hundreds of ng/L). Though NDEA FP and NMOR FP were relatively persistent during AS treatment, they were still low (i.e., <10-20 ng/L) in secondary effluents because of less occurrence (i.e., <20-30 ng/L) in influents. From an epidemiologic perspective, NDEA has a much lower $10^{-6}$ cancer risk level (i.e., 0.2 ng/L) than those of NMOR (i.e., 5 ng/L) and NPYR (i.e., 15 ng/L), and thus may require more attention for its presence in secondary effluents. After treatment with AS 1, removal of NDMA FP or NDEA FP was significantly ($p$ <0.05) correlated with removal of ammonia from WW1-WW4, while poorly (i.e., $R^2$ = 0.036) correlated with DOC removal (**Figure D-10**; **Table D-2**). These results suggest a potential role for nitrification affecting the removal of NDMA FP and NDEA FP from wastewaters.

### *Removal of N-Nitrosamine UFC from Urine and Feces Blackwaters*

Before AS treatment, NDMA UFC from raw urine dosed in Effluent 1, Effluent 2 and Effluent 3 (i.e., mineral solutions treated with AS 1-3; urine diluted 250 fold) were undetectable, 197, and 5454 ng/L, respectively, and NDMA UFC from raw feces (dilution factor of 150-fold) were undetectable, undetectable, and 81 ng/L, respectively. The three effluents (i.e., Effluent 1, Effluent 2 and Effluent 3) may contain distinct amounts of bulk organic matters and thus exhibited different impacts on NDMA UFC from raw urine and feces. The presence of different types and amounts of bulk organic matter could result in distinct impacts on NDMA formation rates during UFC tests, mainly via different degrees of complexations with NDMA precursors (Shen and Andrews, 2011b; Selbes et al., 2013).

After 6 and 24-h treatment with AS 1, NDMA UFC from urine increased from undetectable before AS treatment, to 1616 and 1333 ng/L, respectively (**Figure 8.7**). Similarly, the NDMA UFC from feces increased from undetectable before AS treatment, to 19 ng/L after 24-h treatment, although its NDMA UFC was still undetectable after 6-h treatment with AS 1. Such increase in NDMA UFC for urine and feces after AS treatment were likely because of the decreased amounts of bulk organic matter (i.e., DOC; **Table D-3**) from urine and feces after AS treatment; with less complexed NDMA precursors present, NDMA UFC formation was favored (Shen and Andrews, 2011b). Similar results were found with urine diluted in DDW. With the dilution factors of urine increasing from 100 to 250 and 1000-fold in DDW (i.e., DOC decreasing from 5.5 mg/L to 2.0 and 0.5 mg/L), the corresponding NDMA UFC increased from 332 ng/L to 868 and 10084 ng/L, respectively.

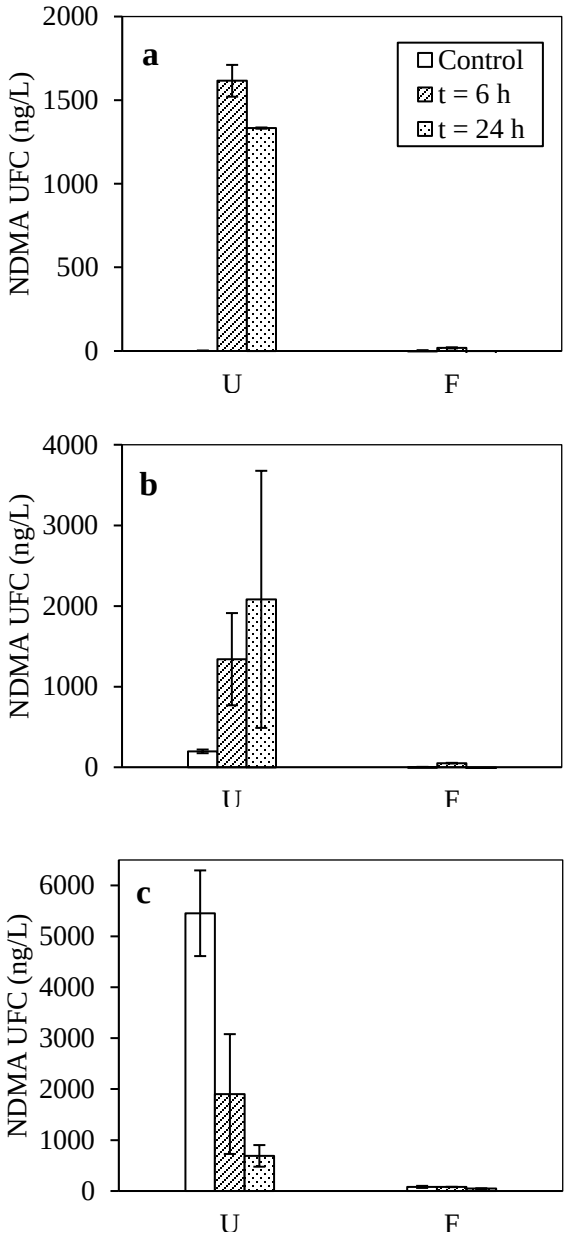



**Figure 8.7.** Removal of NDMA UFC from urine (U) and feces (F) blackwaters during 6 and 24-h treatment with (a) AS 1, (b) AS 2, and (c) AS 3.

After 6 and 24-h treatment with AS 2, NDMA UFC from urine increased from 197 ng/L before AS treatment, to 1340 and 2083 ng/L, respectively. NDMA UFC from feces increased from undetectable before AS treatment, to 52 ng/L after 6-h treatment, although decreased to undetectable after 24-h treatment with AS 2. In contrast, the NDMA UFC from urine decreased from 5454 ng/L to 1904 and 690 ng/L, respectively, after 6 and 24-h treatment with AS 3. The NDMA UFC from feces decreased from 81 ng/L to 79 and 47 ng/L, respectively. These decreases in NDMA UFC after treatment with AS 3 are likely because of the biodegradation of NDMA precursors. The removal of NDMA UFC from urine and feces blackwaters may strongly depend on the type of AS.

### *Removal of N-Nitrosamine UFC from Greywaters*

Before AS treatment, all seven *N*-nitrosamine UFC were detected (i.e., 7-36 ng/L) in laundry greywater. After treatment for 6 and 24 h with AS 1, NDMA UFC from laundry greywater increased from 11 ng/L before AS treatment, to 71 and 157ng/L, respectively (**Figure 8.8**). Similarly, after 6 and 24-h treatment with AS 2, NDMA UFC from laundry greywater increased from 11 ng/L to 165 and 253 ng/L, respectively. However, NDMA UFC from laundry greywater decreased from 11 ng/L to below the detection limit (i.e., <3 ng/L) after 6 and 24-h treatment with AS 3. While the increases in NDMA UFC after treatment with AS 1 and AS 2 were likely because of the decreased bulk organic matters (i.e., DOC; **Table D-3**) that exhibited less complexation with NDMA precursors, the decreases in NDMA UFC after treatment with AS 3 were presumably because of biodegradation of NDMA precursors. Compared to AS 1 and AS 2, AS 3 may exhibit a

higher level of activity for biodegrading NDMA precursors from laundry greywater (i.e., dyes shedding from clothes, surfactants in laundry detergent; Zeng and Mitch, 2015). Textile wastewater has been known to contain abundant (i.e., tens to hundreds of mg/L) dyes and surfactants (Yaseen and Scholz, 2019). Acclimated to textile wastewaters, AS 3 may harbor a distinct microbial community, with two abundant microbial species -*phyla Planctomycetes* (Bacteria) and *Thaumarchaeota* (Archaea) possibly playing important roles in biodegradation of NDMA precursors from laundry greywater (Meerbergen et al., 2017).

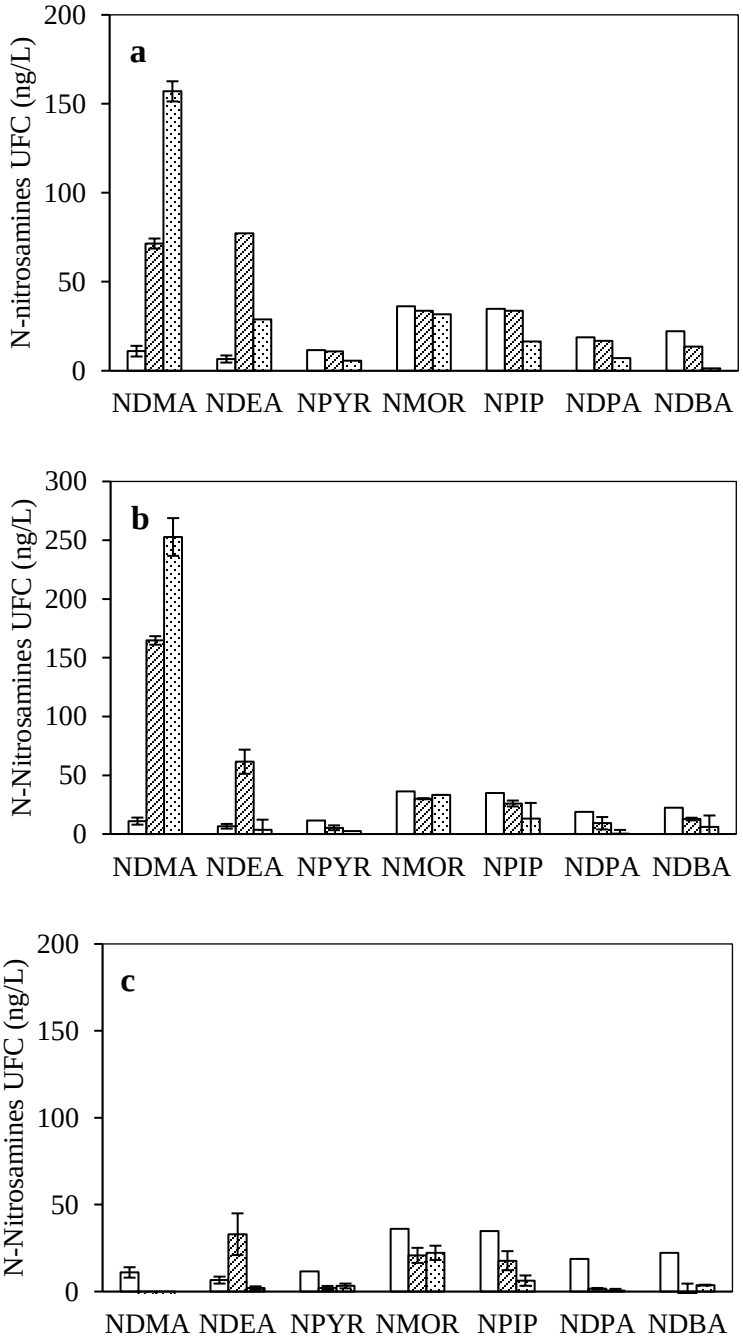



**Figure 8.8.** Removal of *N*-nitrosamine UFC from laundry greywater after 6 and 24-h treatment with (a) AS 1, (b) AS 2, and (c) AS 3.

NDEA UFC from laundry greywater increased from 7 ng/L before AS treatment, to 29-77 ng/L after 6-h and 24-h treatment with the three sources of AS (i.e., AS 1-3). Increases in NDEA UFC were likely because of the decreased DOC, which exhibited less complexation with NDEA precursors after AS treatment, and/or the formation of DEA-based biodegradation products during AS treatment of laundry greywater, which exhibited higher NDEA UFC yields than the parent compounds (**Figure D-5**). For *N*-nitrosamines other than NDMA and NDEA, their UFC all decreased after treatment with the three types of AS, among which NPYR, NDPA and NDBA UFC were relatively removable (i.e., >50%, >70% and nearly 100% removal, respectively). Increasing the incubation time from 6 to 24 h enhanced removal of NPYR, NDPA and NDBA UFC, with their UFC levels all <6 ng/L after the 24-h treatment with the three types of AS. In comparison, NPIP and NMOR UFC were relatively persistent. After 6-h treatment with AS 1 and AS 2, the NPIP UFC decreased only slightly from 35 ng/L before AS treatment, to 34 and 26 ng/L, respectively, and further to 16 and 13 ng/L after 24-h treatment, respectively. AS 3 showed relatively higher removal of NPIP UFC than AS 1 or AS 2, with NPIP UFC decreasing from 35 ng/L before AS treatment, to 18 and 6 ng/L, respectively, after 6 and 24-h treatment. NMOR UFC were even more persistent, decreasing from 36 ng/L before AS treatment, to 34, 30 and 21 ng/L after 6-h treatment with AS 1, AS 2 and AS 3, respectively. Further incubation to 24 h negligibly increased removal of NMOR UFC, which were still 32, 33 and 22 ng/L after 24-h treatment with AS 1, AS 2 and AS 3, respectively.

Before AS treatment, NDBA UFC from shower, bathroom washbasin, and dishwashing greywaters were 28, 33 and 12 ng/L, respectively. After 6 or 24-h treatment

with AS 1, AS 2 and AS 3, NDBA UFC from shower greywater decreased from 28 ng/L to undetectable, 7-13 and 3-7 ng/L, respectively (**Figure D-11**). And NDBA UFC from bathroom washbasin greywater decreased from 33 ng/L to 19-40, 6-8 and <6 ng/L, respectively. After 24-h treatment with the three types of AS, NDBA UFC from dishwashing greywater decreased to below the detection limit (i.e., <3 ng/L). These results suggest that removal of NDBA UFC during the AS process was dependent on sewage components and AS types. Before AS treatment, NPYR UFC from dishwashing greywater was 18 ng/L. After 6 and 24-h treatment with the three types of AS, NPYR UFC decreased to <4 ng/L (**Figure D-12**), suggesting that NPYR UFC from dishwashing greywater was readily removable during AS treatment.

### *Effects of Selected Factors on the Removal of N-Nitrosamine UFC*

Selected factors (i.e., addition of EEDs, diurnal variations in urine concentrations) were investigated for their potential impacts on the removal of *N*-nitrosamine UFC from selected sewage components. To evaluate the effects of adding EEDs, 144 mg/L glucose and 133 mg/L yeast extract (equivalent to 265 mg/L COD and 25 mg/L organic nitrogen) were added into AS liquor during batch treatment tests with AS 1 and AS 2. With EEDs added, NDMA UFC from urine and feces blackwaters still increased after AS treatment (**Figure 8.9**), but the increases in NDMA UFC (i.e., up to 772 ng/L) were significantly lower than those (i.e., >1333 ng/L) observed without any EEDs added (**Figure 8.7**). The addition of EEDs caused higher amounts of bulk organic matters present after AS treatment which may exhibit stronger competition with chloramines to react with NDMA precursors

(or, a possibly higher complexation between organic matters and NDMA precursors), thus causing less NDMA UFC increases than those without EEDs added.

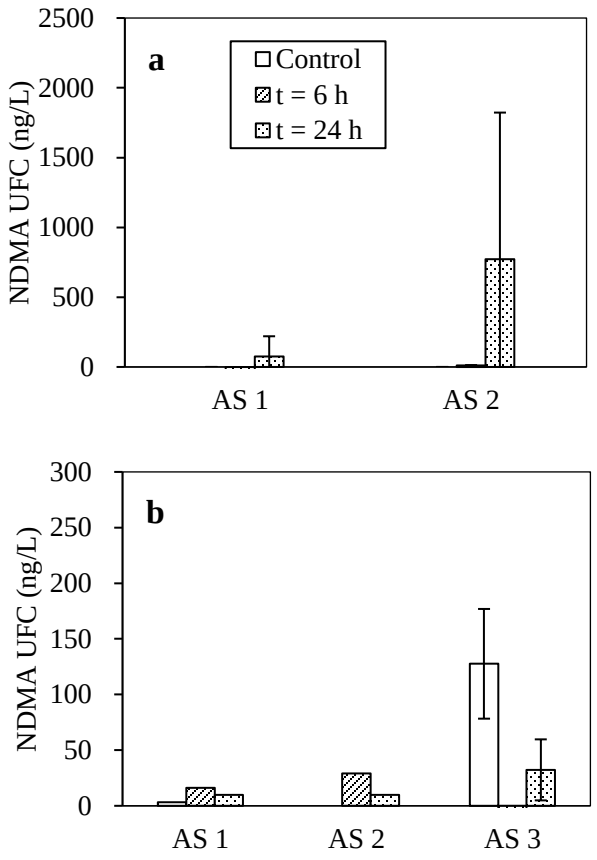



**Figure 8.9.** Removal of NDMA UFC from urine blackwater after treatment with AS 1, AS 2, and/or AS 3 (a) with an addition of EEDs, and (b) with a dilution factor of 125 times. AS 3 was not tested for NDMA UFC removal in the presence of EEDs.

With EEDs added, NDMA UFC from laundry greywater increased from 11 ng/L before AS treatment, to 175 and 228 ng/L, respectively, after 6-h treatment with AS 1 and AS 2 (**Figure D-13**). After 24-h treatment, NDMA UFC increased to 74 and 191 ng/L, respectively. These increases in NDMA UFC were generally similar to those observed

without any EEDs added. Different from urine or feces blackwaters containing limited amounts of bulk organic matters (i.e., <6 mg/L DOC), laundry greywater has a relatively high DOC (i.e., 26 mg/L), and adding EEDs may thus cause less impacts on its NDMA UFC before or after AS treatment. NDEA UFC from laundry greywater also exhibited comparable increases after AS treatment with and without EEDs added. However, the UFC of other *N*-nitrosamines (not including NDMA and NDEA) all decreased after treatment with AS 1 or AS 2, and their removal could be different with and without EEDs added. After 6-h treatment with AS 1, removal of *N*-nitrosamine UFC (not including NDMA and NDEA) were higher with EEDs added, while after 6-h treatment with AS 2, removal of *N*-nitrosamine UFC was lower with EEDs added (**Figure 8.10**). Adding EEDs is considered to have two distinct effects on biodegradation efficiencies of trace organic compounds (such as *N*-nitrosamine precursors), including (i) increasing their removal rates via promoting the production of reductive cofactors (e.g., NADH) that are required for the oxidization of target contaminants, and (ii) decreasing the removal rates of target contaminants by competing for available binding sites on enzymes. Such inconsistent effects of adding EEDs may thus cause different changes of *N*-nitrosamine UFC removal after EEDs were added, likely depending on AS types (i.e., AS 1 vs AS 2).

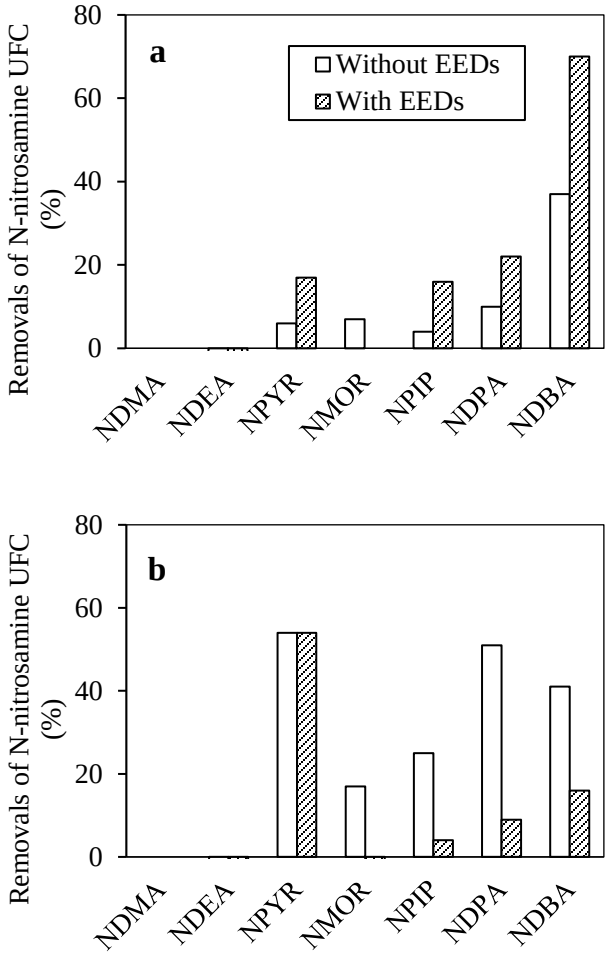



**Figure 8.10.** Removal of *N*-nitrosamine UFC from laundry greywater without and with an addition of EEDs after 6-h treatment with (a) AS 1, and (b) AS 2.

Urine may have different dilution factors (e.g., 100-300 times) in domestic wastewaters, varying with diurnal urine production and wastewater flows (Henze et al., 2008; Friedler et al., 2013). The different dilution factors caused inconsistent amounts of bulk organic matters present during AS treatment, may impact NDMA UFC removal from urine. With a low dilution factor (i.e., 125 times), NDMA UFC from urine increased only slightly from undetectable before AS treatment, to 16, 29 and <3 ng/L after 6-h treatment

with AS 1, AS 2 and AS 3, respectively, or to 10, 10 and 32 ng/L, respectively, after 24-h treatment (**Figure 8.9**). These increases in NDMA UFC were significantly lower than those (i.e., >1333 ng/L) found under a high dilution factor (i.e., 250 times) of urine, probably because the higher amounts of bulk organic matters present with a smaller dilution factor of urine inhibited NDMA UFC more strongly after AS treatment.

## Conclusions

Among the sewage components tested, urine blackwater was the major source of NDMA, NPYR and NMOR FP in raw domestic sewage (i.e., before AS treatment), and laundry greywater was the major source of NDEA, NPIP, NDPA and NDBA FP. After treatment with three type of AS (i.e., domestic rural, domestic urban, and textile AS), urine blackwater was still the major source of NDMA and NPYR FP in secondary effluents, and laundry greywater was the major source of other *N*-nitrosamine (NDEA, NPIP, NMOR, NDPA and NDBA) FP. Removing urine blackwater and laundry greywater (i.e., via urine diversion and laundry greywater separation systems) is one approach for reducing *N*-nitrosamine precursors from wastewater effluents.

Among the three types of AS (i.e., domestic rural, domestic urban, and textile AS) tested, AS 1 exhibited higher removal of *N*-nitrosamine FP from sewage components containing biologically originated organic matters (i.e., human urine, human sweat, food leachates). The textile AS exhibited higher removal of *N*-nitrosamine FP from sewage components containing personal care products (i.e., shampoo, body wash), laundry and dishwashing detergents. Increasing incubation time from 6 to 24 h enhanced the removal

of *N*-nitrosamine FP from all sewage components. At WWTPs, increasing HRT may thus favor the removal of *N*-nitrosamine precursors from secondary effluents.

Among the seven *N*-nitrosamines examined, NDMA and NDEA precursors were relatively persistent during AS treatment of sewage components, likely because of the diverse sources and components of NDMA and NDEA precursors present in wastewaters. Different from NDMA and NDEA precursors which could have diverse sources, morpholine is the only NMOR precursor identified so far (Glover et al., 2019). Although morpholine is biodegradable, NMOR FP from sewage components were still persistent because it was present in wastewaters mainly in the form of NMOR rather than its precursor (i.e., morpholine). NMOR is bio-refractory during biological treatment. In contrast, NPYR, NPIP, NDPA and NDBA precursors were readily removable. NDMA and NPYR were major *N*-nitrosamine species after AS treatment of wastewater influents, followed by NMOR and NDEA. Because NDMA and NDEA have lower $10^{-6}$ cancer risk levels (i.e., 0.7 and 0.2 ng/L, respectively) than other *N*-nitrosamines (i.e., 3-15 ng/L), they may require more attentions for the removal during the AS process. The potential correlations between NDMA or NDEA FP removal and the removal of ammonia from wastewater influents may suggest a potential role of nitrification affecting the removal of NDMA and NDEA FP during the AS process.

After adding EEDs or deceasing the dilution factors of urine, the amounts of bulk organic matters increased, and NDMA UFC were lowered after AS treatment. However, adding EEDs may have negligible effects on NDMA UFC removal and NDEA UFC removal from laundry greywater, and decrease the removal of other *N*-nitrosamine UFC

(not including NDMA and NDEA) from laundry greywater. In general, addition of EEDs (or biostimulation) may not be effective to enhance the removal of *N*-nitrosamine UFC from sewage components. After the DOC of secondary effluents is further reduced via dilutions in receiving water body, NDMA UFC from wastewater effluents may further increase due to enhanced removal of DOC. Further, secondary wastewater effluents could be further purified through advanced treatment trains (such as membrane filtration), during which DOC was removed, and NDMA UFC may thus increase.

Urine blackwater is the predominant source of NDMA UFC in AS-treated effluents, based on its estimated volume fractions in domestic wastewater, and laundry greywater is the major source of other *N*-nitrosamine UFC (not including NDMA). Increasing the incubation time (or HRT) of AS treatment could generally increase NDMA UFC removal from urine and *N*-nitrosamine UFC removal from laundry greywater. Strategies to increase HRT (i.e., extended aeration, biofiltration) may thus be helpful improving the removal of *N*-nitrosamine UFC from wastewaters. Among the three AS tested, the textile AS exhibited the highest deactivation efficiencies of *N*-nitrosamine precursors from laundry greywater, probably because textile AS harbors a higher population of microorganisms actively biodegrading *N*-nitrosamine precursors (i.e., synthetic dyes and surfactants) from laundry greywater. Bioaugmentation with such microorganisms from textile AS may provide a potential strategy to enhance the removal of *N*-nitrosamines UFC from wastewaters, which requires further investigation.

# CHAPTER IX

# CONCLUSIONS AND RECOMMENDATIONS

## Conclusions

***Objective 1:*** ***To examine the formation of N-nitrosamines from different sewage components (i.e., blackwaters and greywaters) under the UFC and FP tests.***

- Even diluted in tap water for 250 times, NDMA yields from human urine were still relatively high (i.e., >10000 ng/L) under the FP test, but could be negligible (i.e., <3 ng/L) under the UFC test.
- Under either the FP or UFC test, laundry greywater was found to be a main contributor to most *N*-nitrosamine (not including NDMA) precursors.

***Objective 2:*** ***To investigate (i) the effects of selected factors (i.e., pH, $Br^-$, DOC, $SUVA_{254}$) on NDMA UFC from model compounds and surface waters, (ii) effects of the selected factors on NDMA FP, and (iii) potential correlations between NDMA UFC and FP.***

- All the tested model compounds yielded peak NDMA UFC at pH 6.8-7.8, while peak NDMA FP were achieved at higher pH than NDMA UFC.
- In surface waters with a higher DOC or $SUVA_{254}$, NDMA UFC tended to be lower.
- While NDMA UFC decreased with increasing DOC, NDMA FP tended to increase with increasing DOC in surface waters.
- The effects of a high level (i.e., 1000 µg/L) of $Br^-$ on NDMA UFC from model compounds strongly depended on pH.

- In the tested surface waters, NDMA UFC were poorly correlated with NDMA FP. However, NDMA UFC/DOC was significantly correlated with NDMA FP/DOC.

***<u>Objective 3:</u> To determine (i) the deactivation efficiencies of model NDMA precursors during the AS process, (ii) the factors (i.e., AS sources, incubation time or HRT, SRT of AS, seasonal variations in AS activity) that influence the removal of model NDMA precursors, (iii) the different deactivation pathways (i.e., biodegradation, biosorption and volatilization) of NDMA precursors, and (iv) the roles of biostimulation and non-specific oxygenase affecting the deactivation efficiencies of model NDMA precursors.***

- TMA and MNCL were readily removable during the AS process, while RNTD and SMTR were more persistent, likely because of the bio-refractory functional groups (i.e., -$NO_2$, -NH-$SO_2$-) in RNTD and SMTR.
- Compared to the textile and lab-grown AS, the municipal AS showed higher deactivation efficiencies of the tested NDMA precursors, likely because of their distinct microbial community structures.
- Increasing incubation time (or HRT) and SRT could enhance the deactivation efficiency of RNTD, while increasing HRT negligibly affected the removal of TMA, MNCL or SMTR.
- The rural domestic AS (AS 1) collected in summer showed higher biodegradation efficiencies of RNTD than that in spring, and the lab-grown AS with an 8-d SRT showed higher biodegradation rates of RNTD than that with a 24-d SRT.

- Biosorption played a key role deactivating the NDMA precursors with large molecular weights (i.e., the three amine-based pharmaceuticals tested), while biodegradation was the major deactivation pathway of TMA.
- Adding selected biostimulants (i.e., benzoate, ammonia, glucose and ammonia, acetate and ammonia, ethanol and ammonia) insignificantly influenced the removal of NDMA FP from RNTD, and the selected non-specific oxygenase (i.e., phenol 2-monooxygenase) played an insignificant role enhancing the removal of NDMA FP from RNTD.

- ***<u>Objective 4:</u> To evaluate (i) the deactivation efficiencies of N-nitrosamine precursors from different sewage components and WWTP influents; (ii) the differences in deactivation efficiencies measured under the UFC and FP tests, and (iii) the effects of biostimulation (i.e., adding EEDs) and other selected factors (i.e., AS types, incubation time or HRT) on the deactivation efficiencies of N-nitrosamine precursors.***
  - *N*-Nitrosamine FP from sewage components generally decreased after treatment with three types of AS (i.e., domestic rural, domestic urban, and textile AS), except that NDMA FP and NDEA FP from laundry greywater increased after AS treatment. These increases were likely attributed to the formation of DMA and DEA-based biodegradation products.
  - The rural domestic AS (AS 1) exhibited higher removal of *N*-nitrosamine FP from the sewage components which contain biologically originated organic matters (e.g., human urine, human sweat, food leachates), while the textile AS exhibited higher

removal of *N*-nitrosamine FP from the sewage components containing surfactants or personal care products (i.e., laundry and dishwashing detergents, shampoo, body wash).

- Urine blackwater was the predominant source of NDMA and NPYR precursors in domestic sewage and secondary effluents, while laundry greywater was the major source of most of the other *N*-nitrosamine (i.e., NDEA, NPIP, NDPA and NDBA) FPs.
- Different from NDMA FP, NDMA UFC from most sewage components increased after 6 or 24-h AS treatment, presumably because the lower amounts of bulk organic matters after AS treatment exhibited less competition with $NHCl_2$ to react with NDMA precursors (or, less complexations between organic matters and NDMA precursors), which favored NDMA UFC.
- Adding EEDs (i.e., glucose and yeast extract) negligibly affected the removal of NDMA FP from different sewage components. However, NDMA UFC from urine and feces blackwaters exhibited less increases after AS treatment in the presence of EEDs.

## Recommendations for Practical Applications

- The occurrences of *N*-nitrosamine precursors are distinct among different sewage components. Urine blackwater was the predominant contributor to NDMA FP in domestic sewage and secondary effluents, while laundry greywater was the most important contributor to most of the other *N*-nitrosamine FPs. Strategies for controlling the discharge of urine blackwater and laundry greywater, such as via urine separation system and/or laundry greywater non-potable reuse management, would lead to decreases in *N*-nitrosamine precursors' levels in wastewater influents, and reduce their occurrences in secondary effluents.
- The effects of different factors on NDMA UFC were distinct from that on NDMA FP in surface waters. NDMA UFC tends to be lower in waters with higher DOC or $SUVA_{254}$, although NDMA FP could increase with increasing DOC. In treated surface waters, NDMA UFC could be comparable or even higher than that in raw waters. The presence of NOM can reduce NDMA UFC in surface waters via complexation with NDMA precursors and thus inhibiting the reactions between chloramines and NDMA precursors. Water utilities may need to reconsider the roles of NOM and strike a balance between the removal of NOM and NDMA UFC in finished waters.
- The biodegradability of NDMA precursors may depend on compound structures. Compounds containing bio-refractory groups could be relatively persistent during AS treatment. Especially, strategies for controlling the discharge of compounds with those bio-refractory groups may lead to decreases in NDMA formation in

secondary effluents. Increasing HRT or SRT may favor the removal of such compounds at WWTPs.

- *N*-nitrosamine UFC exhibited distinct trends from *N*-nitrosamine FP during AS treatment of sewage components. Under the practical chloramination condition (i.e., UFC), the formation of *N*-nitrosamines could be higher in secondary effluents than in influents, and possibly, further higher in tertiary effluents after advanced wastewater treatment for potable reuse. The enhanced removal of bulk organic matters (e.g., DOC) during advanced treatment may favor *N*-nitrosamine UFC in effluents. Wastewater reuse facilities may need to pay attention to *N*-nitrosamine formation during each step of treatment process to minimize the increases in *N*-nitrosamine UFC in reused wastewaters.
- Bioaugmentation could be a promising strategy for WWTPs to control *N*-nitrosamine precursors in secondary effluents. Different types of AS have shown distinct removals of *N*-nitrosamine precursors from sewage components and wastewater influents. Manipulating microbial communities in AS, such as via bioaugmentation, may thus help with an effective reduction of *N*-nitrosamine precursors. Although biostimulation can help shaping the microbial community structures in AS, its effects might be limited based on current results, likely because the tested biostimulants insignificantly promoted the growth of microbial species attributable to the biodegradation of *N*-nitrosamine precursors.

## Recommendations for Future Research

- Screening tests of a wide range of bacterial strains can be used to test the biodegradability of different *N*-nitrosamine precursors for the selection of suitable microbial species for potential bioaugmentation applications.
- Quantitative structure-biodegradability relationship (QSBR) model can be developed to assess the biodegradability of different *N*-nitrosamine precursors to minimize experimental tests.
- Further research is needed to identify the intermediate products that can be formed during the biodegradation of *N*-nitrosamine precursors and to determine the according biodegradation pathways.
- Isotope (e.g., $^{14}C$) labelling and metagenome sequencing techniques can be used to identify the key enzymes or genomes attributable for the biodegradation of target *N*-nitrosamine precursors.
- Biosorption could have an important effect on the removal of *N*-nitrosamine precursors. The interactions of *N*-nitrosamine precursors with the surface functional groups of AS flocs can be investigated to gain further insight.

# APPENDICES

# Appendix A

**Table A-1.** Removal of *N*-nitrosamine precursors during biological wastewater treatment.

| WWTP location | Number of WWTPs | Influent type | Biological treatment process | Model *N*-nitrosamine precursors | Concentration in influents (µg/L) | | Removal (%) | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Mean | Range | Mean | |
| Canada | 6 | N.A. | Multiple | 4-Epianhydrochlortetracycline (4-EACTC) | N.A. | <0.08 | N.A. | N.A. | Guerra et al., 2014 |
| Canada | 6 | N.A. | Multiple | 4-Epianhydrotetracycline (4-EATC) | N.A. | <0.4 | N.A. | N.A. | Guerra et al., 2014 |
| Canada | 6 | N.A. | Multiple | 4-Epichlortetracycline (4-ECTC) | N.A. | <0.08 | N.A. | N.A. | Guerra et al., 2014 |
| Canada | 6 | N.A. | Multiple | 4-Epioxytetracycline (4-EOTC) | N.A. | <0.04 | N.A. | N.A. | Guerra et al., 2014 |
| Canada | 6 | N.A. | Multiple | 4-Epitetracycline (4-ETC) | 0.03-0.12 | 0.07 | N.A. | N.A. | Guerra et al., 2014 |
| UK | 1 | Municipal | Biofiltration (BAF) | | 0.31-5.14 | 1.25 | N.A. | 70 | Kasprzyk-Hordern et al., 2009 |
| UK | 1 | Municipal /industrial | CAS | Amitriptyline (AT) | 0.50-6.71 | 2.09 | N.A. | 100 | Kasprzyk-Hordern et al., 2009 |
| Germany | 1 | N.A. | A/O + chemical P removal | | 0.05-0.1 | 0.08 | N.A. | 15[a] | Gurke et al., 2015 |
| Canada | 6 | N.A. | Multiple | Anhydrochlortetracycline (ACTC) | N.A. | <0.2 | N.A. | N.A. | Guerra et al., 2014 |
| Canada | 6 | N.A. | Multiple | Anhydrotetracycline (ATC) | N.A. | <0.09 | N.A. | N.A. | Guerra et al., 2014 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Spain | 5 | Municipal | CAS | | N.D.-0.30 | 0.15 | N.A. | 37 | Gros et al., 2006 |
| Switzerland | 2 | Municipal | CAS | | 0.08-0.32 | 0.15 | N.A. | 7 | Gobel et al., 2005 |
| Singapore | 1 | Municipal | CAS | | 1.54-2.95 | 1.95 | N.A. | 76 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | | 1.54-2.95 | 1.95 | N.A. | 92 | Tran et al., 2016 |
| Switzerland | 2 | Municipal | CAS | | N.A. | N.A. | N.A. | <0-55 | Gobel et al., 2007 |
| US | 1 | Municipal | CAS | | 1.3 (max) | N.A. | N.A. | 48 | Blair et al., 2018 |
| South Africa | 5 | Municipal/ industrial | A/A/O and MLE | | 0.02-0.14 | N.A. | N.A. | -150 | Archer et al., 2017 |
| China | 13 | Multiple | Multiple | | 0.001-1.69 | 0.35 | N.A. | 70 | Ben et al., 2018 |
| China | 4 | Municipal | A/A/O | Azithromycin (AZM) | 0.24-0.27 | 0.26 | N.A. | 67 | Yan et al., 2014 |
| Canada | 1 | CAS | Multiple | | N.A. | 0.064 | N.A. | -42 | Guerra et al., 2014 |
| Canada | 1 | CAS | Multiple/ industrial | | N.A. | 0.034 | N.A. | 29 | Guerra et al., 2014 |
| Croatia | 1 | Municipal /industrial | CAS | | 0.12-1.63 | 0.50 | N.A. | 30 | Senta et al, 2013 |
| Spain | 1 | Municipal /hospital | Tricking filter (TF) | | 0.080-0.30 | 0.19 | <0-67 | 6 | Santos et al., 2013 |
| Spain | 1 | N.A. | N.A. | | N.A. | 0.21 | N.A. | 17 | Gros et al., 2012 |
| Spain | 1 | N.A. | N.A. | | N.A. | 0.044 | N.A. | 30 | Gros et al., 2012 |
| Japan | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | <0 | Yasojima et al., 2006 |
| Japan | 1 | N.A. | CAS | | N.A. | 0.20 | N.A. | <0 | Yasojima et al., 2006 |

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|---|---|---|---|---|---|---|---|---|---|
| Japan | 1 | N.A. | CAS | | N.A. | 0.13 | N.A. | 15 | Yasojima et al., 2006 |
| Italy | 1 | N.A. | CAS | | N.A. | 0.12 | N.A. | <0 | Verlicchi et al., 2014 |
| China | 1 | Hospital | CAS | Chlorpromazine (CPZ) | N.A. | 0.22 | N.A. | 54 | Yuan et al., 2013 |
| China | 1 | Municipal | CAS | | N.A. | N.D. | N.A. | N.A. | Yuan et al., 2013 |
| Singapore | 1 | Municipal | CAS | | 2.33-15.91 | 6.43 | N.A. | 73 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | | 2.33-15.91 | 6.43 | N.A. | 87 | Tran et al., 2016 |
| China | 14 | Multiple | Multiple | | 0.001-0.04 | 0.005 | N.A. | 18 | Ben et al., 2018 |
| China | 8 | N.A. | Multiple | Chlortetracycline (CTC) | N.A. | N.D.-0.09 | N.A. | 94-100[d] | Zhang et al., 2018 |
| Canada | 6 | N.A. | Multiple | | N.A. | <0.03 | N.A. | N.A. | Guerra et al., 2014 |
| South Korea | 1 | Livestock | A/O | | N.A. | 4.0 | N.A. | 0 | Kim et al., 2013 |
| US | 1 | N.A. | CAS | | N.A. | 0.12 | N.A. | 56 | Gao et al., 2012 |
| Spain | 4 | Municipal | CAS | | N.A. | N.A. | N.A. | 0 | Castiglioni et al., 2006 |
| Switzerland | 11 | N.A. | CAS | | 0.5 | 0.25 | N.A. | 50 | Hollender et al., 2009 |
| Singapore | 1 | Municipal | CAS | Clarithromycin (CLA) | 1.20-1.85 | 1.50 | N.A. | 64 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | | 1.20-1.85 | 1.50 | N.A. | 72 | Tran et al., 2016 |
| Switzerland | 2 | Municipal | CAS | | 0.16-0.44 | 0.33 | N.A. | 21 | Gobel et al., 2005 |

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|---|---|---|---|---|---|---|---|---|
| Switzerland | 2 | Municipal | CAS | N.A. | N.A. | N.A. | <0-20 | Gobel et al., 2007 |
| German | 12 | Municipal | Batch AS test | N.A. | N.A. | N.A. | 80 | Krah et al., 2016 |
| US | 1 | Municipal | CAS | 8.1 (max) | N.A. | N.A. | -73 | Blair et al., 2018 |
| South Africa | 5 | Municipal /industrial | A/A/O and MLE | 0.26-1.54 | N.A. | N.A. | 30 | Archer et al., 2017 |
| China | 14 | Multiple | Multiple | 0.005-0.55 | 0.19 | N.A. | 81 | Ben et al., 2018 |
| China | 8 | N.A. | Multiple | N.A. | 0.37-0.66 | N.A. | 43-100[d] | Zhang et al., 2018 |
| Croatia | 1 | Municipal /industrial | CAS | 0.11-0.30 | 0.20 | N.A. | 64 | Senta et al, 2013 |
| Spain | 1 | Municipal /hospital | TF | N.D.-0.052 | 0.022 | <0-55 | <0 | Santos et al., 2013 |
| Spain | 1 | N.A. | N.A. | N.A. | 0.46 | N.A. | 58 | Gros et al., 2012 |
| Spain | 1 | N.A. | N.A. | N.A. | 0.055 | N.A. | 65 | Gros et al., 2012 |
| US | 1 | Municipal /industrial | N.A. | N.D.-0.72 | 0.28 | 16-34 | 18 | Spongberg and Witter, 2008 |
| Japan | 1 | N.A. | CAS | N.A. | 0.42 | N.A. | 17 | Yasojima et al., 2006 |
| Japan | 1 | N.A. | CAS | N.A. | 0.50 | N.A. | 30 | Yasojima et al., 2006 |
| Japan | 1 | N.A. | CAS | N.A. | 0.70 | N.A. | 60 | Yasojima et al., 2006 |
| Japan | 1 | N.A. | CAS | N.A. | 0.48 | N.A. | 27 | Yasojima et al., 2006 |
| Japan | 1 | N.A. | CAS | N.A. | 0.50 | N.A. | 24 | Yasojima et al., 2006 |

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|---|---|---|---|---|---|---|---|---|---|
| Japan | 1 | N.A. | CAS | | N.A. | 0.78 | N.A. | 42 | Yasojima et al., 2006 |
| Italy | 1 | N.A. | CAS | | N.A. | 0.20 | N.A. | <0 | Verlicchi et al., 2014 |
| China | 1 | Hospital | CAS | Clomipramine (CLI) | N.A. | 0.061 | N.A. | 43 | Yuan et al., 2013 |
| China | 1 | Municipal | CAS | | N.A. | N.D. | N.A. | N.A. | Yuan et al., 2013 |
| Canada | 6 | N.A. | Multiple | Demeclocycline (DEM) | N.A. | <0.07 | N.A. | N.A. | Guerra et al., 2014 |
| US | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | N.A. | Gao et al., 2012 |
| South Africa | 5 | Municipal /industrial | A/A/O and MLE | Desvenlafaxine (DVS) | N.D.-0.32 | N.A. | N.A. | -25 | Archer et al., 2017 |
| Canada | 1 | N.A. | A/A/O | | 3.09-3.33 | N.A. | 3-6 | N.A. | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | BAF | | 1.85-3.28 | N.A. | 10-16 | N.A. | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | TF | | N.A. | 2.85 | N.A. | 6 | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | CAS | | N.A. | 1.86 | N.A. | 29 | Lajeunesse et al., 2012 |
| Israel | 1 | N.A. | CAS | | 1.13-1.50 | N.A. | 49-93 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.075-0.11 | N.A. | <0 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.10-0.19 | N.A. | <0 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.12-0.37 | N.A. | <0-3 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.29-0.23 | N.A. | <0-22 | N.A. | Gasser et al., 2012 |

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|---|---|---|---|---|---|---|---|---|---|
| Israel | 1 | N.A. | CAS | | 0.17-0.21 | N.A. | <0-24 | N.A. | Gasser et al., 2012 |
| Singapore | 1 | Municipal | CAS | Diethyltoluamide (DEET) | 1.81-2.41 | 2.34 | N.A. | 94 | Tran and Gin, 2017 |
| Singapore | 1 | Municipal | MBR | | 1.81-2.41 | 2.34 | N.A. | 88 | Tran and Gin, 2017 |
| China | 8 | N.A. | Multiple | | N.A. | 0.05-1.36 | N.A. | 55-94[d] | Zhang et al., 2018 |
| UK | 1 | Municipal | BAF | Diltiazem (DTZ) | 0.23-3.21 | 0.77 | N.A. | 35 | Kasprzyk-Hordern et al., 2009 |
| UK | 1 | Municipal /industrial | CAS | | 0.41-5.26 | 1.56 | N.A. | 75 | Kasprzyk-Hordern et al., 2009 |
| US | 1 | Municipal | CAS | | 0.26 (max) | N.A. | N.A. | 13 | Blair et al., 2018 |
| US | 1 | Municipal | CAS | Diphenhydramine (DPH) | 0.08 (max) | N.A. | N.A. | 91 | Blair et al., 2018 |
| Australia | 1 | N.A. | N.A. | | N.A. | 0.04 | N.A. | 27 | Roberts et al., 2016 |
| Spain | | Municipal /industrial | A/A/O | Diuron (DRN) | 0.03-0.20 | 0.11 | N.A. | 62 | Rosal et al., 2010 |
| Germany | 1 | N.A. | A/O + chemical P removal | Doxepin (DXP) | 0.06-0.10 | 0.09 | N.A. | 9[a] | Gurke et al., 2015 |
| China | 14 | Multiple | Multiple | Doxycycline (DOX) | 0.001-0.024 | 0.006 | N.A. | 75 | Ben et al., 2018 |
| Canada | 6 | N.A. | Multiple | | 0.02-0.08 | 0.04 | N.A. | N.A. | Guerra et al., 2014 |
| US | 1 | N.A. | CAS | | N.A. | 0.44 | N.A. | <0 | Gao et al., 2012 |

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|---|---|---|---|---|---|---|---|---|---|
| UK | 1 | Municipal | BAF | | 0.24-6.76 | 1.61 | N.A. | -100 | Kasprzyk-Hordern et al., 2009 |
| UK | 1 | Municipal /industrial | CAS | | 0.14-10.03 | 2.53 | N.A. | 50 | Kasprzyk-Hordern et al., 2009 |
| Spain | | Municipal /industrial | A/A/O | | N.D.-2.31 | 0.35 | N.A. | 4.3 | Rosal et al., 2010 |
| Spain | 5 | Municipal | CAS | | N.D. | N.D. | N.A. | N.A. | Gros et al., 2006 |
| Italy | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | N.A. | Verlicchi et al., 2014 |
| US | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | N.A. | Gao et al., 2012 |
| Spain | | Municipal | CAS | | N.A. | N.A. | N.A. | 0 | Castiglioni et al., 2006 |
| Spain | 1 | Municipal /industrial | CAS | Erythromycin·$H_2O$ (ERY) | 0.09-0.25 | 0.15 | N.A. | 24 | Radjenovic et al., 2007 |
| Spain | 1 | Municipal /industrial | MBR | | 0.09-0.25 | 0.15 | N.A. | 67 | Radjenovic et al., 2007 |
| Spain | 9 | Municipal /industrial | A/O | | 0.32-2.7 | 0.82 | N.A. | 35±50 | Radjenovic et al., 2009 |
| Spain | 9 | Municipal /industrial | Flat sheet MBR | | 0.32-2.7 | 0.82 | N.A. | 43±52 | Radjenovic et al., 2009 |
| Spain | 9 | Municipal /industrial | Hollow fiber MBR | | 0.32-2.7 | 0.82 | N.A. | 25±109 | Radjenovic et al., 2009 |
| Lab test | 7 | Synthetic feeding | Nitrification | | 80-320 | N.A. | N.A. | 55-95 | Fernandez-Fontaina et al., 2012, 2014 |
| Singapore | 1 (4) | Municipal | CAS | | 0.11-0.40 | 0.27 | N.A. | 64 | Tran et al., 2016 |
| Singapore | 1 (4) | Municipal | MBR | | 0.11-0.40 | 0.27 | N.A. | 57 | Tran et al., 2016 |

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|---|---|---|---|---|---|---|---|---|
| Switzerland | 2 | Municipal | CAS | 0.04-0.19 | 0.08 | N.A. | 0 | Gobel et al., 2005 |
| Switzerland | 2 | Municipal | CAS | N.A. | N.A. | N.A. | <0-6 | Gobel et al., 2007 |
| German | 12 | Municipal | Batch AS test | N.A. | N.A. | N.A. | 50 | Krah et al., 2016 |
| Lab test | N.A. | Synthetic feeding | CAS | N.A. | N.A. | N.A. | 75 | Alvarino et al., 2014, 2018 |
| China | 14 | Multiple | Multiple | 0.001-1.15 | 0.22 | N.A. | 81 | Ben et al., 2018 |
| China | 4 | Municipal | A/A/O | 0.27-0.29 | 0.26 | N.A. | 44 | Yan et al., 2014 |
| China | 8 | N.A. | Multiple | N.A. | 0.25-0.41 | N.A. | 51-100[d] | Zhang et al., 2018 |
| Greek | 4 | Municipal /hospital /industrial | Nitrification/ denitrification | N.D.-0.32 | 0.07 | N.A. | N.A. | Papageorgiou et al., 2016 |
| Canada | 1 | Multiple | CAS | N.A. | 0.028 | N.A. | -4 | Guerra et al., 2014 |
| Canada | 1 | Multiple/ industrial | CAS | N.A. | 0.004 | N.A. | -17 | Guerra et al., 2014 |
| Croatia | 1 | Municipal /industrial | CAS | 0.025-0.073 | 0.044 | N.A. | 18 | Senta et al, 2013 |
| Spain | 1 | Municipal /industrial | TF | 0.010-0.22 | 0.093 | <0-39 | 21 | Santos et al., 2013 |
| Spain | 1 | N.A. | N.A. | N.A. | 0.063 | N.A. | 73 | Gros et al., 2012 |
| Spain | 1 | N.A. | N.A. | N.A. | 0.035 | N.A. | 60 | Gros et al., 2012 |
| US | 1 | N.A. | CAS | N.A. | 0.48 | N.A. | 44 | Karthikeyan and Meyer, 2006 |

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|---|---|---|---|---|---|---|---|---|---|
| US | 1 | N.A. | CAS | | 0.43-1.2 | 0.82 | 75-79 | 76 | Karthikeyan and Meyer, 2006 |
| Canada | 6 | N.A. | Multiple | Isochlortetracycline (ICTC) | N.A. | <0.03 | N.A. | N.A. | Guerra et al., 2014 |
| Singapore | 1 | Municipal | CAS | Meropenem (MEM) | 0.26-0.43 | 0.32 | N.A. | 85 | Tran et al., 2016 |
| US | 1 | Municipal | CAS | | 99 (max) | N.A. | N.A. | 99 | Blair et al., 2015 |
| Singapore | 4 | Municipal | MBR | Metformin (MET) | 0.26-0.43 | 0.32 | N.A. | 86 | Tran et al., 2016 |
| South Africa | 5 | Municipal /industrial | A/A/O and MLE | | 3.59-9.23 | N.A. | N.A. | 95 | Archer et al., 2017 |
| Singapore | 1 | Municipal | CAS | | 0.73-3.81 | 1.23 | N.A. | 38 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | Minocycline (MNCL) | 0.73-3.81 | 1.23 | N.A. | 100 | Tran et al., 2016 |
| Canada | 6 | N.A. | Multiple | | N.A. | <0.24 | N.A. | N.A. | Guerra et al., 2014 |
| China | 1 | N.A. | CAS | Octyl dimethyl-p-aminobenzoic acid (OD-PABA) | 0.043-0.14 | N.A. | 27-31 | N.A. | Tsui et al., 2014 |
| China | 1 | N.A. | CAS | | 0.074-0.35 | N.A. | 27-31 | N.A. | Tsui et al., 2014 |
| Singapore | 1 | Municipal | CAS | | 1.63-30.05 | 4.89 | N.A. | 70 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | | 1.63-30.05 | 4.89 | N.A. | 92 | Tran et al., 2016 |
| China | 7 | Multiple | Multiple (AS, OD, A/A/O) | Oxytetracycline (OTC) | 0.04-0.4 | 0.16 | N.A. | 85 | Wang et al., 2018 |
| China | 7 | Municipal | A/A/O | | 0.08-0.39 | 0.29 | N.A. | 92 | Ashfaq et al., 2017 |
| China | 14 | Multiple | Multiple | | 0.004-0.63 | 0.11 | N.A. | 97 | Ben et al., 2018 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Canada | 6 | N.A. | Multiple | | N.A. | <0.03 | N.A. | N.A. | Guerra et al., 2014 |
| Luxembourg | 1 | N.A. | BAF | | N.D.-0.007 | N.A. | N.A. | N.A. | Pailler et al., 2009 |
| South Korea | 1 | Livestock | A/O | | N.A. | 0.8 | N.A. | <0 | Kim et al., 2013 |
| US | 1 | N.A. | CAS | | N.A. | 0.026 | N.A. | 23 | Gao et al., 2012 |
| Lab test | 7 | Synthetic feeding | Nitrification | Roxithromycin (ROX) | 80-320 | N.A. | N.A. | 50-95 | Fernandez-Fontaina et al., 2012, 2014 |
| Switzerland | 2 | Municipal | CAS | | 0.01-0.05 | 0.02 | N.A. | 0 | Gobel et al., 2005 |
| Switzerland | 2 | Municipal | CAS | | N.A. | N.A. | N.A. | <0-38 | Gobel et al., 2007 |
| Lab test | N.A. | Synthetic feeding | CAS | | N.A. | N.A. | N.A. | 82 | Alvarino et al., 2014, 2018 |
| US | 1 | Municipal | CAS | | 0.12 | N.A. | N.A. | 81 | Blair et al., 2018 |
| China | 14 | Multiple | Multiple | | 0.039-1.04 | 0.41 | N.A. | 73 | Ben et al., 2018 |
| China | 4 | Municipal | A/A/O | | 0.34-0.44 | 0.39 | N.A. | 20 | Yan et al., 2014 |
| China | 8 | N.A. | Multiple | | N.A. | 0.22-0.46 | N.A. | 43-100[d] | Zhang et al., 2018 |
| Greek | 4 | Municipal /hospital /industrial | Nitrification/ denitrification | | N.D. | 0.05 | N.A. | N.A. | Papageorgiou et al., 2016 |
| Canada | 6 | N.A. | Multiple | | 0.001-0.004 | 0.002 | N.A. | N.A. | Guerra et al., 2014 |
| Italy | 1 | N.A. | CAS | | N.A. | 0.065 | N.A. | <0 | Verlicchi et al., 2014 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Spain | | Municipal | CAS | Spiramycin (SPI) | N.A. | N.A. | N.A. | 0 | Castiglioni et al., 2006 |
| Spain | 7 | Multiple | CAS | Tetracycline (TCN) | <0.02-1.3 | 0.02 | N.A. | 71±33 | Gros et al., 2010 |
| Singapore | 1 | Municipal | CAS | | 1.24-12.34 | 3.60 | N.A. | 79 | Tran et al., 2016 |
| Singapore | 1 | Municipal | MBR | | 1.24-12.34 | 3.60 | N.A. | 93 | Tran et al., 2016 |
| China | 7 | Multiple | Multiple (AS, OD, A/A/O) | | 0.02-0.2 | 0.08 | N.A. | 90 | Wang et al., 2018 |
| China | 7 | Municipal | A/A/O | | N.D.-0.28 | 0.18 | N.A. | 90 | Ashfaq et al., 2017 |
| China | 14 | Multiple | Multiple | | 0.002-0.11 | 0.015 | N.A. | 90 | Ben et al., 2018 |
| Canada | 1 | Multiple | CAS | | N.A. | 0.008 | N.A. | 68 | Guerra et al., 2014 |
| Canada | 1 | Multiple/ industrial | CAS | | N.A. | 0.003 | N.A. | 100 | Guerra et al., 2014 |
| Luxembourg | 1 | N.A. | BAF | | N.D.-0.085 | N.A. | N.A. | N.A. | Pailler et al., 2009 |
| Italy | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | N.A. | Verlicchi et al., 2014 |
| US | 1 | N.A. | CAS | | N.A. | 0.15 | N.A. | 0 | Gao et al., 2012 |
| Spain | 1 | Municipal /hospital | TF | | N.D.-0.032 | 0.012 | <0-96 | <0 | Santos et al., 2013 |
| Spain | 2 | N.A. | N.A. | | N.A. | N.D. | N.A. | N.A. | Gros et al., 2012 |
| US | 1 | Municipal /industrial | N.A. | | N.D.-0.039 | 0.023 | <0-12 | 4 | Spongberg and Witter, 2008 |
| US | 1 | N.A. | CAS | | 0.51-0.79 | 0.65 | 80-90 | 83 | Karthikeyan and Meyer, 2006 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| US | 1 | N.A. | CAS | | N.A. | 0.24 | N.A. | 71 | Karthikeyan and Meyer, 2006 |
| US | 1 | N.A. | OD | | N.A. | 0.48 | N.A. | <0 | Karthikeyan and Meyer, 2006 |
| US | 1 | N.A. | Activated lagoon | | N.A. | 0.09 | N.A. | <0 | Karthikeyan and Meyer, 2006 |
| US | 1 | N.A. | Activated lagoon | | N.A. | 0.53 | N.A. | 68 | Karthikeyan and Meyer, 2006 |
| US | 1 | N.A. | Activated lagoon | | N.A. | 1.20 | N.A. | 76 | Karthikeyan and Meyer, 2006 |
| UK | 1 | Municipal | BAF | | 8.51-89.03 | 36.75 | N.A. | -20 | Kasprzyk-Hordern et al., 2009 |
| UK | 1 | Municipal /industrial | CAS | | 23.04-85.84 | 48.49 | N.A. | 40 | Kasprzyk-Hordern et al., 2009 |
| South Africa | 5 | Municipal /industrial | A/A/O and MLE | Tramadol (TRA) | N.D.-0.50 | N.A. | N.A. | -55 | Archer et al., 2017 |
| Germany | 1 | N.A. | A/O + chemical P removal | | 0.38-0.83 | 0.67 | N.A. | 1[a] | Gurke et al., 2015 |
| Canada | 1 | N.A. | CAS | | N.A. | 0.058 | N.A. | <0 | Rodayan et al., 2014 |
| Singapore | 4 | Municipal | CAS, MBR | | N.D. | N.D. | N.A. | N.A. | Tran et al., 2016 |
| China | 14 | Multiple | Multiple | Tylosin (TYL) | N.D. | N.D. | N.A. | N.A. | Ben et al., 2018 |
| China | 8 | N.A. | Multiple | | N.A. | N.D.-0.49 | N.A. | 87[d] | Zhang et al., 2018 |

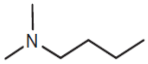
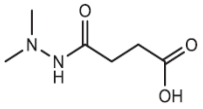
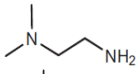
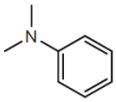
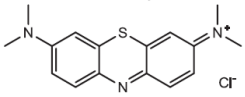
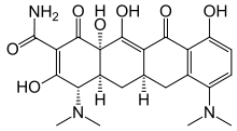
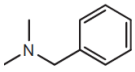
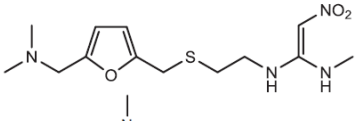
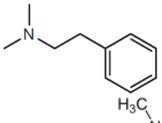
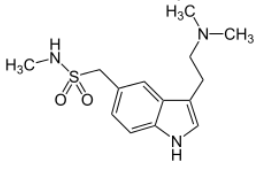
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Canada | 6 | N.A. | Multiple | | N.A. | <0.02 | N.A. | N.A. | Guerra et al., 2014 |
| US | 1 | N.A. | CAS | | N.A. | N.D. | N.A. | N.A. | Gao et al., 2012 |
| South Africa | 5 | Municipal /industrial | A/A/O and MLE | | N.D.-0.46 | N.A. | N.A. | 55 | Archer et al., 2017 |
| Greek | 4 | Municipal /hospital /industrial | Nitrification/ denitrification | | n.d.-0.07 | 0.06 | N.A. | N.A. | Papageorgiou et al., 2016 |
| Germany | 1 | N.A. | A/O + chemical P removal | | 0.33-0.74 | 0.59 | N.A. | 8[a] | Gurke et al., 2015 |
| Spain | 1 | Municipal /hospital | TF | | 0.068-0.27 | 0.18 | <0-11 | <0 | Santos et al., 2013 |
| Spain | 1 | N.A. | N.A. | | N.A. | 0.53 | N.A. | 29 | Gros et al., 2012 |
| Spain | 1 | N.A. | N.A. | | N.A. | 0.58 | N.A. | 37 | Gros et al., 2012 |
| Canada | 1 | N.A. | A/A/O | Venlafaxine (VLFX) | 1.62-1.7 | N.A. | 12-18 | N.A. | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | BAF | | 0.79-0.11 | N.A. | 17-24 | N.A. | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | TF | | N.A. | 1.28 | N.A. | 8 | Lajeunesse et al., 2012 |
| Canada | 1 | N.A. | CAS | | N.A. | 1.46 | N.A. | 39 | Lajeunesse et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.9-41.7 | N.A. | 28-73 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.075-0.11 | N.A. | <0 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.10-0.16 | N.A. | <0 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | | 0.12-0.31 | N.A. | <0-3 | N.A. | Gasser et al., 2012 |

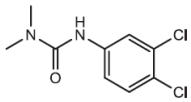
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Israel | 1 | N.A. | CAS | 0.29-0.41 | N.A. | <0-22 | N.A. | Gasser et al., 2012 |
| Israel | 1 | N.A. | CAS | 0.17-0.25 | N.A. | <0-24 | N.A. | Gasser et al., 2012 |

[a]: Calculated based on mean mass loads in influents and effluents.

# Appendix B

**Table B-1.** Chemical structures and pKa values of selected model compounds.

| Compound | Chemical structure | pKa | Source |
|---|---|---|---|
| Dimethylamine (DMA) |  | 10.7 | Perrin, 1965 |
| Trimethylamine (TMA) |  | 9.8 | Perrin, 1965 |
| *N*,*N*-dimethylethylamine (DMEA) |  | 10.2 | Perrin, 1965 |
| *N*,*N*-dimethylbutylamine (DMBA) |  | 10.0 | Perrin, 1965 |
| *N*,*N*-dimethylisopropylamine (DMiPA) |  | 10.4 | Juranic, 2014 |
| *1,1*-dimethylhydrazine (UDMH) |  | 7.2 | Braun and Zirrolli, 1983 |
| Daminozide (DMNZD) |  | 4.7 | Tomlin, 1997 |
| *N*,*N*-dimethylethylenediamine (DMEDA) |  | 9.5 | Albert, 1979 |
| *N*,*N*-dimethylaniline (DMAN) |  | 8.9 | Cox, 2013 |
| Methylene blue (MB) |  | 3.8 | Kim et al., 2013 |
| Minocycline (MNCL) |  | 9.3 | Jain et al., 2007 |
| *N*,*N*-dimethylbenzylamine (DMBzA) |  | 8.8 | Backtorp et al., 2015 |
| Ranitidine (RNTD) |  | 8.2 | Kortejarvi et al., 2005 |
| *N*,*N*-dimethylphenethylamine (DMPhA) |  | 9.3 | ChemAxon[a] |
| Sumatriptan (SMTR) |  | 9.5 | Shen and Andrews, 2013 |

| | | | |
|---|---|---|---|
| Diuron (DRN) |  | 13.6 | Environment Canada, Health Canada, 2011 |

[a]: Computational prediction.

**Table B-2.** Selected water quality parameters measured in raw and treated surface waters.

| Category | Water samples | DOC (mg/L) | $SUVA_{254}$ ($L \cdot mg^{-1} \cdot m^{-1}$) | DN (mg/L) | $Br^-$ (μg/L) |
|---|---|---|---|---|---|
| Raw surface waters[a] | RA | 4.9 | 3.1 | 0.4 | 41 |
| | RB | 5.2 | 3.8 | 0.4 | 53 |
| | RC | 8.9 | 4.7 | 0.5 | 67 |
| | RD | 21.7 | 4.9 | 0.8 | 46 |
| | RE | 25.0 | 5.1 | 0.9 | 50 |
| Treated waters[b] | TA | 2.4 | 1.7 | 0.2 | 39 |
| | TB | 2.7 | 1.9 | 0.4 | 52 |
| | TCD | 3.6 | 2.0 | 0.4 | 65 |
| | TE | 5.9 | 2.1 | 0.3 | 49 |

[a]: Five raw surface water samples (RA, RB, RC, RD and RE) were collected from five watersheds in southeast US. [b]: Four treated surface waters (TA, TB, TCD and TE) were collected from water utilities after conventional treatment of RA-RE prior to disinfection process (RC and RD were mixed before treatment in a water utility.

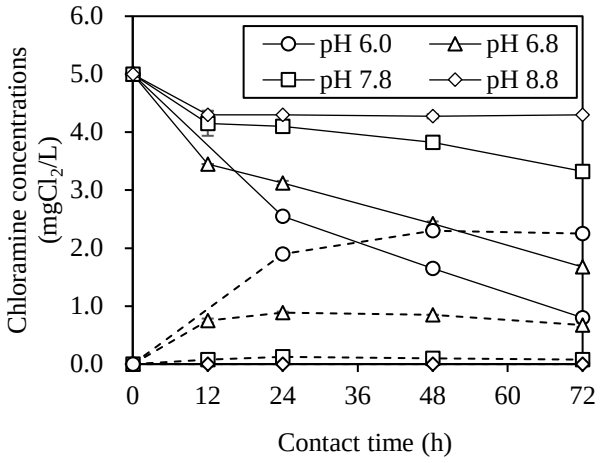



**Figure B-1.** Monochloramine ($NH_2Cl$) and dichloramine ($NHCl_2$) concentrations during 3-d uniform UFC tests in DDW. Solid lines hereafter represent $NH_2Cl$ concentrations, while dashed lines hereafter represent $NHCl_2$ concentrations.

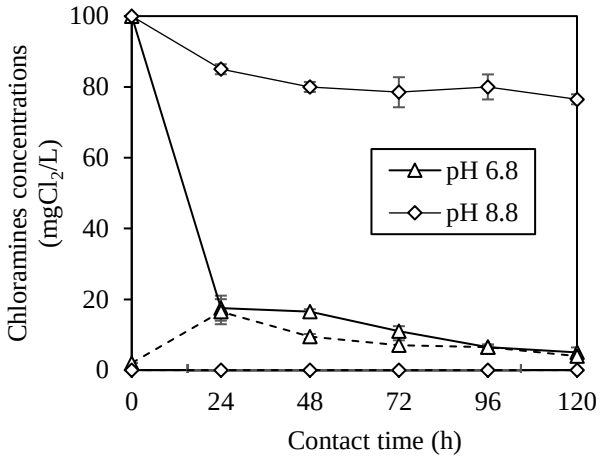



**Figure B-2.** $NH_2Cl$ and $NHCl_2$ concentrations during 5-d FP tests in DDW.

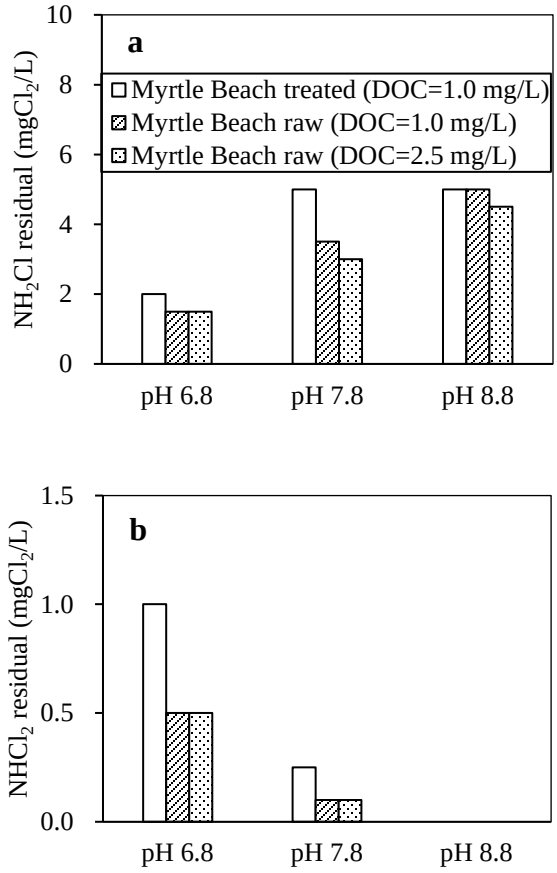



**Figure B-3.** Residual concentrations of (a) $NH_2Cl$ and (b) $NHCl_2$ measured after 3-d UFC tests in different water matrices.

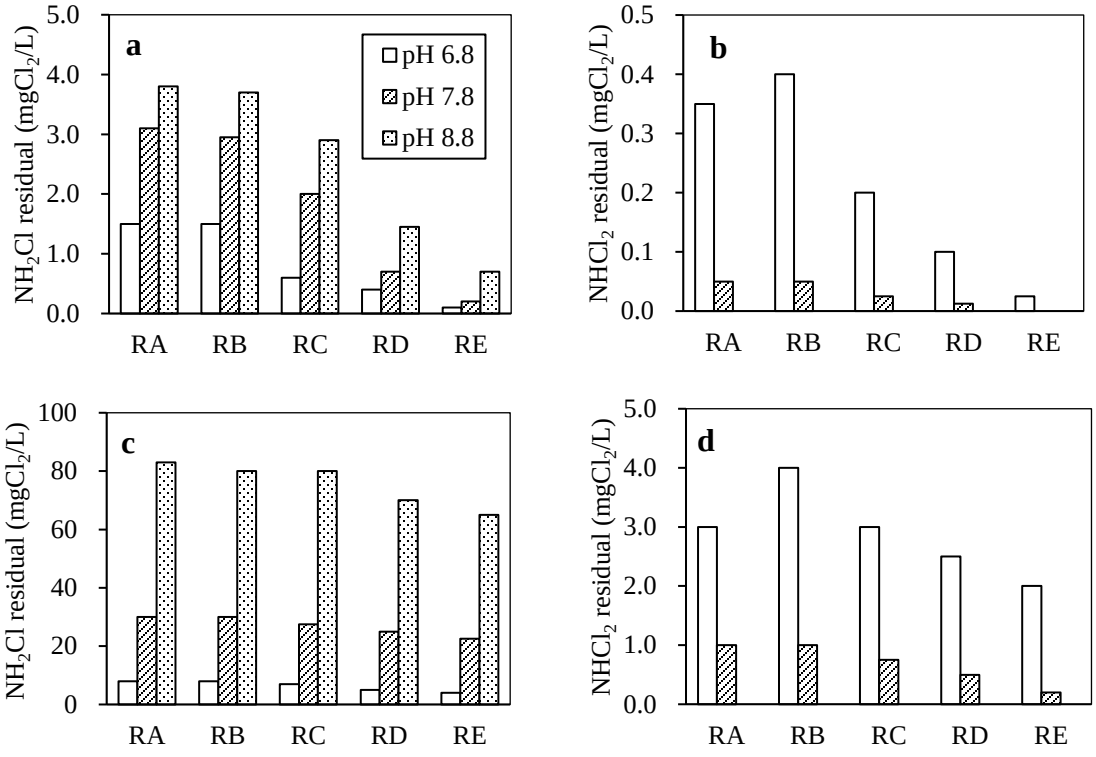



**Figure B-4.** $NH_2Cl$ and $NHCl_2$ residual concentrations after (a)-(b): 3-d UFC tests, and (c)-(d): 5-d FP tests in raw surface waters.

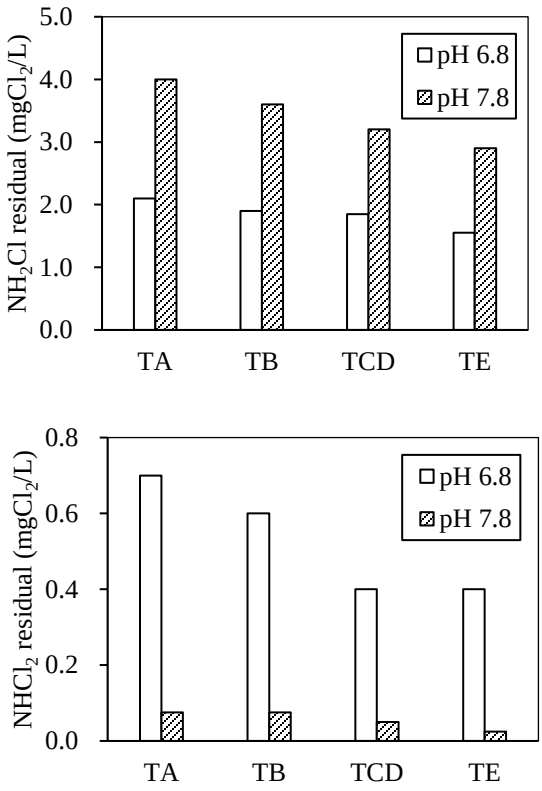



**Figure B-5.** $NH_2Cl$ and $NHCl_2$ residual concentrations after 3-d UFC tests in treated surface waters.

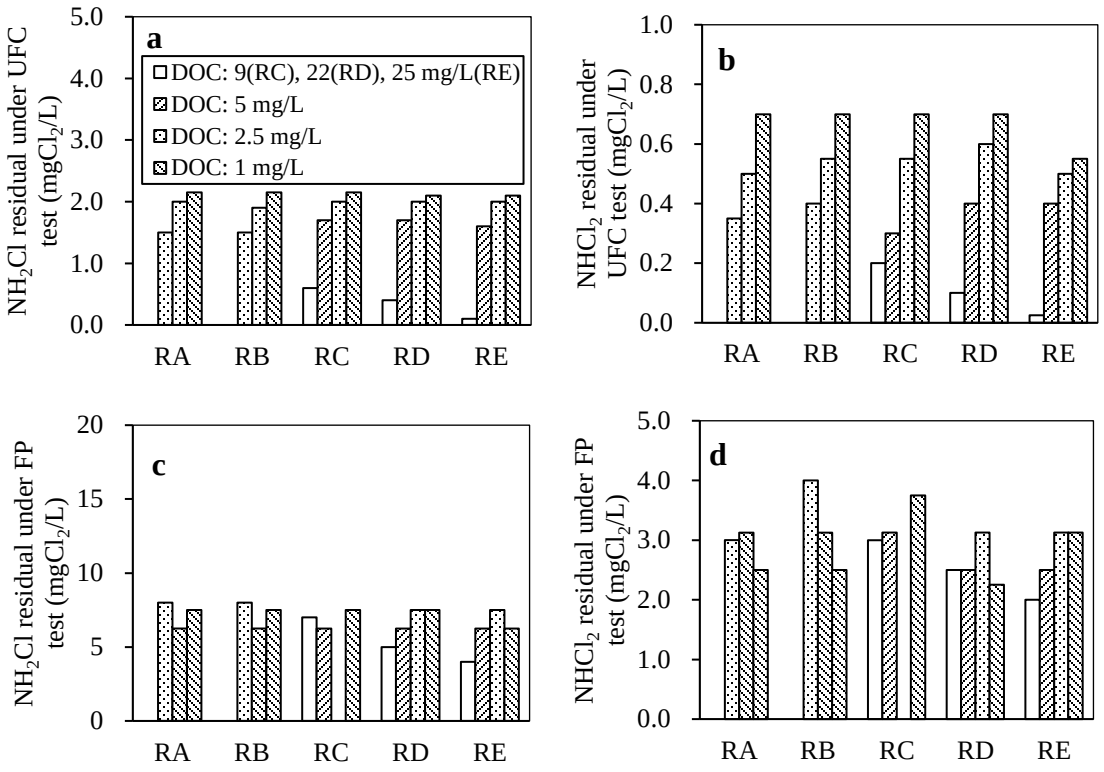



**Figure B-6.** $NH_2Cl$ and $NHCl_2$ residual concentrations after (a)-(b): 3-d UFC tests, and (c)-(d): 5-d FP tests in raw surface waters (RC water with 2.5 mg/L DOC was not measured for FP).

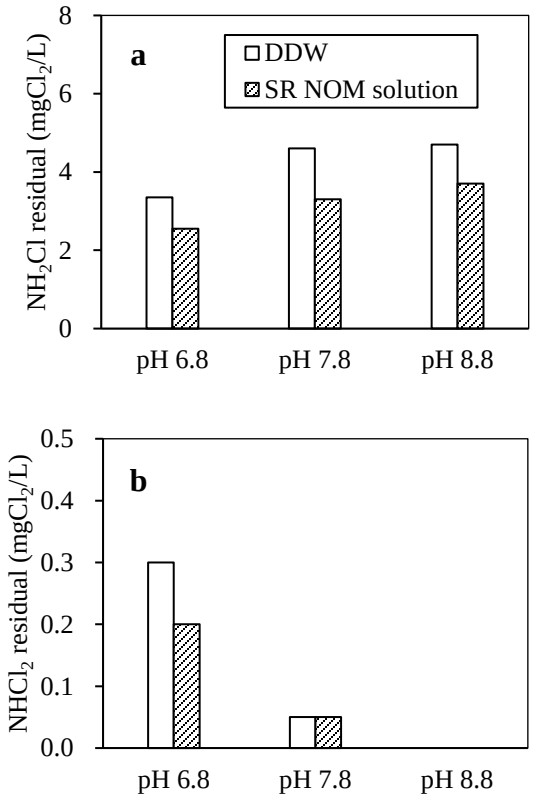



**Figure B-7.** Residual concentrations of (a) $NH_2Cl$ and (b) $NHCl_2$ after 3-d UFC tests in DDW and in Suwannee River (SR) NOM solution (i.e., 10 mg/L).

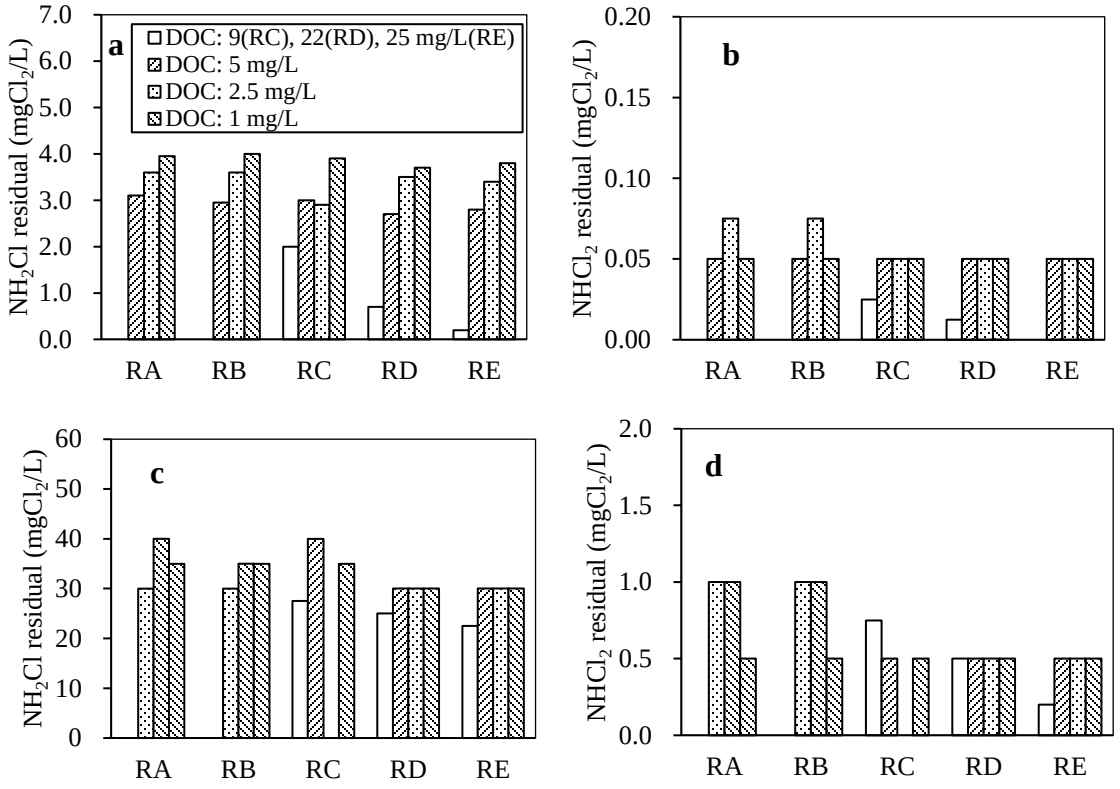



**Figure B-8.** $NH_2Cl$ and $NHCl_2$ residual concentrations measured after (a)-(b): UFC tests, and (c)-(d): FP tests in raw surface waters at pH 7.8 (RC water with 2.5 mg/L DOC was not measured for FP).

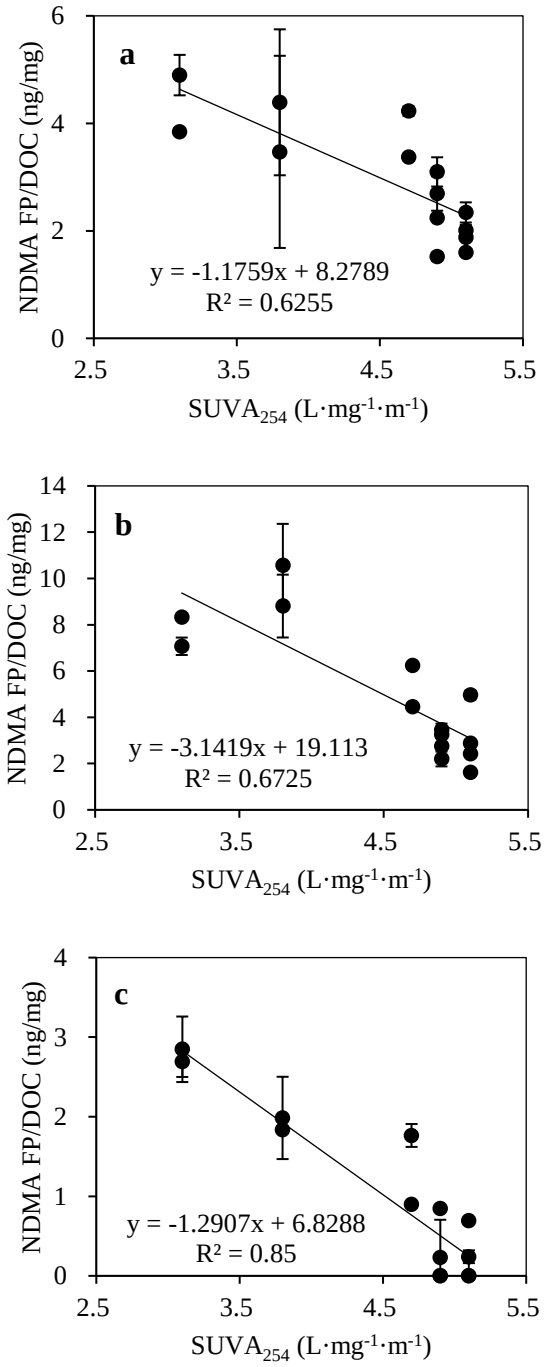



**Figure B-9.** Correlations between NDMA FP/DOC and $SUVA_{254}$ in different surface waters at (a) pH 6.8, (b) pH 7.8, and (c) pH 8.8. n = 12.

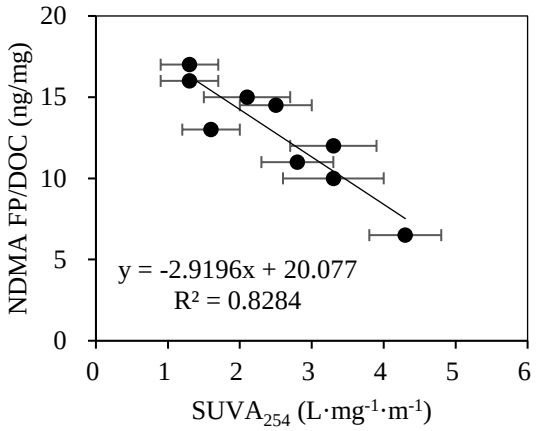



**Figure B-10.** Linear correlations between the mean NDMA FP/DOC and mean $SUVA_{254}$ in surface waters collected from nine different watersheds in southeastern US. pH = 7.8. Data were cited from Uzun et al. (2015).

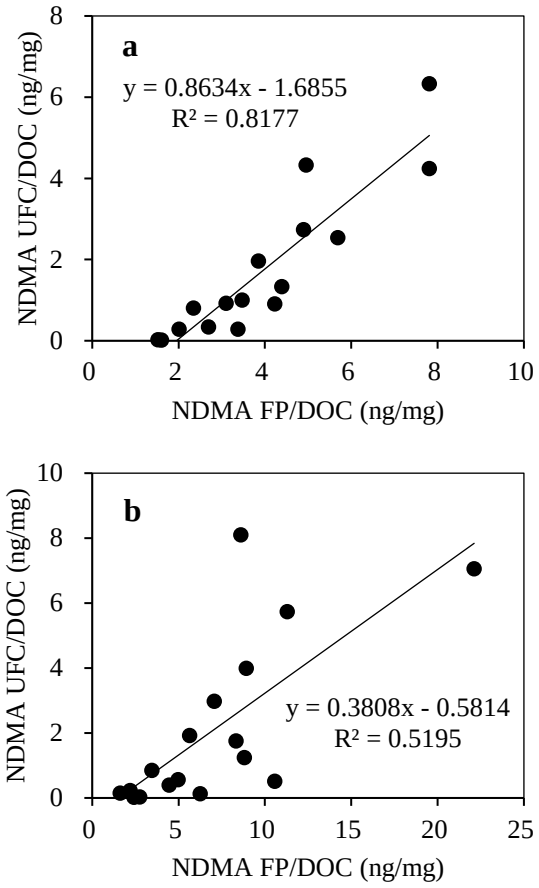



**Figure B-11.** Correlations between NDMA UFC/DOC and NDMA FP/DOC in different surface waters at (a) pH 6.8 and (b) pH 7.8.

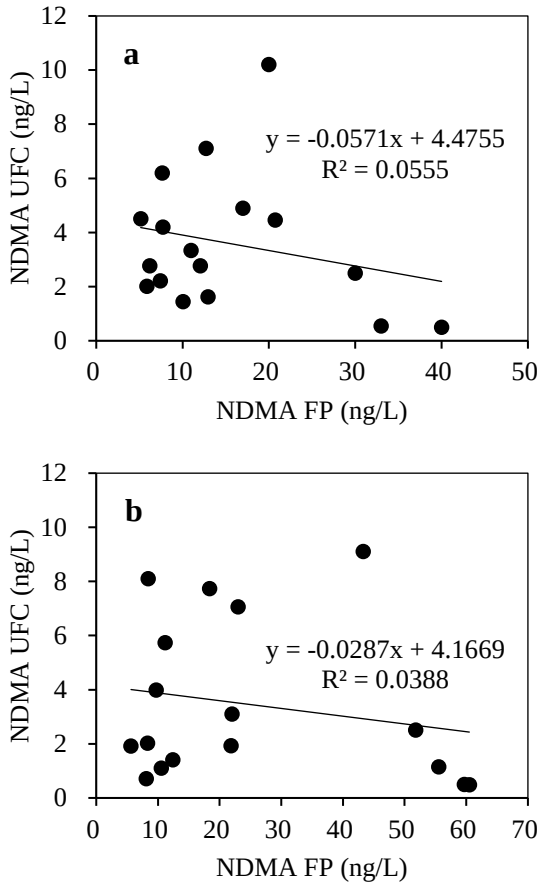



**Figure B-12.** Correlations between NDMA UFC and NDMA FP in different surface waters at (a) pH 6.8 and (b) pH 7.8.

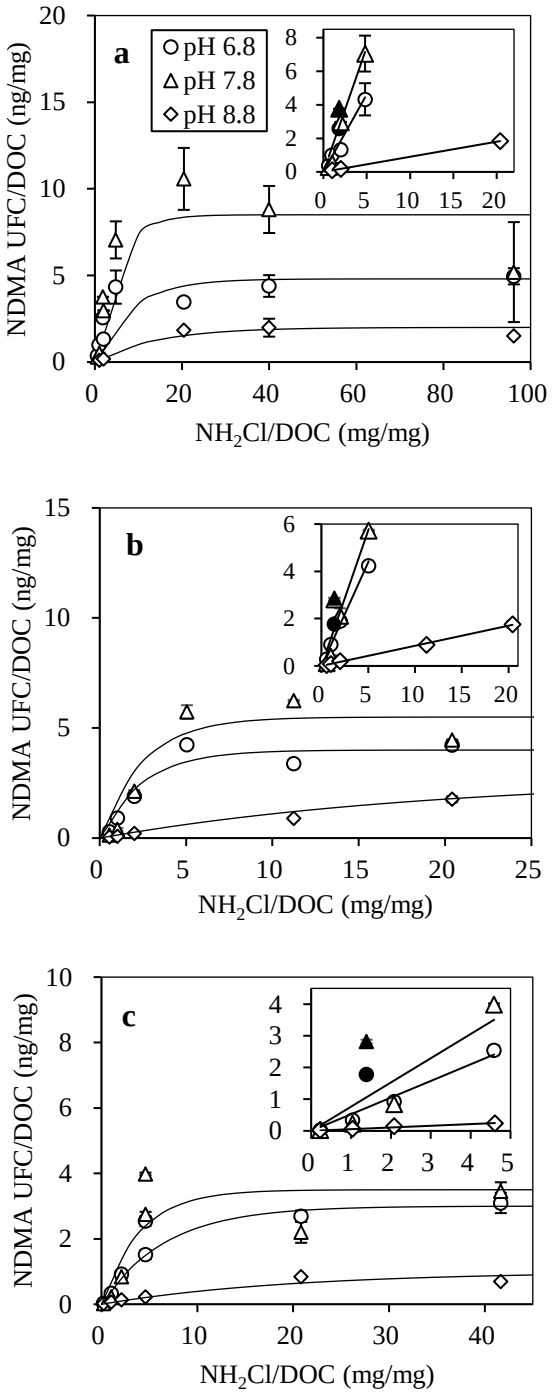



**Figure B-13.** Effects of DOC-normalized $NH_2Cl$ dosage (i.e., $NH_2Cl$/DOC) on NDMA UFC/DOC and NDMA FP/DOC in (a) RA, (b) RB, (c) RC, (d) RD and (e) RE waters. The solid points represent values measured in (a) TA, (b) TB, (c)-(d) TCD, and (e) TE waters.

# Appendix C

**Table C-1.** Recipe of synthetic wastewater used to feed lab-scale SBR.

| Ingredients | Concentrations in PDS[a] (g/L) | Concentrations in synthetic wastewater (mg/L) |
|---|---|---|
| Glucose | 28.1 | 216 |
| Yeast | 26 | 200 |
| $NH_4Cl$ | 12.5 | 96 |
| $MgSO_4 \cdot 7H_2O$ | 2.47 | 19 |
| $FeSO_4 \cdot 7H_2O$ | 0.09 | 0.7 |
| $MnSO_4 \cdot H_2O$ | 0.09 | 0.7 |
| $CaCl_2 \cdot 2H_2O$ | 3.0 | 23 |
| $KH_2PO_4$ | 51.0 | 280 |
| $Na_2HPO_4$ | 116.8 | 642 |

[a]: Primary dilution solution.

**Operations of laboratory SBR**

Four lab-scale SBRs were set up at Clemson University and run concurrently at room temperature (20±2°C). Each reactor was made of a polymethyl methacrylate cylinder with an effective volume of 4.0 L. The SBRs were seeded with freshly collected AS 1 and operated on a 2-h cycle which included feed addition (5 min), aerobic reaction (90 min), settling (30 min), and discharge of settled effluent (2 min). The synthetic wastewater that was used to feed SBRs was prepared by dissolving glucose, yeast extract and various inorganic nutrients in tap water (**Table C-1**), which has a COD of 400 mg/L, $NH_3$-N 25 mg/L and pH 7.0±0.1 (buffered with 6.5-mM phosphate) to mimic the ingredients in domestic sewage. The dissolved oxygen (DO) during aerobic reaction was maintained above 2 mg/L through aeration with compressed air (~20 L/min in each reactor). The effective HRT was 6 h, and the nominal SRT was 8 d. After acclimation for at least three SRTs, a composite AS sample was collected from the four bioreactors and used for AS treatment tests. The MLSS concentrations in reactors, the DOC, $NH_3$-N, dissolved nitrogen (DN) and total suspended solids (TSS) in discharged effluents were monitored on a daily basis during the two weeks prior to AS collection (**Table C-2**).

**Table C-2.** Mixed liquor suspended solids (MLSS) measured in SBR and selected water quality parameters measured in discharged effluents before AS collection.

| Date | MLSS in bioreactor (mg/L) | TSS in effluent (mg/L) | Actual SRT[b] (d) | DOC in feed (mg/L) | DOC in effluent (mg/L) | DOC Removal (%) | DN in feed (mg N/L) | $NH_3$-N in effluent (mg/L) | Removal of $NH_3$-N[c] (%) |
|---|---|---|---|---|---|---|---|---|---|
| 03/20/2015 | | | | | 35.3 | | | 8.4 | 66 |
| 03/21/2015 | | | | | 34.5 | | | 9.2 | 63 |
| 03/22/2015 | 2110 (42)[a] | 24 (28) | 6.3 | 97.5 | 34.3 | 65 | 39.6 | 8.4 | 66 |
| 03/23/2015 | 2465 (120) | 6 (6) | 7.6 | | 32.5 | | | 8.2 | 67 |
| 03/24/2015 | 2210 | 8 (7) | 7.4 | 168.3 | 29.3 | 83 | 45.5 | 6.6 | 74 |
| 03/25/2015 | 1850 (71) | 27 (4) | 5.9 | 156.9 | 27.4 | 83 | 41.9 | 3.4 | 86 |
| 03/26/2015 | 2395 (92) | 5 (0) | 7.6 | 135.6 | 28.0 | 79 | 45.0 | 6.8 | 73 |
| 03/27/2015 | 2410 (198) | 6 | 7.5 | 145.5 | 13.4 | 91 | 37.6 | <0.4 | >98 |
| 03/28/2015 | 2420 (283) | 13 (12) | 7.1 | 126.9 | 17.9 | 86 | 39.5 | 2.0 | 92 |
| 03/29/2015 | 2980 | 18 | 7.0 | 103.1 | 25.8 | 75 | 39.4 | 5.8 | 77 |
| 03/30/2015 | | | | | 25.4 | | | | |
| 03/31/2015 | 2905 (587) | 16 (6) | 7.1 | 91.6 | | | 44.4 | | |
| 04/01/2015 | 3145 (21) | 5 (6) | 7.7 | | 32.4 | | | 9.4 | 62 |
| 04/02/2015 | | | | | 28.0 | | | 6.4 | 74 |
| 04/03/2015 | 3055 (49) | 10 (1) | 7.4 | | 9.2 | | | <0.4 | >98 |

[a]: Data in parenthesis are standard deviations of duplicate samples. [b]: TSS in discharged effluents were taken into account to calculate the actual SRT of the SBR. [c]: Removal of $NH_3$-N was evaluated based on dosed $NH_3$-N concentrations in feed (i.e., 25 mg/L $NH_3$-N) and $NH_3$-N concentrations measured in effluents.

**Table C-3.** Recipe of mineral salts solution used to wash and resuspend AS.[a].

| Stock solutions[b] | Chemicals | Concentrations (g/L)[c] |
|---|---|---|
| A | $KH_2PO_4$ | 8.50 |
| | $KFHPO_4$ | 21.75 |
| | $Na_2HPO_4 \cdot 2H_2O$ | 33.40 |
| | $NH_4Cl$ | 0.5 |
| B | $CaCl_2 \cdot 2H_2O$ | 36.40 |
| C | $MgSO_4 \cdot 7H_2O$ | 22.50 |
| D | $FeCl_3 \cdot 6H_2O$ | 0.25 |

[a]: To prepare 1 L of mineral solution, 10 mL of solution A, 1 mL of solution B, C and D, respectively, were added to 987 mL of dechlorinated tap water. [b]: One drop of concentrated HCl was added to 1 L of stock solution B, C and D, respectively, to prohibit precipitation during storage at 4ºC. [c]: Cited from OECD (1992).

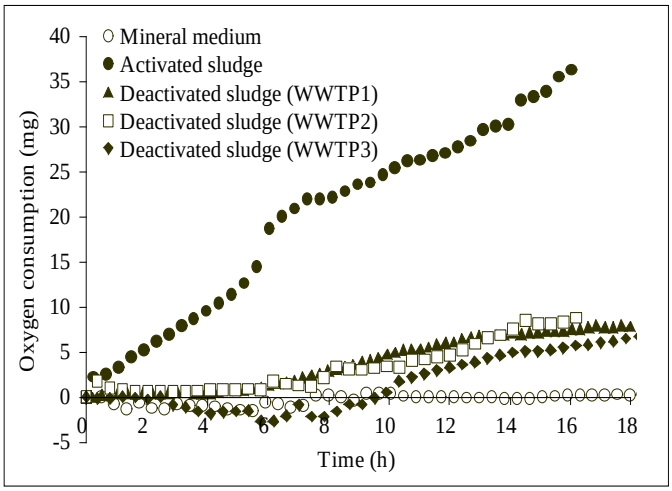



**Figure C-1.** Oxygen uptake curves measured during biosorption tests with fresh and $NaN_3$-deactivated AS.

**Table C-4.** Recipe of the mineral salts in nutrient broth (NB) and phenol medium for incubating *P. putida* cells.

| Category | Chemicals | Concentration (mg/L) |
|---|---|---|
| Buffer | $K_2HPO_4 \cdot 3H_2O$ | 4250 |
| | $NaH_2PO_4 \cdot H_2O$ | 1000 |
| Nitrogen source | $NH_4Cl$ | 70 |
| Complexing agent | Ethylenediaminetetraacetic acid (EDTA) | 20 |
| Metal ions | $CaCl_2 \cdot 2H_2O$ | 36 |
| | $MgSO_4 \cdot 7H_2O$ | 200 |
| | $FeSO_4 \cdot 7H_2O$ | 12 |
| | $MnSO_4 \cdot H_2O$ | 3.0 |
| | $ZnSO_4 \cdot 7H_2O$ | 3.0 |
| | $CoCl_2 \cdot 6H_2O$ | 1.0 |
| | $CuSO_4 \cdot 5H_2O$ | 0.2 |

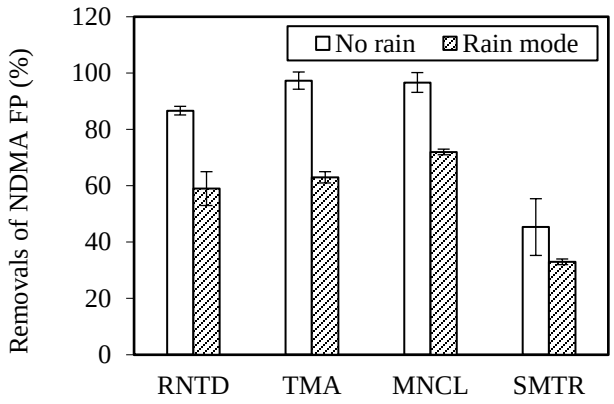



**Figure C-2.** Removal of NDMA FP from model precursor compounds during treatment with AS 1 collected under rain mode (~1000 mg/L MLSS) and without rain (~3000 mg/L MLSS). Initial NDMA FP from each compound ≈ 1000 ng/L, HRT = 24 h. During rain mode, oxygen supply to aeration basin is cut off and AS biomass is settled to the bottom of aeration basin. The AS 1 liquor collected during rain mode had an MLSS (~1000 mg/L) smaller than that (~3000 mg/L) collected under routine operational conditions.

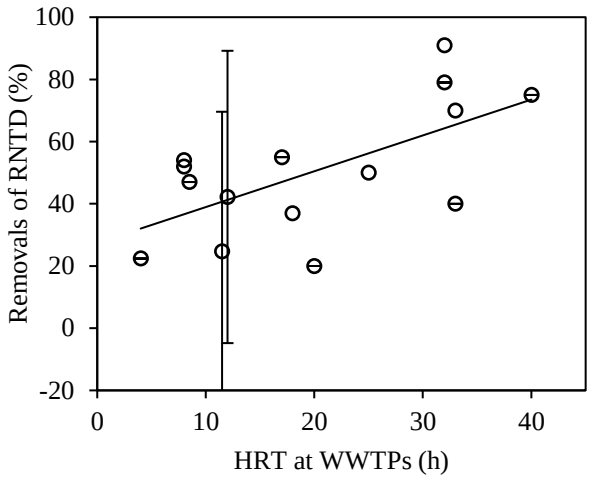



**Figure C-3.** Significant ($p$ <0.05) positive correlations between the removal of RNTD and HRTs of AS process at different WWTPs. Data cited from Castiglioni et al. (2006), Gros et al. (2006), Radjenovic et al. (2007), Kasprzyk-Hordern et al. (2009), Kern et al. (2010), Jelic et al. (2011).

**Table C-5.** Selected water quality parameters measured in secondary effluents from WWTP 1 and corresponding AS 1 characteristics.[a]

| Season | Sample | Atmospheric data | | Primary effluent | | Secondary effluent | | | | AS solids | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rain (inches) | Temperature (°C) | Flow (MGD) | BOD[b] (mg/L) | BOD (mg/L) | $NH_3$-N (mg/L) | $NO_3^-$-N (mg/L) | TKN[c] (mg/L) | MLSS (mg/L) | SVI[d] (mL) |
| Spring | Daily average[e] | 0 | 16.9 | 1.59 | 198 | 2.0 | 0.1 | N.A.[h] | N.A. | 4015 | 117 |
| | Monthly average[f] | 0.22 | 19.4 | 1.71 | 191 | 2.4 | 0.94 | 0.4 | 41 | 3736 | 105 |
| Summer | Daily average | 0 | 25.0 | 2.15 | 207 | N.D.[g] | N.D. | 4.5 | 0.6 | 4130 | 143 |
| | Monthly average | 0.10 | 24.8 | 1.57 | 220 | 1.2 | N.D. | N.A. | N.A. | 3944 | 133 |

[a]: Lower BOD, $NH_3$-N, or TKN concentrations while a higher $NO_3^-$-N concentration were found in secondary effluents during summer than in spring, suggesting a higher AS 1 activity collected in summer than in spring. [b]: Biological oxygen demand. [c]: Total Kjeldahl Nitrogen. [d]: Sludge Volume Index. e: The average values of parameters measured during the day when AS sample was collected. f: The average values of parameters measured during the month when AS sample was collected. [g]: Not detectable. [h]: Not available.

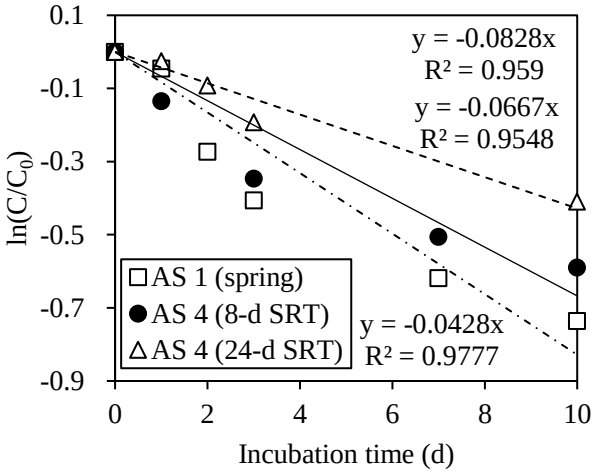



**Figure C-4.** Pseudo-first-order biodegradation kinetics of NDMA FP from RNTD during biodegradation tests with AS 4 (8-d and 24-d SRT) and AS 1 collected in spring. Initial RNTD concentration = 100 nM. MLSS ≈ 200 mg/L.

## Appendix D

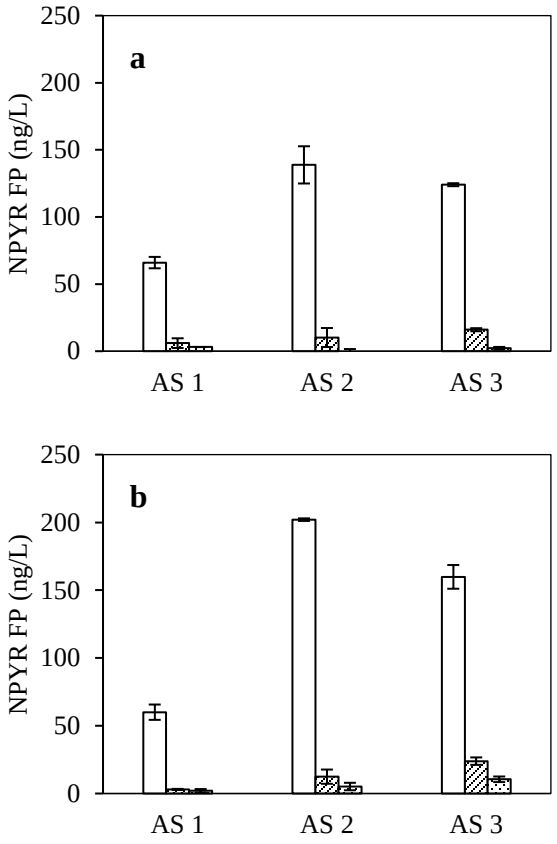



**Figure D-1.** Removal of NPYR FP from urine blackwaters collected (a) before taking Zantac (U), and (b) after taking Zantac (UR) after 6 and 24-h treatment with three types of AS (i.e., AS 1, AS 2 and AS 3).

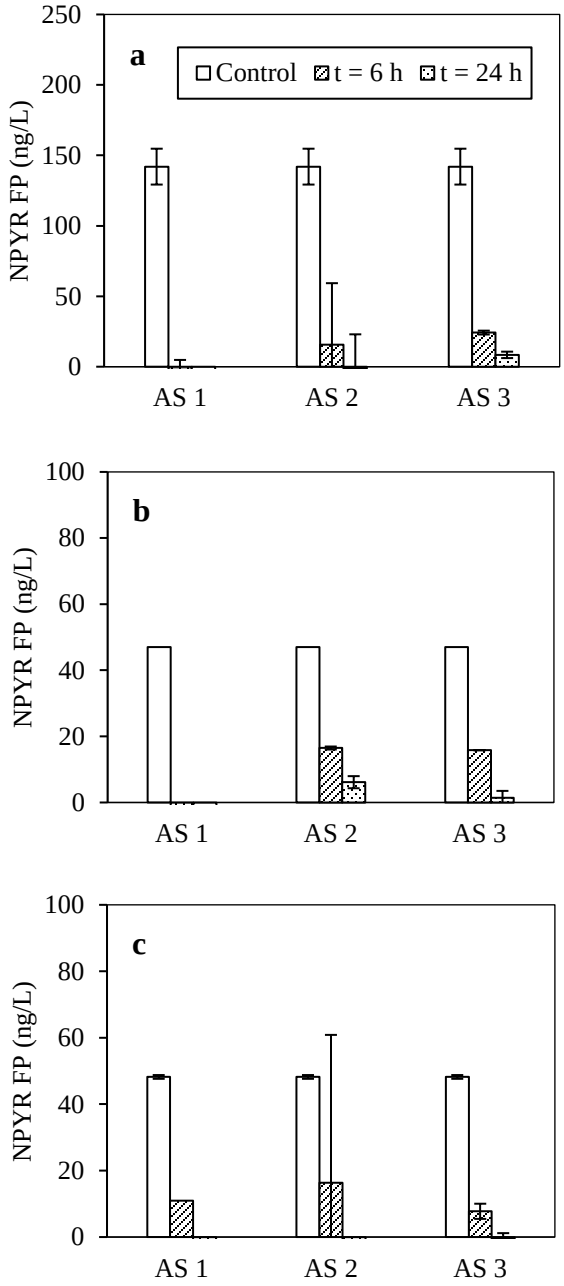



**Figure D-2.** Removal of NPYR FP from shower greywaters containing (a) no personal care products (S), (b) shampoo only (SS), and (c) body wash only (SB) during 6 and 24-h treatment with three types of AS.

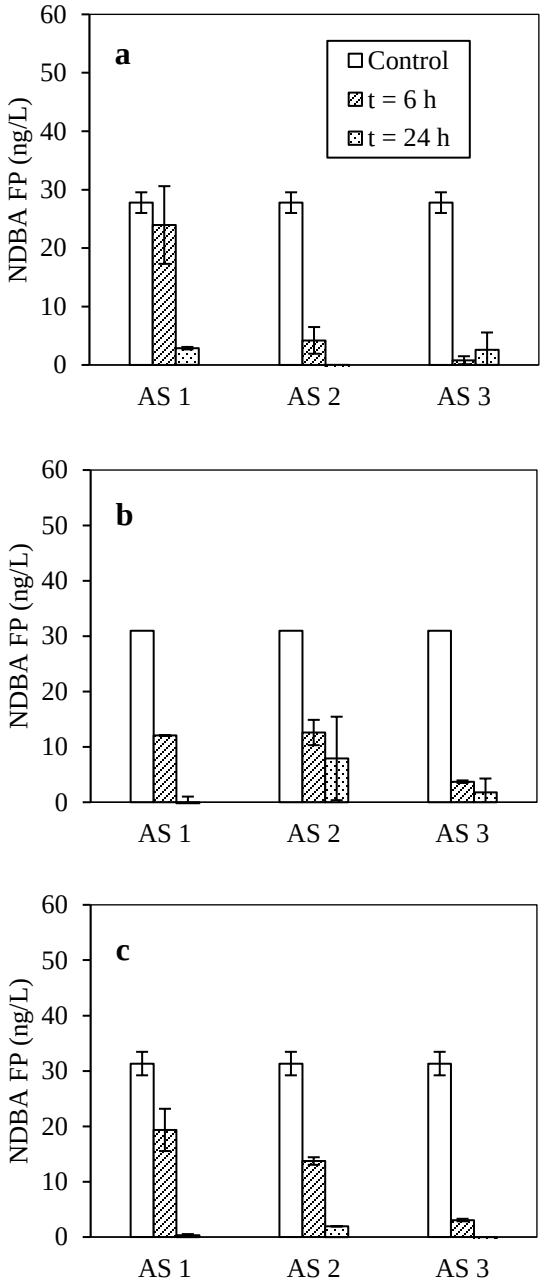



**Figure D-3.** Removal of NDBA FP from shower greywaters containing (a) no personal care products (S), (b) shampoo only (SS), and (c) body wash only (SB) during 6 and 24-h treatment with three types of AS.

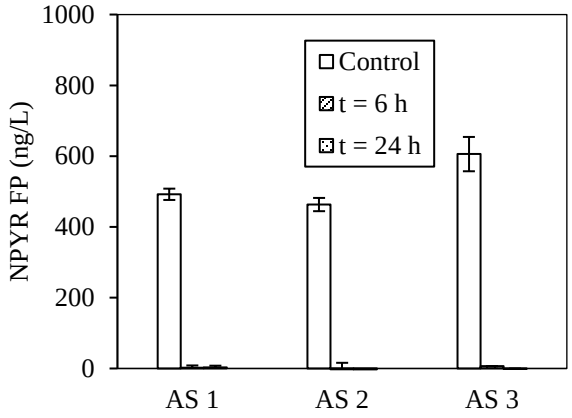



**Figure D-4.** Removal of NPYR FP from kitchen greywater containing food leachates (KF) during 6 and 24-h treatment with three types of AS.

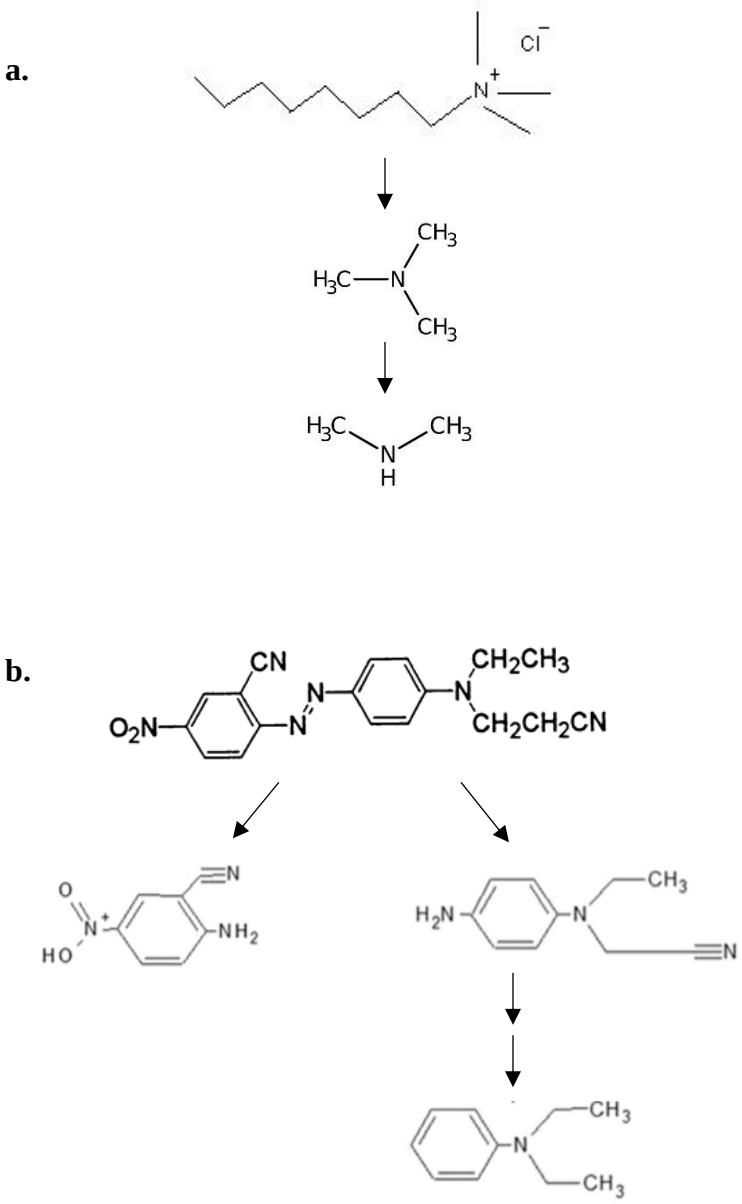



**Figure D-5.** Proposed biodegradation pathways of (a) an NDMA precursor (i.e., octyltrimethylammonium chloride, a quaternary ammonium salt) and (b) an NDEA precursor (i.e., an *N*,*N*-diethyl dye) (Verschueren, 2009; Watharkar et al., 2018).

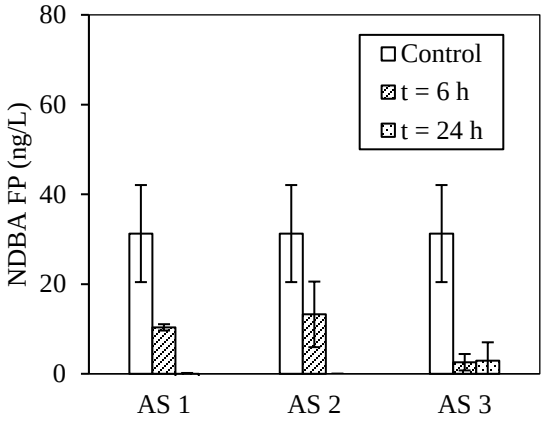



**Figure D-6.** Removal of NDPA FP from kitchen greywater containing dishwashing detergent only (KD) during 6 and 24-h treatment with three types of AS.

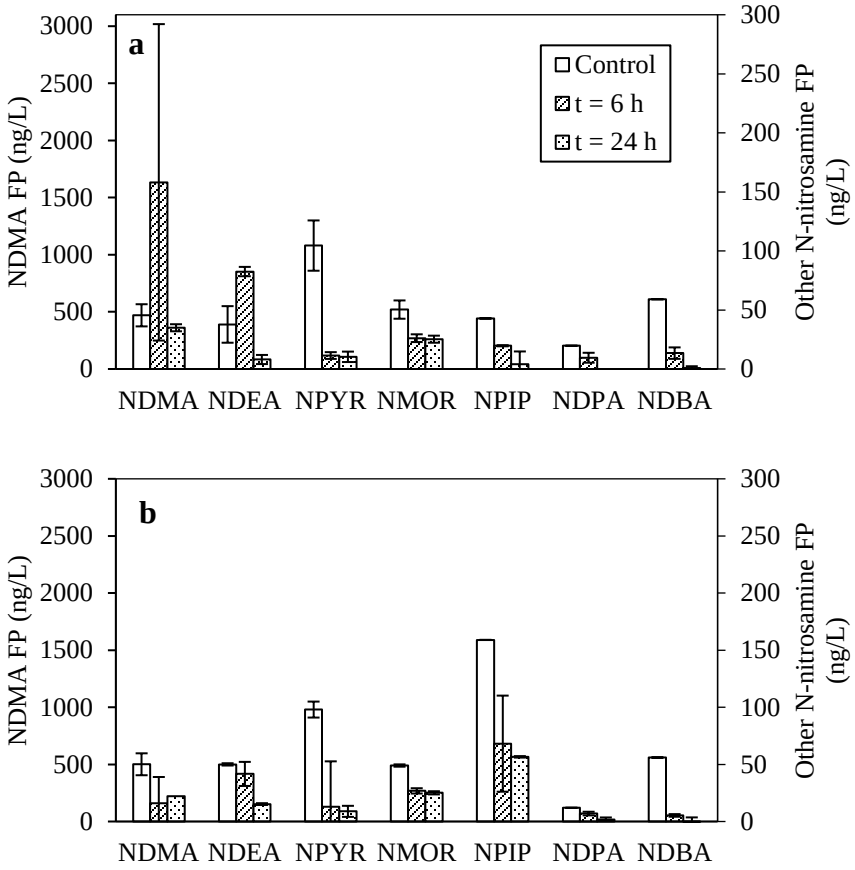



**Figure D-7.** Removal of *N*-nitrosamine FP from laundry greywaters containing (a) detergent only (LD), (b) detergent and fabric softener (LF) during 6 and 24-h treatment with AS 2.

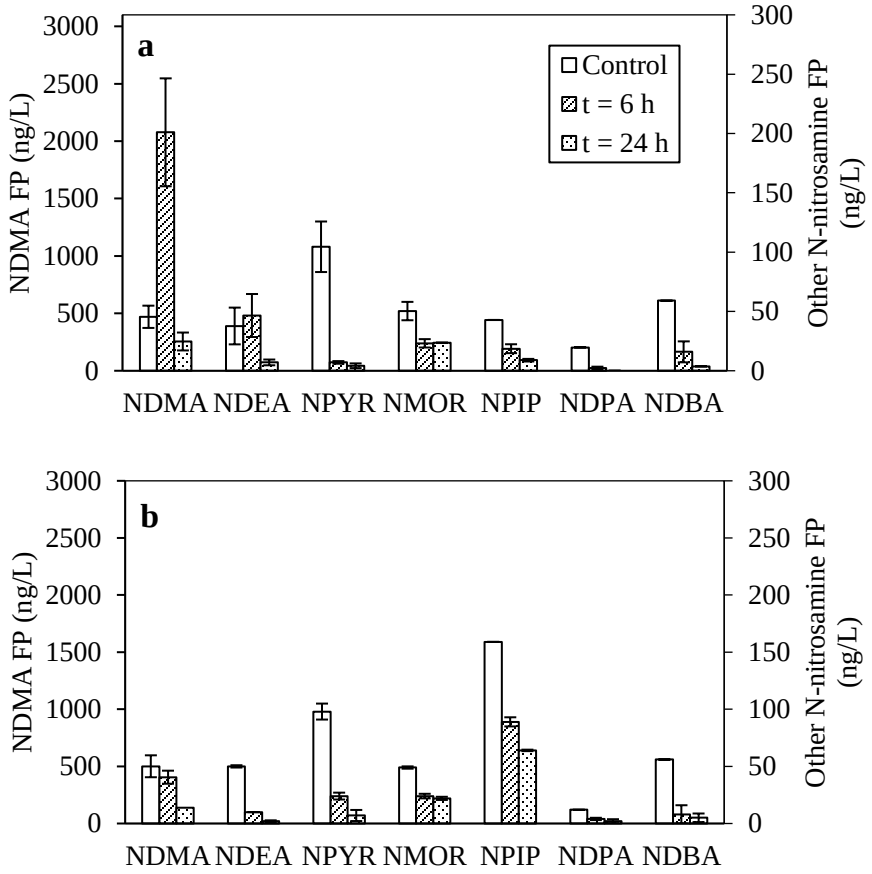



**Figure D-8.** Removal of *N*-nitrosamine FP from laundry greywaters containing (a) detergent only (LD), (b) detergent and fabric softener (LF) during 6 and 24-h treatment with AS 3.

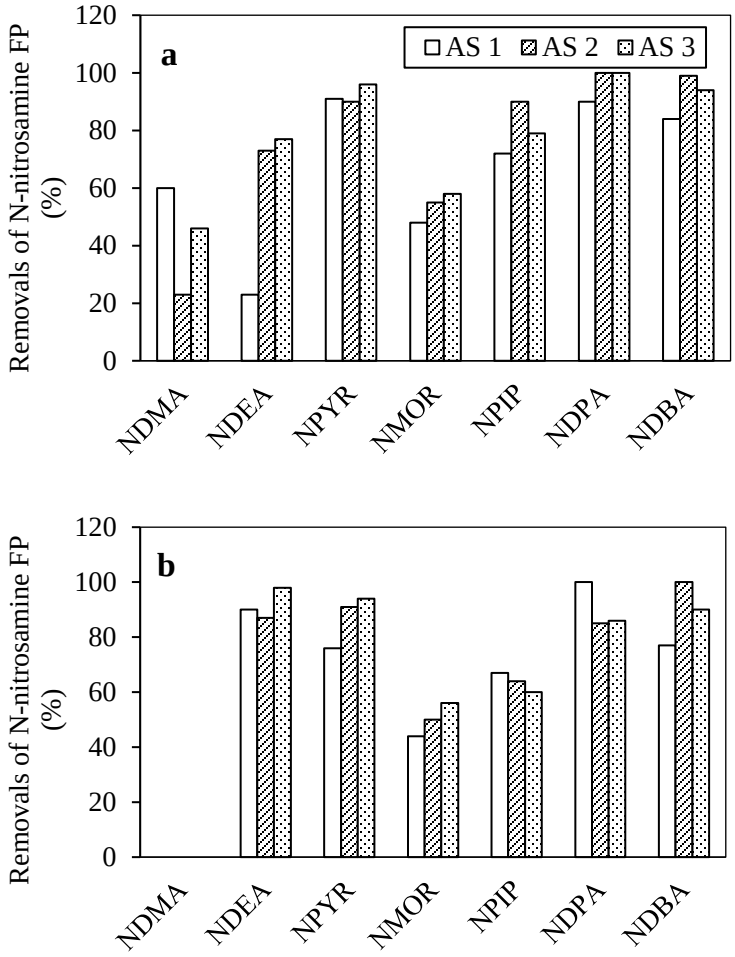



**Figure D-9.** Removal of *N*-nitrosamine FP from laundry greywaters containing (a) detergent only (LD), and (b) detergent and fabric softener (LF) after 24-h treatment with three types of AS.

**Table D-1.** Removal of *N*-nitrosamine FP (other than NDMA) from wastewater influents after 6 and 24-h treatment with AS 1 and AS 2.

| *N*-nitrosamines | Wastewater influents | AS 1 (summer)[a] | | AS 2 (summer)[a] | |
|---|---|---|---|---|---|
| | | 6-h removal (%) | 24-h removal (%) | 6-h removal (%) | 24-h removal (%) |
| NDEA | WW1[b] | N.M.[e] | N.M. | 36 | 87 |
| | WW2[c] | 27 | 94 | 56 | 82 |
| | WW3[d] | N.M. | N.M. | 100 | 71 |
| | WW4[d] | 66 | 92 | 80 | 100 |
| NPYR | WW1 | 91 | 93 | 66 | 90 |
| | WW2 | 94 | 97 | 90 | 99 |
| | WW3 | 97 | 96 | 98 | 93 |
| | WW4 | 93 | 94 | 89 | 96 |
| NMOR | WW1 | 87 | 58 | -38 | 9 |
| | WW2 | -42[f] | -55 | 7 | 81 |
| | WW3 | 28 | -28 | 78 | 64 |
| | WW4 | 64 | 75 | -19 | 7 |
| NPIP | WW1 | 92 | 94 | N.M. | N.M. |
| | WW2 | N.M.[g] | N.M. | 95 | 98 |
| | WW3 | N.M. | N.M. | N.M. | N.M. |
| | WW4 | N.M. | N.M. | N.M. | N.M. |
| NDBA | WW1 | 100 | 100 | 100 | 100 |
| | WW2 | 100 | 100 | 100 | 100 |
| | WW3 | 100 | 100 | 100 | 100 |
| | WW4 | 72 | 100 | 100 | 100 |

[a]: AS 1 and AS 2 were both collected during summer. [b]: WW1 collected during spring and summer were treated with AS 2 and AS 1, respectively. [c]: WW2 collected during spring and summer were treated with AS 2 and AS 1, respectively. [d]: WW3 and WW4 were both collected during summer. [e]: Removal of NDEA FP was not examined because of the extremely low NDEA FP (i.e., <10 ng/L) in WW1 and WW3. [f]: Minus removal indicates an increasein *N*-nitrosamine FP after AS treatment. [g]: NPIP FP from WW1 and WW2 collected during summer, WW3 and WW 4 were not measurable because the target NPIP peak (115 m/z) was interfered with a neighbor peak (114 m/z) on GC spectrum.

**Table D-2.** Selected water quality parameters of wastewater influents measured before and after treatment with AS 1.

| Wastewater influents | Sample | DOC (mg/L) | $NH_3$-N (mg/L) | $SUVA_{254}$ (L/(mg·m)) |
|---|---|---|---|---|
| WW1 | Control[a] | 13 | 26 | 2.0 |
| | 6-h incubation | 6.5 | 8 | 2.5 |
| | 24-h incubation | 4.8 | N.D.[b] | 2.9 |
| WW2 | Control | 15 | 24 | 2.0 |
| | 6-h incubation | 9.6 | 10 | 2.0 |
| | 24-h incubation | 8.2 | N.D. | 2.4 |
| WW3 | Control | 11 | 19 | 2.1 |
| | 6-h incubation | 6.5 | 2 | 2.7 |
| | 24-h incubation | 6.6 | N.D. | 2.9 |
| WW4 | Control | 13 | 7 | 1.8 |
| | 6-h incubation | 9.7 | 1 | 2.0 |
| | 24-h incubation | 9.0 | N.D. | 2.3 |

[a]: Before AS treatment. [b]: Not detectable (i.e., <0.02 mg/L).

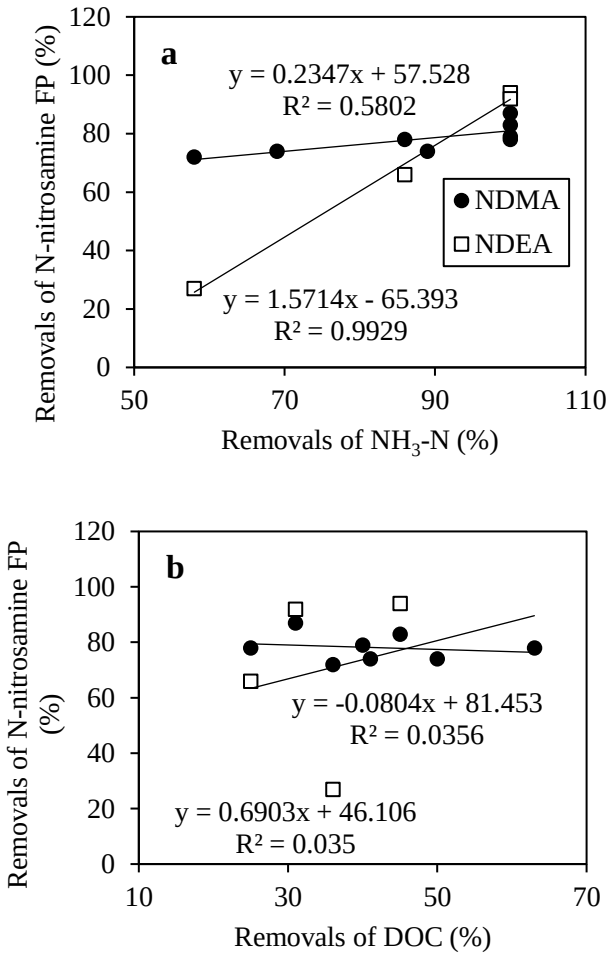



**Figure D-10.** Correlations between the removal of *N*-nitrosamine (i.e., NDMA and NDEA) FP and (a) removal of $NH_3$-N, (b) removal of DOC from wastewater influents during treatment with AS 1.

**Table D-3.** Selected water quality parameters measured in sewage components before and after treatment with AS 1.*

| Sewage components | Incubation duration | DOC (mg/L) | COD (mg/L) | $UV_{254}$ ($cm^{-1}$) | $NH_3$-N (mg/L) |
|---|---|---|---|---|---|
| U | Control[a] | 1.2 | 18 | 0.0263 | 1.4 |
| | 6-h incubation | N.D. | <10 | N.M.[b] | 0.8 |
| | 24-h incubation | N.D. | <10 | N.M. | N.D.[c] |
| F | Control | 5.5 | 15 | 0.0744 | 0.5 |
| | 6-h incubation | 1.6 | <10 | N.M. | 0.3 |
| | 24-h incubation | 0.8 | <10 | N.M. | N.D. |
| LD | Control | 26 | 135 | 0.2709 | 22 |
| | 6-h incubation | 7.2 | <10 | N.M. | 0.2 |
| | 24-h incubation | 5.9 | 11 | N.M. | N.D. |
| S | Control | 33 | N.M. | 0.3743 | 0.2 |
| | 6-h incubation | 4.4 | N.M. | N.M. | 0.8 |
| | 24-h incubation | 6.4 | N.M. | N.M. | 0.1 |
| W | Control | 78 | N.M. | 0.5355 | N.D. |
| | 6-h incubation | 1.9 | N.M. | N.M. | N.D. |
| | 24-h incubation | 1.1 | N.M. | N.M. | N.D. |
| KD | Control | 16 | N.M. | 0.0866 | N.D. |
| | 6-h incubation | 4.2 | N.M. | N.M. | N.D. |
| | 24-h incubation | 0.6 | N.M. | N.M. | N.D. |
| KF | Control | 21 | N.M. | 0.1015 | N.D. |
| | 6-h incubation | 0.4 | N.M. | N.M. | N.D. |
| | 24-h incubation | 1.0 | N.M. | N.M. | 0.1 |

*: The $NH_3$-N, COD and DOC concentrations listed in this table have been subtracted by the concentrations measured in control samples (i.e., without dosing any NDMA precursors).
[a]: Prior to incubation. [b]: Not measured. [c]: Not detectable (i.e., <0.02 mg N/L).

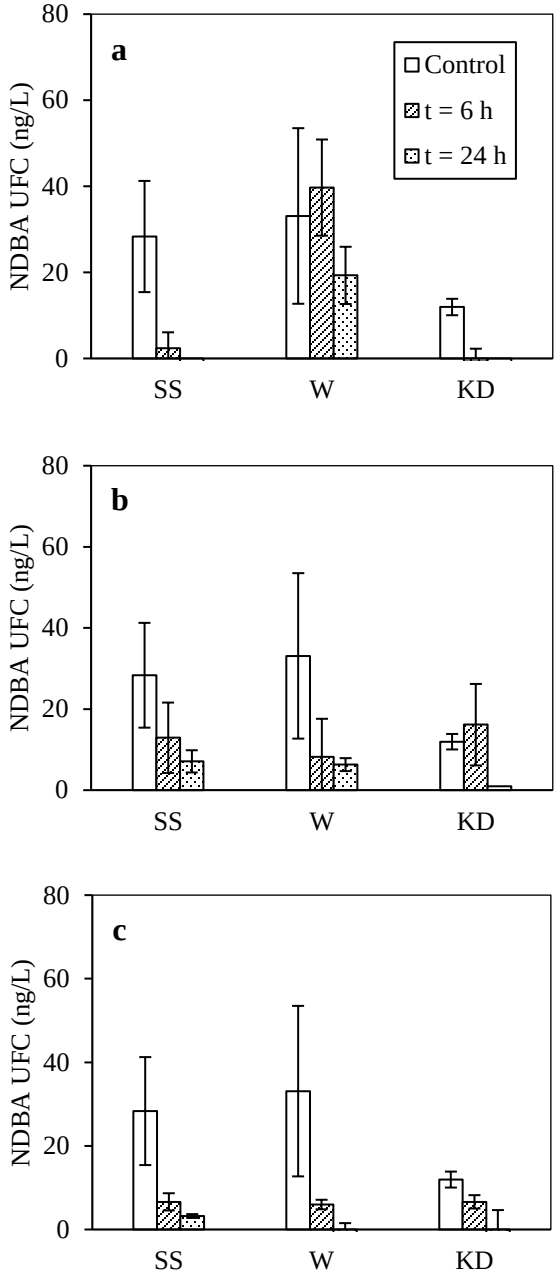



**Figure D-11.** Removal of NDBA UFC from different greywaters after 6 and 24-h treatment with (a) AS 1, (b) AS 2, and (c) AS 3. S: shower greywater not containing any personal care products, W: bathroom washbasin greywater, KD: kitchen greywater containing dishwashing detergent only.

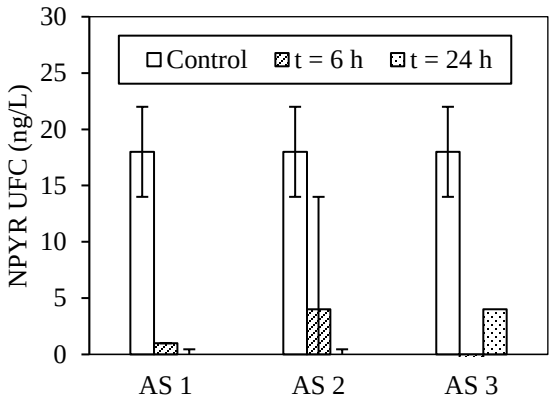



**Figure D-12.** Removal of NPYR UFC from kitchen greywater containing dishwashing detergent (KD) after 6 and 24-h treatment with AS 1, AS 2 and AS 3.

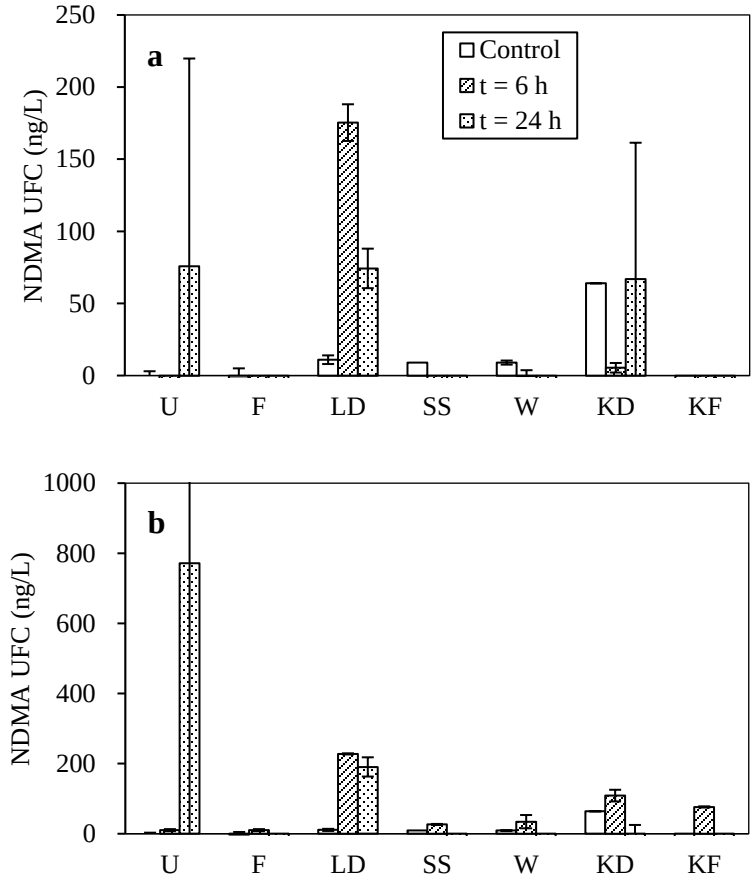



**Figure D-13.** Removal of NDMA UFC from different sewage components in the presence of EEDs after 6 and 24-h treatment with (a) AS 1, and (b) AS 2.

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