JOURNAL OF ENVIRONMENTAL SCIENCES 117 (2022) 242–252

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Effect of activated sludge treatment on the formation of N–nitrosamines under different chloramination conditions

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ARTICLE INFO

Article history:
Received 1 March 2022
Revised 24 April 2022
Accepted 27 April 2022
Available online 8 May 2022

Keywords:
Disinfection by-products
N–Nitrosamine formation potential
Uniform formation condition (UFC)
Sewage components
Biological wastewater treatment

ABSTRACT

Municipal wastewater discharge is considered as one of the main sources of *N*-nitrosamine precursors which can impact the qualities of downstream source waters and reclaimed wastewaters for potable reuse. *N*–Nitrosamine precursors can be removed to various degrees during biological wastewater treatment (e.g., the activated sludge (AS) process). So far, little is known about the impact of the AS process on *N*-nitrosamine formation under practical disinfection condition (e.g., uniform formation condition (UFC)). In this study, *N*-nitrosamine UFC from selected model compounds, sewage components (i.e., blackwaters and greywaters) and sewage samples were comprehensively investigated during batch AS treatment tests. *N*–Nitrosodimethylamine (NDMA) formation from the tested precursor compounds (i.e., trimethylamine (TMA) and sumatriptan (SMTR)) under UFC chloramination decreased mostly after 6 or 24 hr treatment with different types of AS (i.e., domestic rural AS, domestic urban AS, and textile AS), and the reductions in NDMA UFC were comparable to their NDMA formation potential (FP) reductions. In urine and feces blackwaters, NDMA UFC increased after 6 or 24 hr treatment with the domestic (i.e., rural and urban) AS, while NDMA FP decreased substantially. The increases in NDMA UFC after AS treatment was presumably attributed to the removal of bulk organic matters (e.g., dissolved organic carbon (DOC)) which favored NDMA formation under UFC. On the other hand, in laundry greywaters having relatively abundant DOC, *N*-nitrosamine UFC was less affected by DOC removal before or after AS treatment, but decreased to similar degrees with *N*-nitrosamine FP. In sewage samples collected from wastewater treatment plants, *N*-nitrosamines UFC tended to increase or remain constant during AS treatment, despite the decreases in their FPs. These results suggest that biological wastewater treatment (e.g., the AS process) may not effectively reduce *N*-nitrosamine formation (e.g., measured under UFC) partially because the concurrent removal of bulk organic matters (e.g., DOC) favored *N*-nitrosamine formation in s econdary effluents.

Introduction

Chloramination and ozonation during drinking water treatment processes can lead to the formation of *N*-nitrosamines, a group of carcinogenic disinfection by-

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https://doi.org/10.1016/j.jes.2022.04.048
1001-0742/

products (Mitch et al., 2003; Russell et al., 2012). Extremely low levels (e.g., 0.2–15 ng/L) of *N*-nitrosamines in drinking waters are possibly associated with 10^{-6} excess lifetime cancer risks (US EPA, 2001). Due to their adverse health effects, five *N*-nitrosamines (i.e., *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-n-propylamine (NDPA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosodiphenylamine (NPhA)) have been included in the United States Environmental Protection Agency (US EPA) Contaminant Candidate List 4 for possible regulation in future (US EPA, 2016). In California, 10 ng/L notification level has been established for NDMA, NDEA, and NDPA in drinking waters (CDPH, 2010). Similar guidance and guidelines have also been established in Canada, Australia, and the European Union, to address the health issues related to NDMA in drinking water and wastewater for potable reuse (UK DWI, 2000; Health Canada, 2011; NHMRC and NRMMC, 2011).

N–Nitrosamines are formed via reactions between disinfectants (e.g., chloramines or ozone) and organic amine precursors (Mitch and Sedlak, 2002; Selbes et al., 2013; Zeng et al., 2015; Chuang et al., 2019a). *N*–Nitrosamine precursors can originate from wastewater effluents, algal bloom, pharmaceuticals and personal care products (PPCPs) (Krasner et al., 2013; Zeng et al., 2016a; Beita-Sandi et al., 2019). The other major source of precursors involves those added during the treatment process, particularly coagulants (Zeng et al., 2016b). These are important not just for drinking water, but for wastewater as well. The return streams containing amine-based coagulants added in the solids handling units (e.g., thickeners) can account for ~50% of the NDMA formation potential (FP) in secondary effluent despite the small flowrates of the return flows (Mitch and Sedlak, 2004). Moreover, some utilities add amine-based coagulants to the secondary clarifiers or upstream of tertiary filters (Chuang et al., 2019b). Once formed during chloramination, NDMA, a dominant *N*-nitrosamine species typically detected in chloraminated drinking water and wastewater, is difficult to remove, because of its high solubility in water (i.e., 290 g/L at 20 °C) and continuous formation in the distribution system (Shen and Andrews, 2011). Reducing their precursors in raw source waters (i.e., influents of water utilities) would be an efficient way to better control *N*-nitrosamine formation in drinking water. However, conventional water treatment process has been found to exhibit inconsistent removal efficiencies of *N*-nitrosamine precursors (Krasner et al., 2013; Uzun et al., 2015).

Municipal wastewater discharge has been considered one of the major sources of *N*-nitrosamine precursors that can impact downstream source water qualities (Krasner et al., 2013; Zeng et al., 2016a; Sgroi et al., 2018). During biological wastewater treatment (e.g., the activated sludge (AS) process), *N*-nitrosamine precursors can be deactivated to different degrees, depending on wastewater treatment plants (WWTPs) and *N*-nitrosamine species (Mitch and Sedlak, 2004; Sedlak et al., 2005; Krauss et al., 2010; Yoon et al., 2013; Wang et al., 2014). Numerous previous studies have reported changes of *N*-nitrosamine precursors as changes of their FPs. However, the impact of biological wastewater treatment on the *N*-nitrosamine formation under practical chloramination condition (e.g., uniform formation condition (UFC): 2–5 mg Cl_2/L monochloramine dosage, 1–3 days contact time; Krasner et al., 2013) has been far less understood. Unlike the UFC test which evaluates *N*-nitrosamine formation under typical disinfection (i.e., chloramination) condition in drinking water utilities, the FP test is more a tool for evaluating the total amount of *N*-nitrosamine precursors in water samples, by using an excessive monochloramine dosage (i.e., 100–140 mg Cl_2/L) for a long contact time (i.e., 5–10 days) to convert all precursors to *N*-nitrosamines (Mitch et al., 2003; Krasner et al., 2013).

AS sources, precursor types, hydraulic retention time (HRT), and solids retention time (SRT) have been found to affect the removal efficiencies of *N*-nitrosamine precursors during biological wastewater treatment (Grady et al., 2011; Zhang et al., 2020a, 2020b). Such factors may also impact *N*-nitrosamine formation under UFC, which remains largely unexplored. A lower DOC is considered to consume less chloramines (especially dichloramine ($NHCl_2$)) (Vikesland et al., 2001), exhibits less complexation with NDMA precursors, and thus favors the reactions between chloramines and precursors forming NDMA (Shen and Andrews, 2011; Selbes et al., 2013). The reductions in DOC during the AS process may thus favor *N*-nitrosamine UFC in secondary effluents, which requires further elucidation.

The main objective of this study is to investigate the impact of biological wastewater treatment on *N*-nitrosamine formation under UFC chloramination conditions from selected model compounds, sewage components (i.e., blackwaters and greywaters), and different types of sewage samples (containing <1%–25% industrial discharges) during batch AS treatment tests. AS types, incubation time or HRT, and organic loadings (e.g., urine concentration or addition of DOC) were further examined for their potential effects on *N*-nitrosamine UFC during the AS treatment. The results may benefit potential strategies for better controlling *N*-nitrosamine formation in secondary wastewater effluents.

1. Materials and methods

1.1. *Model compounds*

Three NDMA precursor compounds, including trimethylamine (TMA), minocycline (MNCL), and sumatriptan (SMTR), were selected for the AS treatment tests. MNCL and SMTR are active ingredients of the most widely prescribed amine-based pharmaceuticals (i.e., Minocin, and Imitrex, respectively) in the US (Shen and Andrews, 2013). TMA is a known component of human urine and feces in wastewaters (Mitch and Sedlak, 2004; Svensson et al., 1994; Lee et al., 2010). MNCL and SMTR were purchased from TCI (Duncan, US) in solid forms, which were then dissolved in methanol to 0.4 mmol/L, and further diluted in deionized and distilled water (DDW) to 200 μmol/L. TMA was purchased from Sigma-Aldrich (St. Louis, US) in an aqueous solution (4% mass concentration), which was further diluted in DDW to 200 μmol/L.

1.2. *Sewage components*

Sewage components including blackwaters (urine and feces) and greywaters (laundry, shower, bathroom washbasin, and

kitchen greywaters) were collected for the AS treatment tests, with details described elsewhere (Zhang et al., 2020a). In brief, raw human urine (UB) and feces (FB) samples were collected from a volunteer and then diluted 250 and 150 folds in tap water to mimic toilet flush dilutions. Laundry greywaters were collected from washing machine discharges after white and colored clothes were washed using only detergent (LG 1) or using both detergent and fabric softener (LG 2). Shower greywaters were collected after another volunteer took a hot shower not using any personal care products (SG 1), using shampoo only (SG 2), and using body wash only (SG 3). Bathroom washbasin greywater (WG) was a mixture of handwashing, toothbrushing, and face-cleaning wastewaters produced in a routine morning wash. Kitchen greywaters were collected after manual washing of clean dishes using dishwashing detergent (KG 1), or after mixing raw and cooked food waste leachates (i.e., each ~50 g/L of vegetables, grains, meats, and seafood) in 1:4 vol ratio, followed by a 100-fold dilution in tap water (KG 2).

All collected samples were filtered through a 0.7-μm glass fiber membrane followed by a 0.45-μm membrane (Whatman, GE Healthcare Life Sciences, US) before being stored at −20 °C (for urine, feces and KG 2 samples) or 4 °C (for the other greywaters) until used. Selected water quality parameters for the filtered samples were measured based on the Standard Methods (APHA et al., 2005) and are shown in Appendix A Table S1. In brief, urine and feces blackwaters (after dilution) showed lower DOC (i.e., 2.2–3.4 and 3.3–3.4 mg/L, respectively) than greywater samples (i.e., 15–78 mg/L). The ammonia (NH_3–N) concentrations were generally <0.5 mg/L except in urine blackwater (i.e., 1.4–1.6 mg/L) and laundry greywater (i.e., 20–40 mg/L) samples. The specific UV absorbance at 254 nm ($SUVA_{254}$) values in all samples ranged between 0.3–1.5 L/mg/•m.

1.3. *Sewage samples*

Four sewage samples (Sewage 1–4) were collected from the inlet to aeration basins (i.e., primary effluents) at four WWTPs in South Carolina (SC), US. The key operational parameters for these WWTPs are shown in Appendix A Table S2. These WWTPs have different influent compositions (i.e., containing <1%–25% industrial discharges), treatment capacities (i.e., 2.0–70 million gallons per day) and processes (i.e., extended aeration, anaerobic/anoxic/oxic process, membrane bioreactor), and geographical locations (i.e., rural and urban). Approximately 10 L of wastewater samples were grabbed and filtered with 0.45-μm membrane filter (Whatman, GE Healthcare Life Sciences, US) before being stored at 4 °C until used. Selected water quality parameters of the filtered sewage samples were measured based on the Standard Methods (APHA et al., 2005) and are shown in Appendix A Table S3. The DOC of Sewage 1–4 was 11–15 mg/L, NH_3–N 7–26 mg/L, and $SUVA_{254}$ 1.8–2.1 L/mg/m.

1.4. *AS samples*

Three AS samples were collected from a rural domestic WWTP, an urban domestic WWTP, and a textile WWTP in SC, used to treat *N*-nitrosamine precursors. The key operating conditions of the three WWTPs are summarized in Appendix A Table S2. AS liquor (5–10 L) was grabbed from the aeration basin of each WWTP, transferred to the laboratory within 1 hr, and then aerated for less than 12 hr at 23±2 °C for preconditioning. Prior to the AS treatment test, a mineral salt solution (pH 7.4 ± 0.2, Appendix A Table S4, OECD, 2003) was used to wash AS solids three times to remove the residual *N*-nitrosamine precursors from AS liquor. The washing procedure is as follows; (i) AS liquor was centrifuged (2000 × g, 5 min), (ii) the supernatant was decanted, and the pellet portion was harvested and resuspended in the mineral salt solution (i.e., 6000 mg/L mixed liquor suspended solids (MLSS)), and (iii) the same procedures were repeated three times. The final MLSS concentration was adjusted to ~3000 mg/L.

1.5. *Batch AS treatment tests*

500-mL washed and resuspended AS was transferred to 1-L incubation bottles, dosed with a target model compound, urine, feces or KG 2 sample, respectively, under well mixing condition, and then incubated for 6 or 24 hr at 23±2 °C under aerobic condition (i.e., 150 L/(m^3•min) compressed air). The concentration of model compounds was set at 20–1000 nmol/L to achieve ~1000 ng/L of an initial NDMA FP. At the end of incubation, 250-mL mixed liquor was harvested and filtered through 0.45-μm membrane. The filtrate was used for *N*-nitrosamine formation tests.

Two controls (i.e., precursor-free and AS-free) were prepared and run in parallel with the AS treatment tests. Precursor-free controls (i.e., AS filtrates) were prepared by incubating the washed and resuspended AS in the mineral salt solution with no NDMA precursors added. The AS-free controls were prepared by measuring *N*-nitrosamine formation from the target precursors dosed in the filtrates of AS without any biosolids present. Reductions in *N*-nitrosamine UFC or FP during AS treatment were calculated based on the following equation (Eq. (1)). All tests were run in duplicate.

$$R = \frac{\text{NDMA UFC or FP in effluent}}{\text{Control 1} - \text{Control 2}} \times 100\% \quad (1)$$

R is the reduction in NDMA UFC or FP during batch AS treatment test, Control 1 and Control 2 are the NDMA UFC (ng/L) or FP (ng/L) measured in AS-free control and precursor-free control, respectively.

1.6. *N–Nitrosamine formation tests*

Seven selected *N*-nitrosamines, including NDMA, NDEA, NPYR, *N*-nitrosopiperidine (NPIP), NMOR, NDPA, and *N*-nitrosodibutylamine (NDBA) were tested for *N*-nitrosamine formation. Their key physiochemical properties are shown in Appendix A Table S5. These seven *N*-nitrosamines are among the most frequently detected and reported *N*-nitrosamines in wastewater effluents and wastewater-impacted source waters (Zeng et al., 2016a; Sgroi et al., 2018).

For *N*-nitrosamine formation tests, a NH_2Cl stock solution was freshly prepared by adding a sodium hypochlorite solution (NaClO, ~4000 mg Cl_2/L) drop by drop to an ammonium chloride solution (NH_4Cl, ~1000 mg N/L) at pH 9 with a Cl:N

mass ratio at 4:1. A pre-determined volume of NH_2Cl stock solution was then added to the filtered samples to achieve the target dosages (i.e., 5 mg Cl_2/L for the UFC test, 100 mg Cl_2/L for the FP test). The chloraminated samples were incubated at 23±2 °C in the dark for 3 days (UFC test) or 5 days (FP test) before excess sodium thiosulfate ($Na_2S_2O_3$) was added to quench residual chloramines. The chloramine residuals for UFC tests ranged from ~0.5 to 2.5 mg/L in general. The urine, feces samples (diluted) and sewage samples showed higher chlorine residuals (~2.0 mg/L) than greywater samples (~1.0 mg/L) and samples (~0.5 mg/L) with exogenous electron donors including glucose and yeast extract. Due to higher DOC, an initial dose of ~10 mg/L chloramine was used for the laundry greywater samples and residuals ranged from ~0.5 to 1.0 mg/L. *N*–Nitrosamines were then extracted using solid-phase extraction (SPE) according to the US EPA Method 521, separated on a gas chromatograph (GC, Agilent 7890A) equipped with a DB-1701 column (30 *m* × 0.25 mm × 0.25 μm), and analyzed with a tandem mass spectrometer (MS-MS, Agilent, Santa Clara, US) (US EPA, 2004). More details were described elsewhere (Beita-Sandi et al., 2019). The minimum reporting levels (MRLs) were determined to be 2 ng/L (for NDMA and NDEA) or 3 ng/L (for the other *N*-nitrosamines). In the presence of nitrite, an unintentional NDMA formation has been reported during the SPE extraction via the reaction with activated carbon cartridges (Chuang et al., 2019b). To assess the impact of nitrite on the NDMA analysis, influents and effluents of both domestic rural and urban WWTPs were collected and the NDMA occurrence levels were compared: ~15 ng/L in influents and ~10 ng/L in effluents. There was no substantial NDMA increase after AS treatment at the plants, which indicates that our NDMA measurements were not affected by nitrite.

2. Results and discussion

2.1. *NDMA formation from model compounds during as treatment*

Prior to AS treatment, NDMA formation from the three model compounds in AS filtrates was measured (Appendix A Table S6). NDMA UFC yields were 0.2%–0.6% for TMA in the AS filtrates, which are comparable to previously reported values (i.e., 0.4% in DDW; Selbes et al., 2013). NDMA UFC yields (i.e., 0.2%–0.7%) for TMA and SMTR in the AS filtrates were found to be lower than NDMA FP yields (i.e., 1.4%–1.8%). MNCL yielded negligible (i.e., undetectable) NDMA UFC in the AS filtrates, though its NDMA FP yield reached 0.7%–1.3%. TMA, SMTR, and MNCL could be more reactive with dichloramine ($NHCl_2$) than NH_2Cl (Selbes et al., 2013). During both the FP and UFC tests, NH_2Cl is much more abundant. In contrast, absolute amount of $NHCl_2$ is more under the FP test than the UFC test because the difference of initial doses (Selbes et al., 2013). $NHCl_2$ concentration was typically <0.5 mg Cl_2/L during the UFC test with a practical NH_2Cl dosage (i.e., 5 mg Cl_2/L). During the FP test, however, $NHCl_2$ concentration can reach 2–5 mg Cl_2/L (Mitch et al., 2003; Krasner et al., 2013). The decay of NH_2Cl can form $NHCl_2$ (Vikesland et al., 2001). Therefore, probably due to the less available $NHCl_2$ and shorter contact time during the UFC test, NDMA UFC yields from TMA, SMTR, and MNCL were lower than NDMA FP yields.

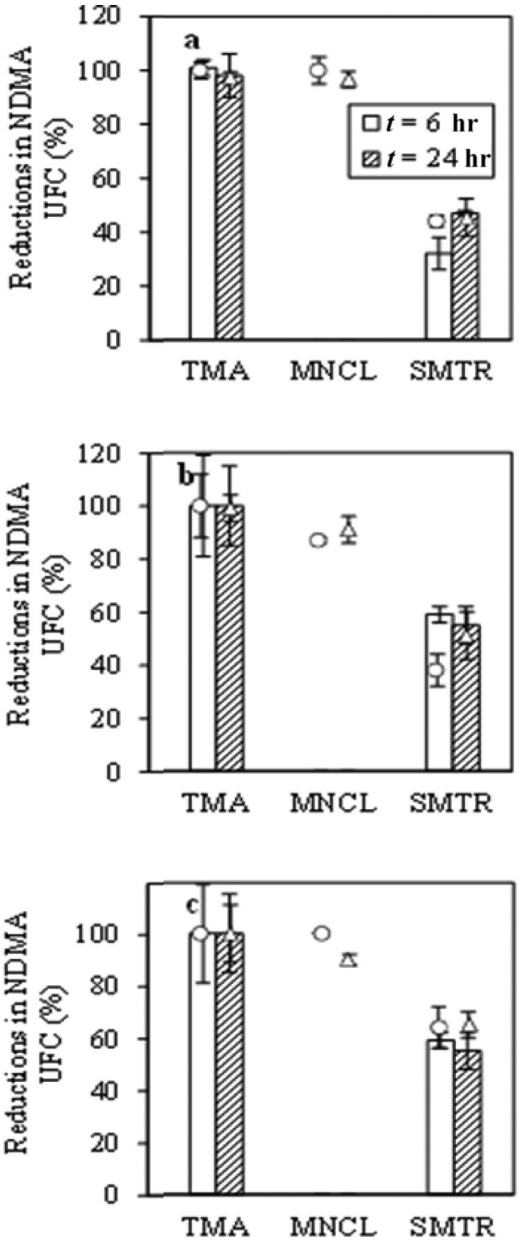

Fig. 1 – Reductions in NDMA UFC and FP from model compounds during treatment with the rural domestic AS (a), the urban domestic AS (b), and the textile AS (c). Bar graph hereafter represents an average value from duplicate tests, dot represents an average reduction in NDMA FP, and error bar hereafter represents the mean deviation from duplicate tests. TMA: trimethylamine, MNCL: minocycline, SMTR: sumatriptan. MNCL showed an undetectable NDMA UFC before or after AS treatment.

After 6 or 24-hr treatment with the three types of AS (i.e., domestic rural, domestic urban and textile AS), NDMA UFC from all tested compounds (except for MNCL with negligible NDMA UFC yield) consistently decreased (Fig. 1). NDMA UFC

from TMA and SMTR decreased by 98%–100% and 32%–59%, respectively, which was comparable to the reductions in NDMA FPs (i.e., 92%–98% and 29%–46%, respectively). This suggests that biological wastewater treatment (e.g., the AS process) reduces NDMA formation under UFC to a similar degree with the removal efficiency of precursor compounds.

2.2. N–Nitrosamine formation from urine and feces blackwaters during AS treatment

Prior to AS treatment, the formation of most *N*-nitrosamines' UFC was as low as <10 ng/L in raw urine and feces blackwaters, bathroom washbasin greywater, and shower greywater (Appendix A Table S7). These findings are generally consistent with previously reported values (Zeng and Mitch, 2015). An exception was for NDBA UFC (28–33 ng/L) in bathroom washbasin and shower greywaters which were higher than reported values. Only for the laundry greywater, the formation of all seven *N*-nitrosamines (7–36 ng/L) were detected under UFC chloramination. In kitchen greywater, NDMA UFC (64 ng/L) and NPYR UFC (18 ng/L) were also detected at relatively high levels.

After 6 and 24-hr treatment with the domestic (rural or urban) AS, NDMA UFC from urine blackwaters increased from undetectable to 1616 ng/L (Fig. 2a), or from 197 to 2083 ng/L (Fig. 2c), though decreased from 5454 to 690 ng/L after treatment with the textile AS (Fig. 2e). In contrast, NDMA FP from urine blackwaters consistently decreased after 6 or 24-hr treatment with the three types of AS (Fig. 2b, 2d, and 2f). The increases in NDMA UFC were presumably because of the removal of bulk organic matters (e.g., DOC; Appendix A Table S8) during the AS treatment, which is considered to favor NDMA formation under UFC. A lower DOC may consume less chloramines (especially $NHCl_2$), complex less with NDMA precursors, and thus enhance the reactions between chloramines and precursors forming NDMA (Vikeland et al., 2001; Shen and Andrews, 2011). However, the reductions in DOC may insignificantly affect NDMA formation under the FP test where excess amount of NH_2Cl is dosed, and thus the biodegradation of NDMA precursors by AS can effectively decrease NDMA FPs.

This was confirmed by further experiments with diluted blackwater samples. With the DOC of urine blackwater decreased from 5.5 to 2.2 and 1.0 mg/L in DDW (i.e., via adjusting raw urine dilution factors from 100 to 250 and 1000 folds), NDMA UFC increased from 332 ng/L to 868 and 10,084 ng/L, respectively (Fig. 3). Dilution would reduce not only the organics in the samples, but presumably also the precursors, yet still formed more NDMA. Furthermore, increasing the DOC of urine blackwater from 2.2 to 4.4 mg/L (i.e., by reducing raw urine dilution factors) during AS treatment caused to lower NDMA UFC (i.e., decreased from ca. 2000 to < 40 ng/L) in secondary effluents (Appendix A Fig. S1). The effect of exogenous electron donors including glucose and yeast extract on the deactivation of *N*-nitrosamine precursors was further investigated. Adding DOC (i.e., 144 mg/L glucose and 133 mg/L yeast extract, equivalent to ~270 mg/L DOC) to urine blackwater during AS treatment caused also decreased NDMA UFC (i.e., from 308–2012 ng/L to 10–825 ng/L) in secondary effluents (Fig. 4). These results clearly suggest a substantial effect of DOC on NDMA UFC from urine blackwater during AS treatment. In contrast, NDMA FP from urine blackwater exhibited a similar degree of reductions with (i.e., 44%–53%) and without (i.e., 60%) added DOC during 24-hr AS treatment.

It has been conceived that an effective removal of NDMA precursors (i.e., FP) could lead to a deceased NDMA formation in secondary effluents. However, our data showed an opposite result. With the secondary effluents further purified during advanced wastewater treatment (e.g., H_2O_2/UV oxidation, membrane filtration processes) for potable reuse, NDMA UFC may further increase due to an enhanced removal of DOC, although NDMA FP can be apparently reduced (Sgroi et al., 2018; Takeuchi et al., 2018). Such increases in NDMA UFC may pose further health risks, especially in reused waters, which requires further elucidation.

After 6-hr incubation with the rural and urban domestic AS, NDMA UFC from feces blackwaters increased from 3 ng/L to 19 and 52 ng/L, respectively, though stayed around 80 ng/L after 6-hr treatment with the textile AS (Fig. 2a, 2c and 2e). In contrast, NDMA FP from feces blackwaters consistently decreased during 6-hr treatment with the three types of AS (Fig. 2b, 2d and 2f). Increasing the incubation time from 6 to 24 hr promoted the reductions in both NDMA UFC and FP. After 24-hr treatment with the three types of AS, NDMA UFC decreased up to undetectable levels. At a shorter HRT (e.g., 6 hr), the removal efficiency of *N*-nitrosamine precursors (i.e., reduction in *N*-nitrosamine FP) was lower than the removal of DOC during AS treatment of blackwaters and greywaters (Appendix A Fig. S2). However, the removal of DOC may promote NDMA UFC in effluents at the same time. At a prolonged HRT (e.g., 24 hr), on the other hand, the removal of *N*-nitrosamine precursors was substantially enhanced (Appendix A Fig. S2), and *N*-nitrosamine UFC may decrease due to a substantially reduced amount of NDMA precursors in effluents. Therefore, HRT can play an important role in the formation of *N*-nitrosamines in secondary effluents. At a typical HRT (i.e., 5–14 hr) of WWTPs (Gray, 2004), *N*-nitrosamine UFC may possibly increase after AS treatment due to the removal of DOC, rather than decrease caused by the removal of precursors. UFC of the other *N*-nitrosamines from urine and feces blackwaters was undetectable, and thus not examined for batch AS treatment test.

2.3. N–Nitrosamine formation from greywaters during AS treatment

During the UFC test, all the seven *N*-nitrosamine species (i.e., 7–36 ng/L) were detected in laundry greywater before AS treatment. In laundry greywater containing detergent (LG 1), NDMA UFC increased from 11 to 71 and 253 ng/L after 6-hr treatment with the rural and urban AS, respectively, though decreased after treatment with the textile AS (Fig. 5, Appendix A Figs. S3 and S4). Similarly, NDEA UFC increased from 7 ng/L to 77 and 29 ng/L, respectively. The increases in NDMA UFC and NDEA UFC were likely attributed to an increased amount of NDMA and NDEA precursors (i.e., measured as FPs) (Fig. 5, Appendix A Figs. S3 and S4) through biodegradation of detergent ingredients (e.g., surfactants, emulsifiers) and synthetic dyes in LG 1. They may form intermediate products containing either DMA or diethylamine (DEA) group (Appendix A Fig.

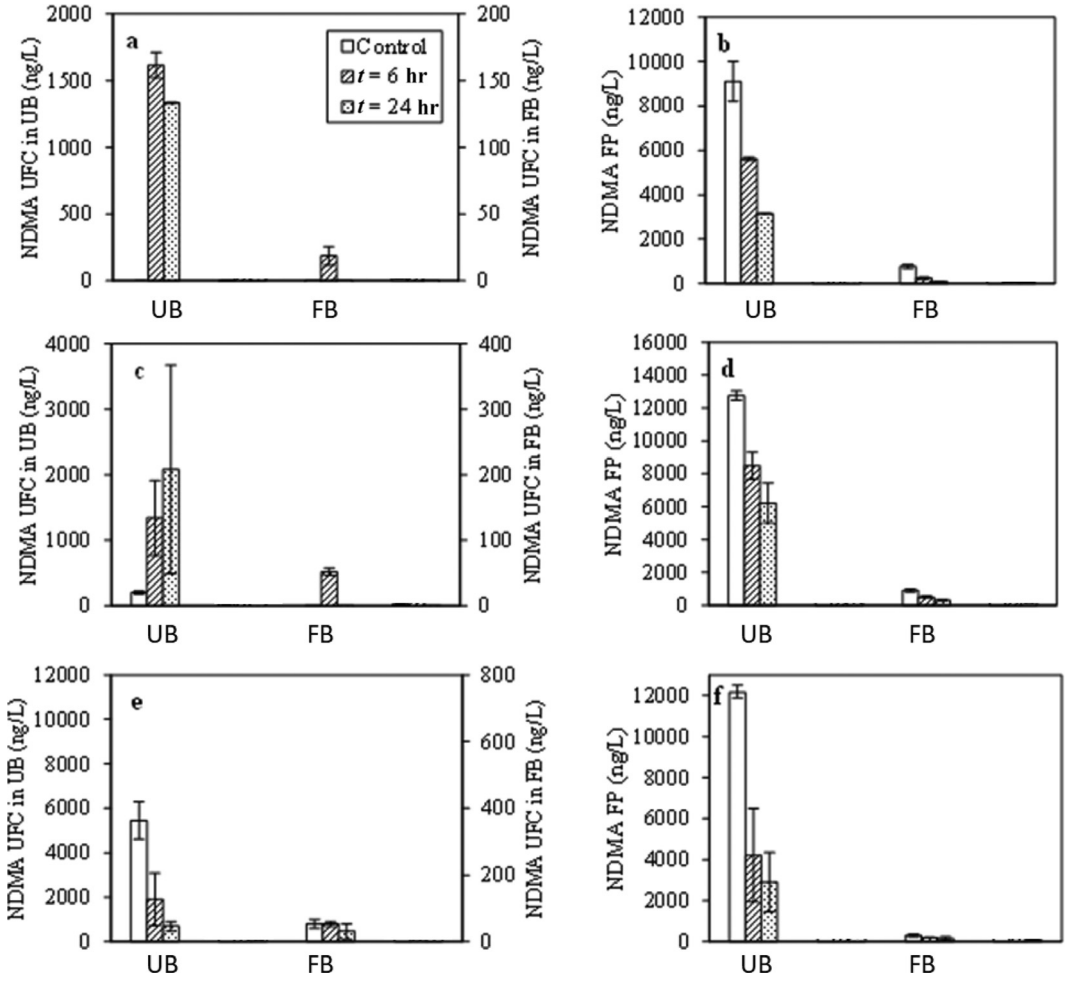

Fig. 2 – NDMA UFC and FP from urine (UB) and feces (FB) blackwaters after treatment with the rural domestic AS (a and b), the urban domestic AS (c and d), and the textile AS (e and f). UB: urine diluted 250 folds in tap water; FB: feces diluted 100 folds. Control test: *N*-nitrosamine UFC and FP before any AS treatment.

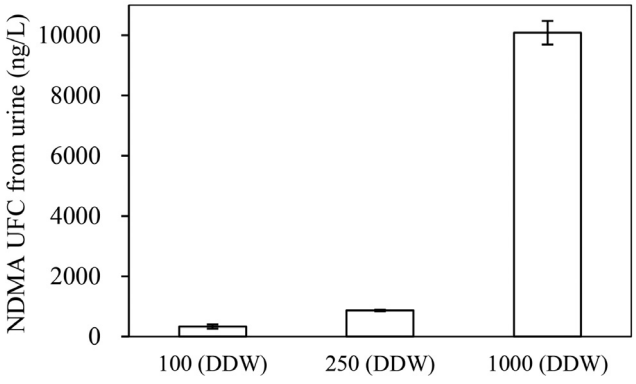

Fig. 3 – NDMA UFC from raw urine diluted in deionized and distilled water (DDW). 100 (DDW): urine diluted 100 folds in DDW; 250 (DDW): urine diluted 250 folds in DDW; 1000 (DDW): urine diluted 1000 folds in DDW.

S5). Such biodegradation products may exhibit higher NDMA or NDEA yields than their parent compounds.

In laundry greywater containing both detergent and fabric softener (LG 2), both NDMA UFC and NDEA UFC decreased after 6- or 24-hr treatment with the three types of AS. Their precursors (i.e., measured as NDMA FP and NDEA FP) in LG 2 also decreased after AS treatment. The presence of fabric softener in LG 2 may impact biodegradation activities of AS (e.g., inhibitory effects of quaternary amines present in fabric softener), and thus NDMA UFC and NDEA UFC (and their FPs) from LG 2 showed a distinct changing trend after AS treatment compared to LG 1. Different from urine or feces blackwaters which had limited amounts of DOC after AS treatment (i.e., <0.8 mg/L), laundry greywater contained relatively high DOC (i.e., 26–55 mg/L) even after AS treatment (i.e., 6–16 mg/L DOC), thus the removal of DOC from laundry greywater did not affect *N*-nitrosamine UFC. *N*–Nitrosamine UFC generally decreased after treatment with the three types of AS (Fig. 5, Appendix A Figs. S3 and S4). Further increasing DOC (i.e., adding glucose and yeast extract with 270 mg/L DOC) to laundry greywater little impacted NDMA UFC after AS treatment (i.e., <228 and <253 ng/L before and after adding DOC, respectively; Appendix A Fig. S6). Among the seven *N*-nitrosamines tested, NDPA UFC and NDBA UFC from laun-

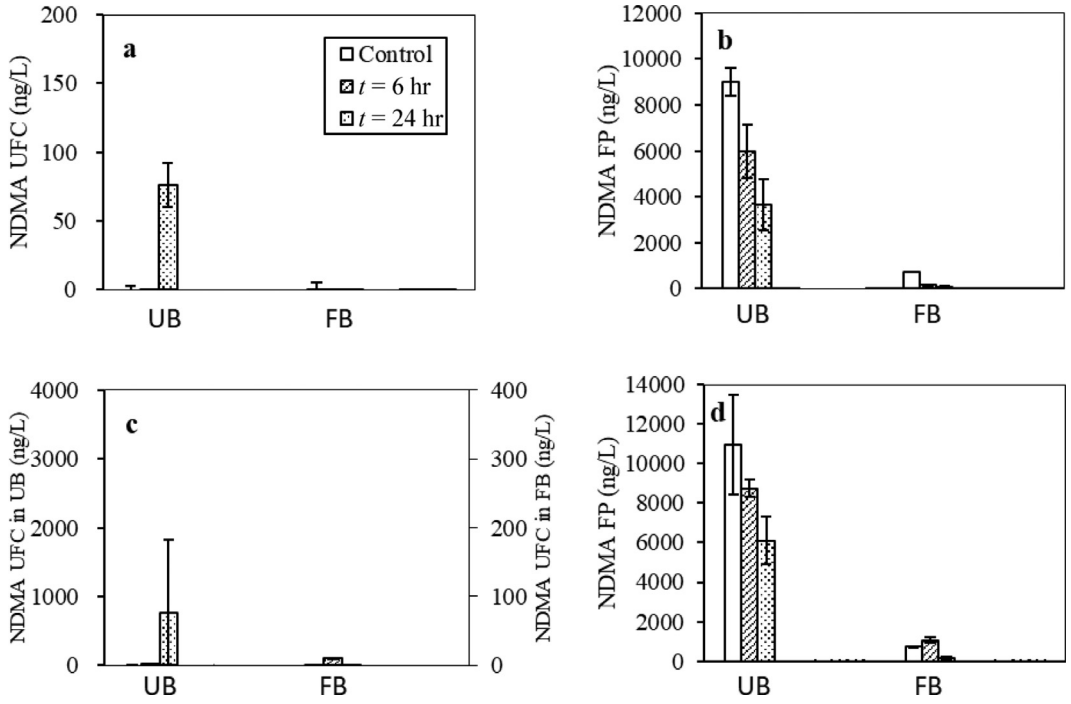

Fig. 4 – NDMA UFC and FP from blackwaters during treatment with the rural domestic AS (a and b) and the urban domestic AS (c and d) with an addition of glucose and yeast extract (i.e., 270 mg/L DOC).

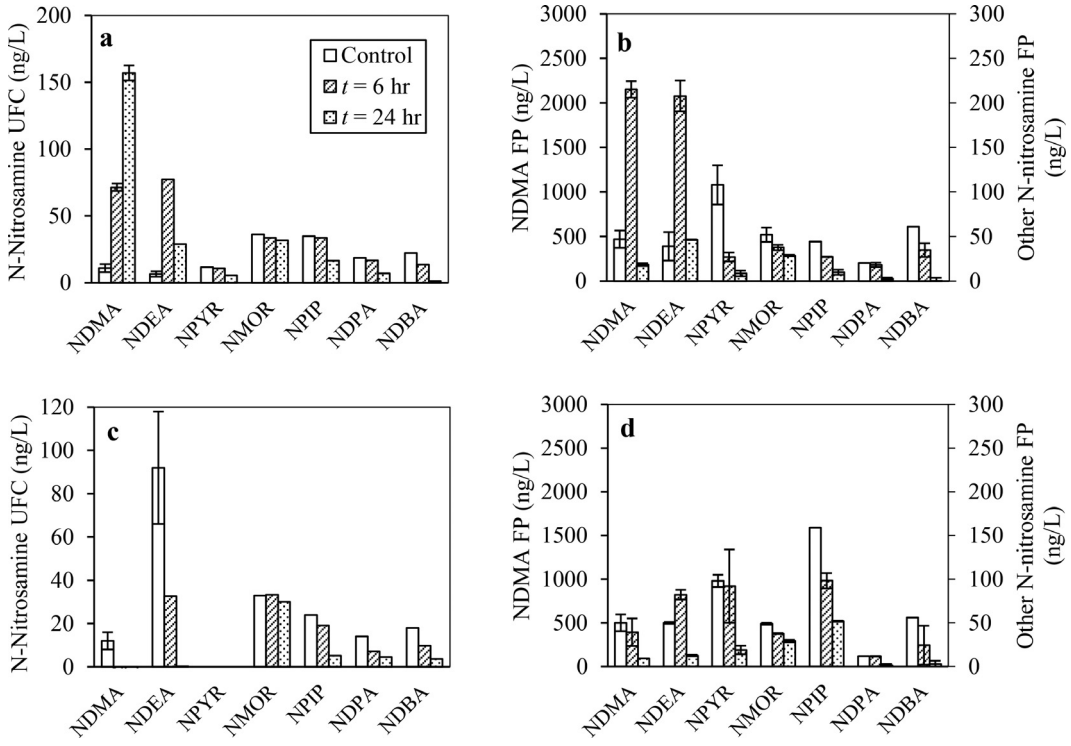

Fig. 5 – N–Nitrosamine UFC and FP from laundry greywater containing detergent only (LG 1, a and b) and detergent plus fabric softener (LG 2, c and d) during treatment with the rural domestic AS. Figures (b) and (d) are reprinted from Zhang et al. (2020a).

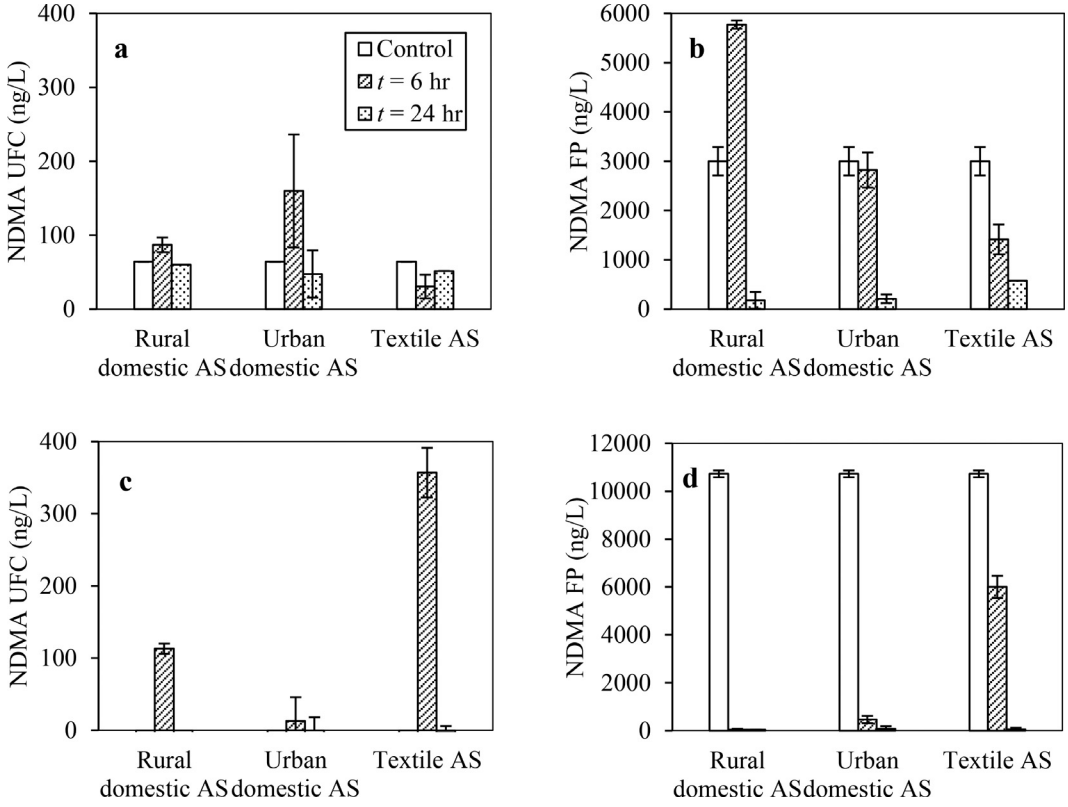

Fig. 6 – NDMA UFC and FP from kitchen greywater containing dishwashing detergent (KG 1, a and b), and food leachates (KG 2, c and d). Figures (b) and (d) are reprinted from Zhang et al. (2020a).

dry greywater were substantially reduced (i.e., 70%–100%), followed by NPYR UFC and NPIP UFC (i.e., 50%–92%). NMOR UFC was reduced by only 3%–42% after 24-hr AS treatment. Such reductions in *N*-nitrosamine UFC were generally consistent with their FP removals. A significant (p <0.05) correlation between the reductions in *N*-nitrosamine UFC and FP was observed after AS treatment of laundry greywaters (Appendix A Fig. S7).

In kitchen greywater containing food leachates (KG 2), NDMA UFC was undetectable before AS treatment. After 6-hr treatment with the three types of AS, however, NDMA UFC from KG 2 increased to 13–357 ng/L, though decreased to undetectable after 24-hr treatment (Fig. 6). Similar to urine and feces blackwaters, the increases in NDMA UFC from KG 2 are likely associated with a substantial removal of DOC (i.e., from 21 to 0.4 mg/L) which may enhance NDMA UFC after AS treatment. In contrast, NDMA FP from KG 2 consistently decreased (i.e., from 10,723 ng/L to 32–6003 ng/L and 32–73 ng/L, respectively) after 6- and 24-hr AS treatment (Fig. 6).

NDMA UFC from kitchen greywater containing dishwashing detergent (KG 1) increased (i.e., by 36%–150%) or remained relatively constant after AS treatment (Fig. 6). However, the increase in NDMA UFC was unlikely caused by the removal of DOC from KG 1. Different from KG 2 with a low DOC (i.e., 0.4 mg/L) after 6-hr AS treatment, KG 1 showed higher DOC (i.e., 4.2–16 mg/L) before and after 6-hr AS treatment. The increase in NDMA UFC was likely because of an increased amount of NDMA precursors (i.e., FP) in KG 1. NDMA FP from KG 1 increased (or remained relatively constant) after 6-hr AS treatment (Fig. 6). For the other *N*-nitrosamine species (i.e., NPYR and NDBA), their UFC decreased after 6-hr AS treatment of KG 1 (Appendix A Fig. S8). The reductions in NPYR UFC and NDBA UFC were generally consistent with their FP reductions (Appendix A Fig. S8).

NDMA UFC from the other types of greywaters (i.e., shower and bathroom washbasin greywaters) was generally low (i.e., <14 ng/L) before or after AS treatment, and thus was not examined for batch AS treatment test. NDBA UFC from shower greywater containing shampoo (SG 2; 28 ng/L) and bathroom washbasin greywater (WG; 33 ng/L) decreased after 6- or 24-hr treatment with the three types of AS (Appendix A Fig. S9). The decreases in NDBA UFC were comparable to the removal efficiencies of its precursors (i.e., measured as NDBA FPs) (Appendix A Fig. S9). In SG 2 or WG, the DOC levels were high before (i.e., 33–78 mg/L) or after (i.e., 1.1–6.4 mg/L) AS treatment, and NDBA UFC was thus less affected by the removal of DOC. Rather, NDBA UFC decreased to a similar degree with its FP removal. UFC of the other *N*-nitrosamines (e.g., NDEA, NMOR, NPIP and NDPA) in shower and bathroom greywaters was undetectable before or after AS treatment, and thus was not examined for the batch AS treatment test.

These results suggest that *N*-nitrosamine formation (UFC) in secondary effluents could depend on different factors, including the ingredients in greywaters (e.g., food leachates, detergent, personal care products such as shampoo), operating conditions (e.g., AS types, HRT) of the AS process, and *N*-nitrosamine species.

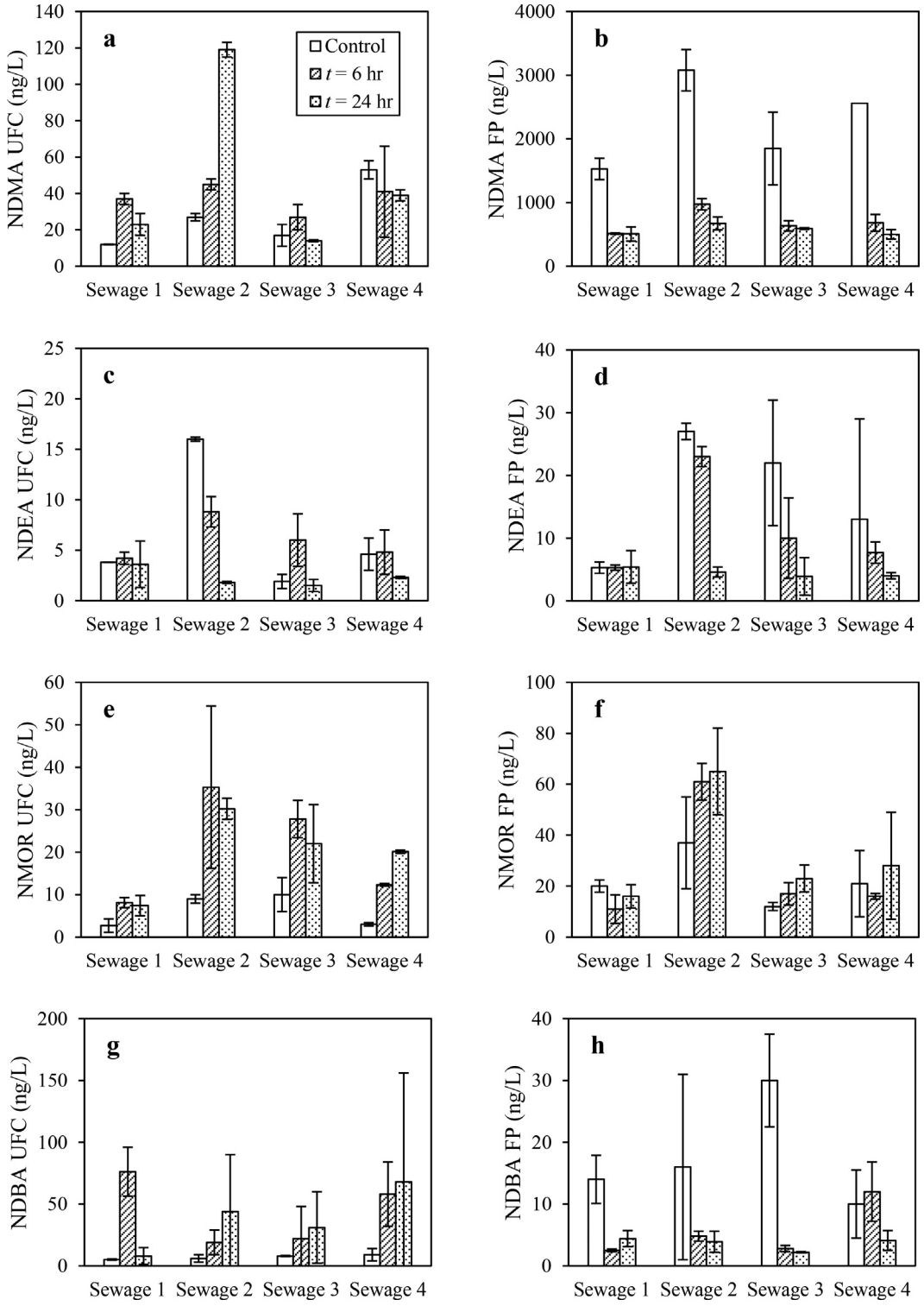

Fig. 7 – N–Nitrosamine UFC and FP from sewage samples during treatment with the rural domestic AS. Sewage 1–4: municipal wastewater samples containing <1–25% industrial discharges, collected from inlets to aeration basins at four WWTPs in SC, USA.

2.4. N–Nitrosamine formation from sewage samples during AS treatment

Four sewage samples (Sewage 1–4) containing <1%–25% industrial discharges were grabbed from local WWTPs, and their *N*-nitrosamine UFC and FP were monitored during batch treatment test with the rural domestic AS. The rural domestic AS was selected because of its superior capability of removing NDMA precursors from most model compounds and sewage components tested. Before AS treatment, measured NDMA UFC from sewage samples was 12–57 ng/L, NDEA UFC 2–16 ng/L, NMOR UFC 3–10 ng/L, and NDBA UFC 5–9 ng/L. UFC of the other *N*-nitrosamines (i.e., NPYR, NPIP and NDPA) was generally undetectable (i.e., <3 ng/L), and thus was not examined for the batch AS treatment test.

As expected, NDMA UFC increased or remained relatively constant after 6 or 24 hr treatment with the rural domestic AS (Fig. 7a). The largest increase in NDMA UFC (i.e., by 441%) was achieved after 24-hr treatment of Sewage 2 (containing a 25% industrial discharge). Similar to what was observed in most blackwaters and greywaters, NDMA FPs from sewage samples deceased (i.e., by 66%–80%) after 6 or 24 hr treatment with the rural domestic AS (Fig. 7b). Although the DOC levels in sewage samples (i.e., 11–15 mg/L) were relatively high after AS treatment (i.e., 4.8–9.7 mg/L), NDMA UFC was found to still increase. This was likely because the characteristics of bulk organic matters (e.g., $SUVA_{254}$) may also impact NDMA formation (UFC) after AS treatment in addition to the concentration of DOC. As a mixture of blackwaters and greywaters, domestic sewage has been retained (for hours or even days) in the sewer system before entering WWTPs. The anaerobic/anoxic condition in a typical sewer system may transform the components of bulk organic matters, causing a substantially altered characteristics of DOC. The $SUVA_{254}$ in all the four sewage samples (i.e., 1.8–2.1 L/mg/m) was consistently higher than any of the sewage components (i.e., 0.3–1.1 L/mg/m) which showed relatively high DOC levels after AS treatment.

NDEA UFC changed differently after AS treatment depending on sewage samples (Fig. 7c). In Sewage 1 and Sewage 3, NDEA UFC largely remained constant or increased after AS treatment, while in Sewage 2 and Sewage 4, NDEA UFC even decreased after AS treatment. In contrast, NDEA FP generally decreased after AS treatment except that NDEA FP in Sewage 1 remained constant (Fig. 7d). Different from NDMA precursors which predominately originate from urine blackwater, NDEA precursors in sewage samples could mainly come from laundry greywater (Zhang et al., 2020a), and thus may have distinct chemical structures, physiochemical properties, and biodegradabilities than NDMA precursors. NDEA UFC thus exhibited a distinct changing trend than NDMA UFC despite an identical AS testing protocol used. NMOR UFC from all the four tested sewage samples consistently increased after 6 or 24 hr treatment with the domestic AS (Fig. 7e). The increases in NMOR UFC (i.e., by 120%–567%) were larger than NMOR FP (i.e., by <76%; Fig. 7f), suggesting a potential effect of the DOC removal (i.e., 25%–63%) which partially favored NMOR UFC after AS treatment. NDBA UFC in all four sewage samples increased after treatment with the rural domestic AS, while NDBA FP consistently decreased (Fig. 7g and 7h).

These results suggest that biological wastewater treatment (i.e., via the AS process) may slightly decrease, or even increase *N*-nitrosamine UFC in secondary effluents, although *N*-nitrosamine precursors (i.e., measured as FPs) could be substantially removed from sewage. There is no single factor that can fully explain the changing patterns of *N*-nitrosamine UFC during the AS treatment. The sources of precursors (e.g., model compounds, blackwaters and greywaters), AS types (e.g., domestic vs textile), organic loadings (e.g., DOC levels, $SUVA_{254}$) in influents, and AS treatment conditions (e.g., HRT) can make combined contributions to the *N*-nitrosamine formation in secondary effluents.

3. Conclusions

NDMA UFC from TMA and SMTR decreased to similar degrees with their NDMA FP reductions, after 6 or 24 hr treatment with domestic rural, domestic urban, and textile AS. To reduce NDMA formation (measured under UFC) from trace-level precursor compounds (such as amine-based pharmaceuticals), increasing HRT or increasing biodegradation activities of AS might be useful ways.

NDMA formation under the UFC chloramination from urine and feces blackwaters and kitchen greywater containing food leachates substantially increased after AS treatment, which was primarily linked to a substantial removal of bulk organic matters (e.g., DOC) after AS treatment. A lower DOC consumes less chloramines (especially $NHCl_2$), complexes less with NDMA precursors, and thus enhances the reactions between chloramines and precursors forming NDMA. The total amounts of NDMA precursors which were measured as FPs, however, typically decreased after AS treatment and were negligibly affected by the DOC removal. In laundry greywater with relatively high DOC (i.e., >4 mg/L) before or after AS treatment, most *N*-nitrosamine UFC (not including NDMA UFC) decreased to similar degrees with their FP removals, suggesting a less impact of the DOC removal on *N*-nitrosamine UFC after AS treatment.

In domestic sewage (containing <1%–25% industrial discharge), *N*-nitrosamine UFC increased or remained constant after AS treatment, although their precursors (i.e., measured as FPs) were generally reduced. The precursor sources, organic loadings in influents, and operating conditions of the AS treatment could make combined impacts on *N*-nitrosamine UFC in secondary effluents. Strategies of controlling *N*-nitrosamine UFC in wastewater discharge may include enhancing the efficiencies of AS biodegrading *N*-nitrosamine precursors, increasing HRT of the AS process, or even increasing organic loadings in influents, depending on WWTPs (e.g., AS types) and *N*-nitrosamine species

Acknowledgments

Authors would like to acknowledge Water Research Foundation (Project #4591) for their financial supports of this work. We would like to thank Dr. Christophe Darnault for allowing us to use biosafety cabinet, Anne Cumming for her assistance in instrument maintenance. We appreciate Drs. Teng Zeng and

William Mitch for their collecting and processing blackwaters and laundry greywater samples. We also want to thank the three WWTPs in SC, USA, for supporting our AS collections.

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