Supporting Information

Source Characterization and Removal of *N*-Nitrosamine Precursors During Activated Sludge Treatment

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Table S1 Selected water quality parameters in wastewater components (after dilution)

Samples[a]	Dilution factors	DOC[b] (mg/L)	SUVA[c] ($L \cdot mg^{-1} \cdot m^{-1}$)	NH_3-N[d] (mg/L)	TN[e] (mg/L)
U1	250	2.2	1.2	1.4	2.5
U2	250	3.4	1.2	1.6	3.9
F1	150	3.3	1.1	0.5	1.6
F2	150	3.4	1.5	0.1	0.6
L1	N.A.[f]	26	1.0	22	20
L2	N.A.	55	1.3	40	41
S1	N.A.	15	0.3	0.3	6.0
S2	N.A.	33	1.1	0.2	1.8
S3	N.A.	15	0.3	0.2	1.5
K1	N.A.	16	0.5	N.D.[g]	0.6
K2	100	16	0.5	N.D.	0.6

[a]: U1-U2: raw urine samples collected before (U1) and after (U2) taking Zantac, both diluted 250 times in tap water; F1-F2: raw feces samples collected before (F1) and after (F2) taking Zantac, both diluted 150 times in tap water; L1-L2: laundry greywaters collected from washing machine discharges containing laundry detergent only (L1), or laundry detergent plus fabric softener (L2); S1-S3: shower greywater not containing any personal care products (S1), containing shampoo (S2) or body wash only (S3); K1-K2: kitchen greywater containing dishwashing detergent (K1), or mixed raw and cooked food waste leachates diluted 100 times in tap water (K2). [b]: Dissolved organic carbon. [c]: Specific ultraviolet absorbance measured at 254 nm. [d]: Ammonia nitrogen. [e]: Total nitrogen. [f]: Not applicable because there was no dilution. [g]: Not detectable (i.e., < 0.02 mg/L).

Table S2 Key information of the selected wastewater treatment plants (WWTPs)

WWTPs	Activated sludge (AS) samples	Wastewater samples	Influent type	Treatment capacity (mgd)[a]	HRT[b] (h)	SRT[c] (d)	Treatment Process	Nutrients Removal	Industrial impact[d]
WWTP1	Domestic AS	WW 1	Domestic wastewater	2.0	22-24	20	Extended aeration	Nitrification	< 1%
WWTP2	Municipal AS	WW 2	Domestic wastewater and industrial discharge	70	13.5	12	Anaerobic/anoxic/oxic	Nitrogen and phosphorus removal	25%
N.A.[e]	Textile AS	Not collected	Textile wastewater	1.7	5	26	Extended aeration	None (N is added)	100%
WWTP3	Not collected	WW 3	Predominantly domestic wastewater	3.0	N.A.	N.A.	N.A.	N.A.	8-15%
WWTP4	Not collected	WW 4	Predominantly domestic wastewater	4.0	N.A.	N.A.	Membrane bioreactor	Nitrification	8-15%

[a]: Million gallons per day. [b]: Hydraulic retention time. [c]: Solids retention time. [d]: The volume fraction of industrial discharge in WWTP influent. [e]: Not applicable or not available.

Table S3 Selected water quality parameters in wastewater samples

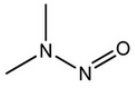
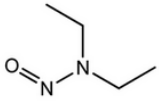
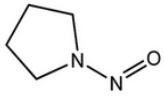
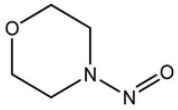
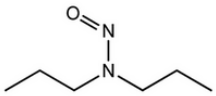
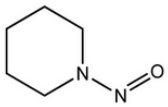
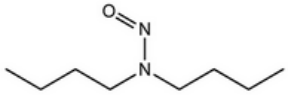
Wastewater samples	DOC (mg/L)	NH_3-N (mg/L)	SUVA ($L \cdot mg^{-1} \cdot m^{-1}$)
WW1	13	26	2.0
WW2	15	24	2.0
WW3	11	19	2.1
WW4	13	7	1.8

Table S4 Recipe of the mineral solution used to wash and resuspend AS solids

Stock solutions[a]	Chemicals	Concentrations (g/L)
A	KH_2PO_4	8.50
	K_2HPO_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 the 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 (OECD, 1992).

Table S5 Key physiochemical properties of the seven selected *N*-nitrosamines

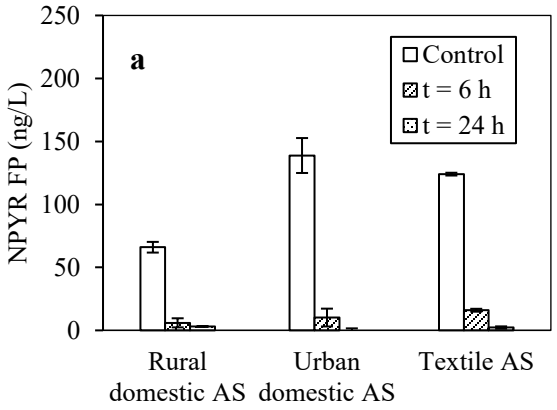
N-nitrosamines	Chemical structures	Molecular weight (g/mol)	log K_{ow}[a]	10^{-6} cancer risk level[b] (ng/L)
N-Nitrosodimethylamine (NDMA)		74.0	-0.57	0.7
N-Nitrosodiethylamine (NDEA)		102.1	0.48	0.2
N-Nitrosopyrrolidine (NPYR)		100.1	-0.19	15
N-Nitrosomorpholine (NMOR)		116.1	-0.44	5
N-Nitrosodi-n-propylamine (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]: US NLM (2019). [b]: US EPA (2001).

Table S6 Volume fractions of blackwaters and greywaters in domestic sewage[a]

Wastewater components	Volume fractions (%)
Urine blackwater	22
Feces blackwater	8
Laundry greywater	26
Shower greywater	24
Kitchen greywater	9

[a]: Zeng and Mitch (2015).



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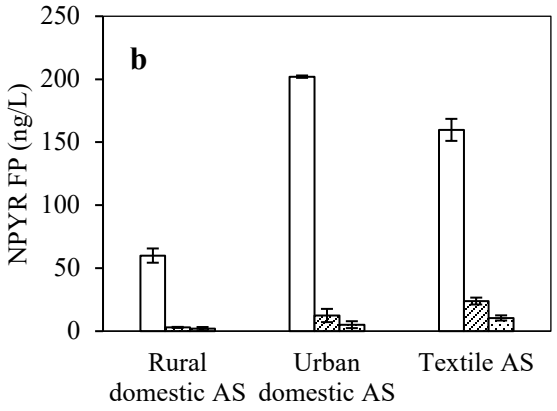

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47 **Fig. S1** Reductions in NPYR FPs during 6 and 24-h AS treatment with urine blackwaters collected
48 (a) before and (b) after taking RNTD. Bar graph hereafter represents the average values from
49 duplicate measurements, and error bars hereafter represent the standard deviations of duplicate
50 measurements.

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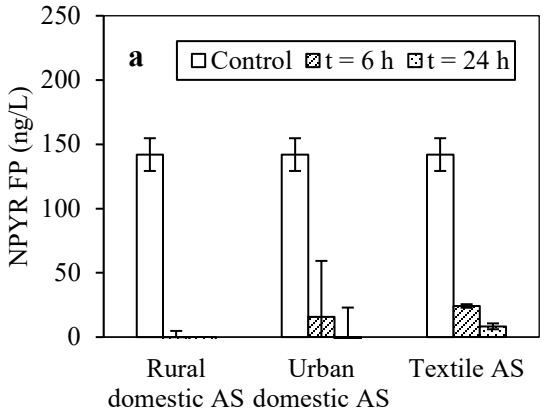

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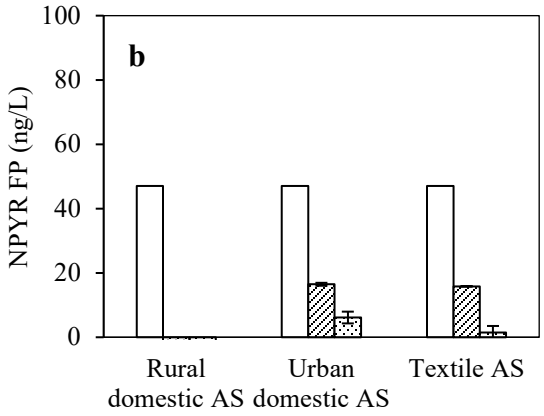

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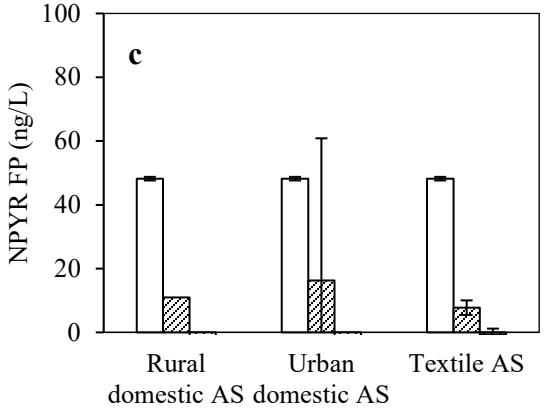

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57 **Fig. S2** Reductions in NPYR FPs during AS treatment with shower greywaters containing (a) no
58 personal care products (S1), (b) shampoo only (S2), and (c) body wash only (S3).

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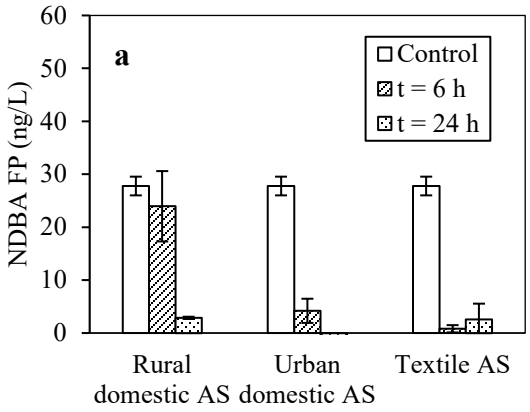

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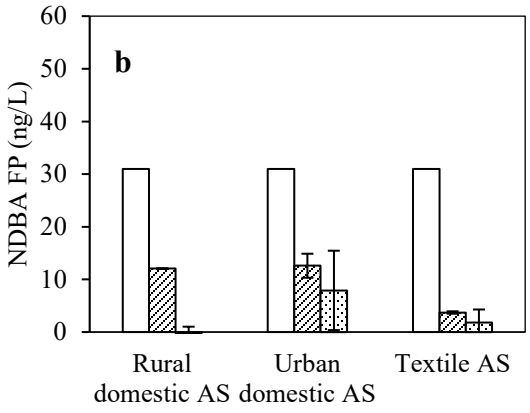

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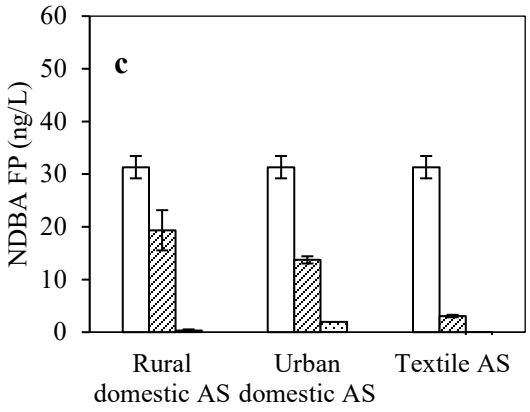

64 **Fig. S3** Reductions in NDBA FPs during AS treatment with shower greywaters containing (a) no
65 personal care products (S1), (b) shampoo only (S2), and (c) body wash only (S3).

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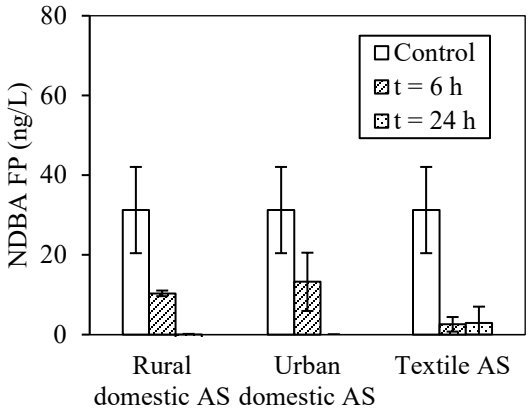

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69 **Fig. S4** Reductions in NDBA FP during AS treatment with kitchen greywater containing

70 dishwashing detergent (K1).

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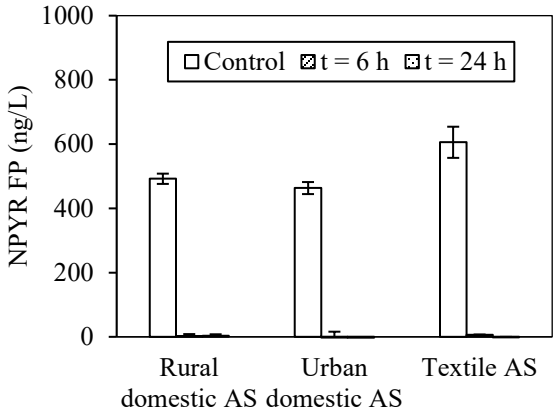

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73 **Fig. S5** Reductions in NPYR FP during AS treatment with kitchen greywater containing food

74 leachates (K2).

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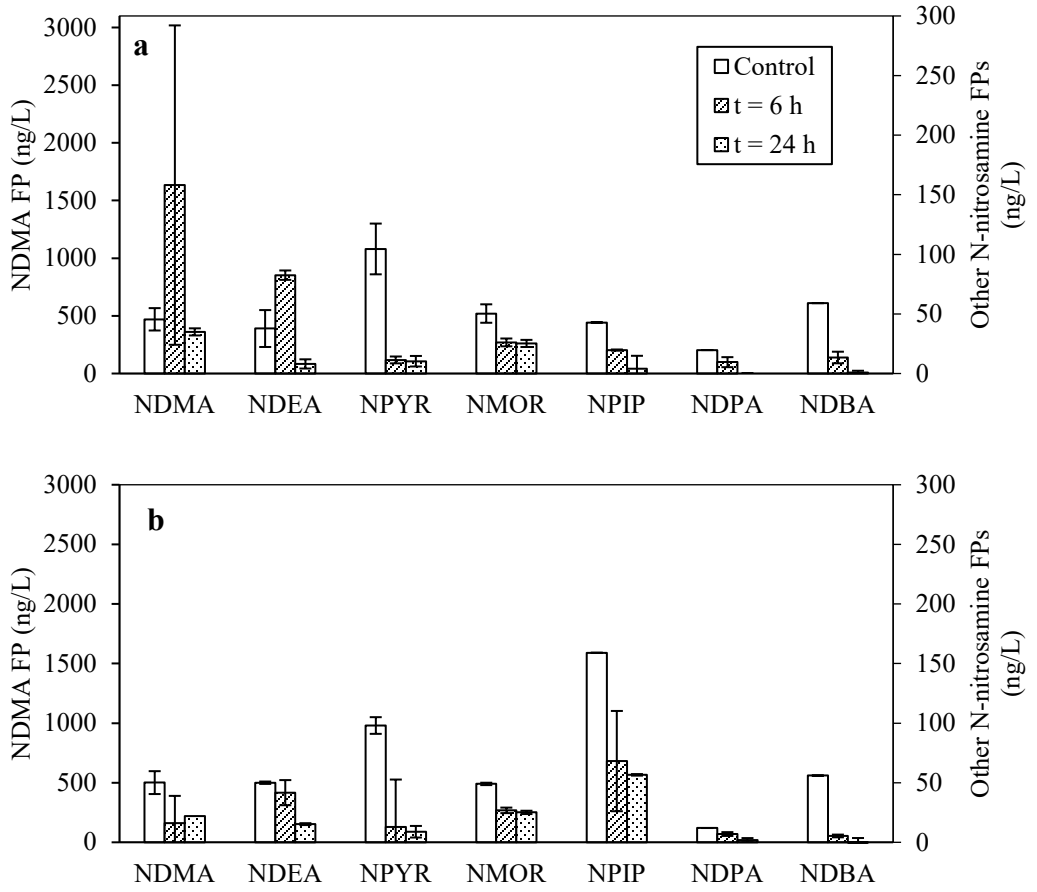


Fig. S6 Reductions in *N*-nitrosamine FPs from laundry greywaters containing (a) laundry detergent only (L1), and (b) laundry detergent and fabric softener (L2) during treatment with the urban domestic AS.

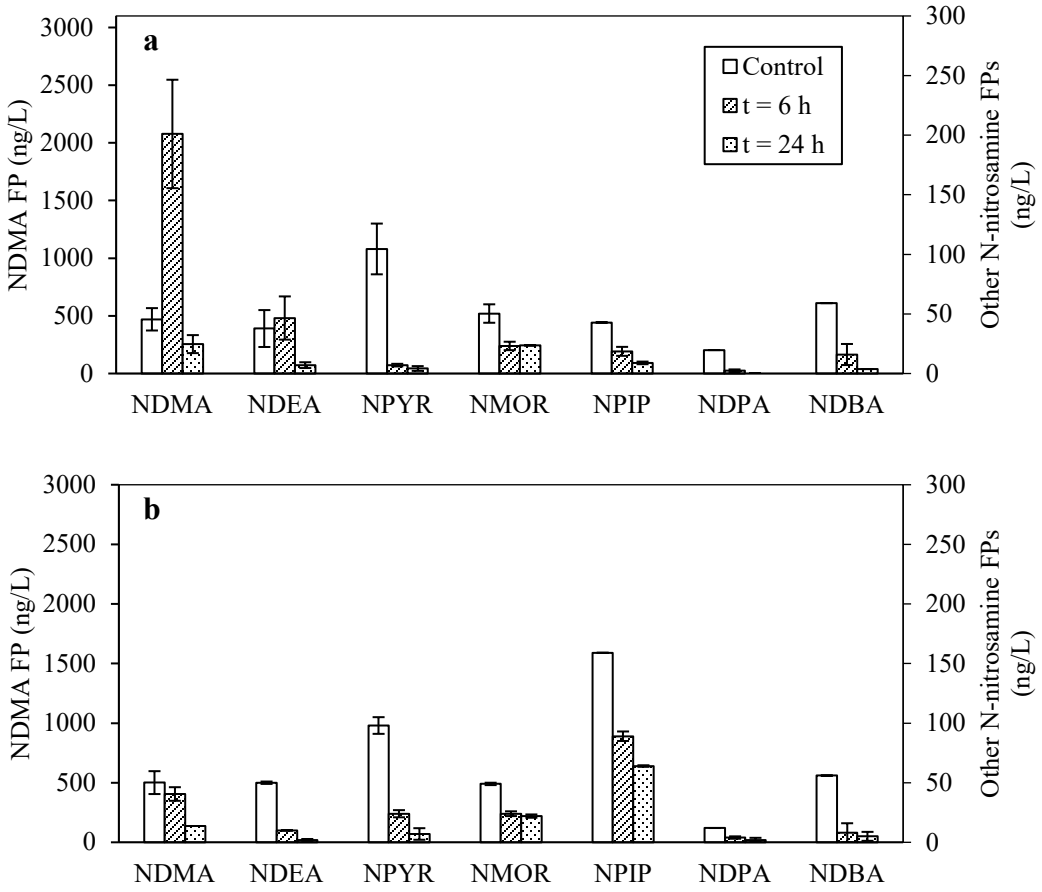


Fig. S7 Reductions in *N*-nitrosamine FPs from laundry greywaters containing (a) laundry detergent only (L1), and (b) laundry detergent and fabric softener (L2) during treatment with the textile AS.

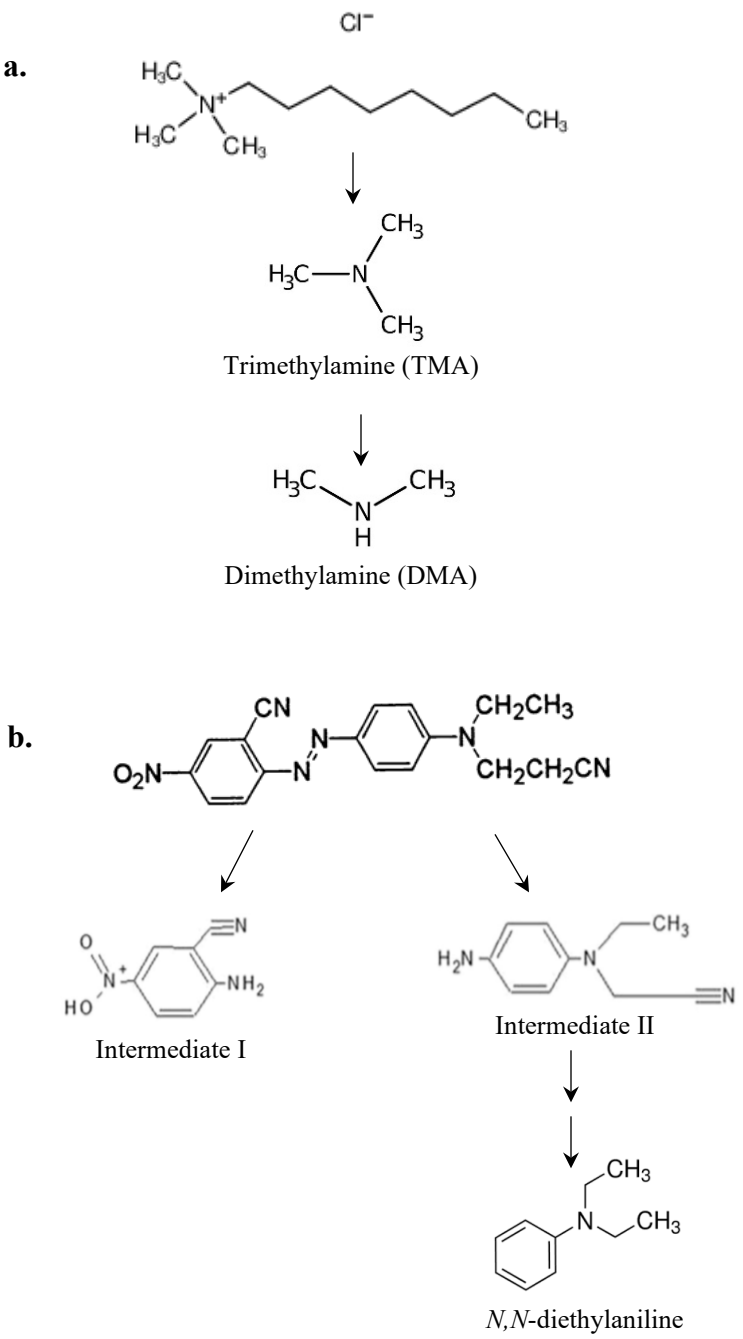


Fig. S8 Biodegradation pathways of (a) an NDMA precursor (i.e., octyltrimethylammonium chloride, a quaternary ammonium salt) widely used in fabric softener, and (b) an NDEA precursor (i.e., an *N,N*-diethyl dye) during biodegradation with (a) a municipal AS inoculum or (b) a pure culture of selected bacterial strain (Verschueren, 2009; Watharkar et al., 2018).

Table S7 *N*-nitrosamine FPs in raw wastewater samples without any AS treatment

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

Table S8 Reductions in NDMA FPs during AS treatment with wastewater samples

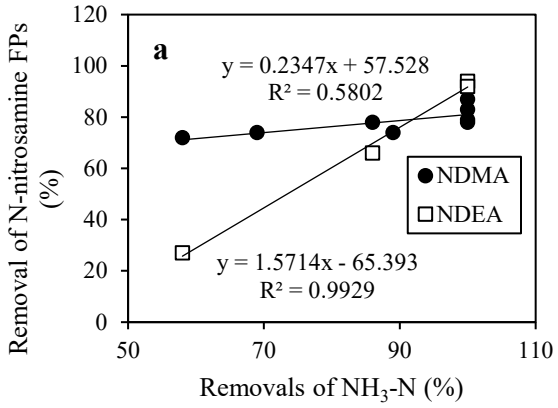
Wastewater samples	Season[a]	Domestic AS (summer)[b]		Municipal AS (spring)[c]		Municipal AS (summer)[d]		Removal at WWTPs[e] (%)
		6-h removal (%)	24-h removal (%)	6-h removal (%)	24-h removal (%)	6-h removal (%)	24-h removal (%)	
WW1	Spring	80	93	63	69	73	85	92
	Summer	74	78					71
WW2	Spring	73	86	74	79	69	87	77
	Summer	72	83					62
WW3	Summer	74	79			77	83	67
WW4		78	87			76	82	51

[a]: Seasons during which the wastewater samples were collected. [b]: AS 1 collected in summer. [c]: AS 2 collected in spring. [d]: AS 2 collected in summer. [e]: Determined via measuring NDMA FPs in WWTP primary and secondary effluents.

Table S9 Reductions in the other *N*-nitrosamine (not including NDMA) FPs during AS treatment with wastewater samples

N-nitrosamines	Wastewater samples	Rural domestic AS (summer)[a]		Urban domestic AS (summer)[a]	
		6-h incubation (%)	24-h incubation (%)	6-h incubation (%)	24-h incubation (%)
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-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 collected in summer. [b]: WW1 collected in spring and summer were treated with the rural and urban domestic AS, respectively. [c]: WW2 collected in spring and summer were treated with the rural and urban domestic AS, respectively. [d]: WW3 and WW4 were collected in summer. [e]: NDEA FPs in WW1 and WW3 were extremely low (i.e., < 10 ng/L), thus not examined during the AS treatment test. [f]: Increased after the AS treatment. [g]: NPIP FP from WW1 and WW2 collected in 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.



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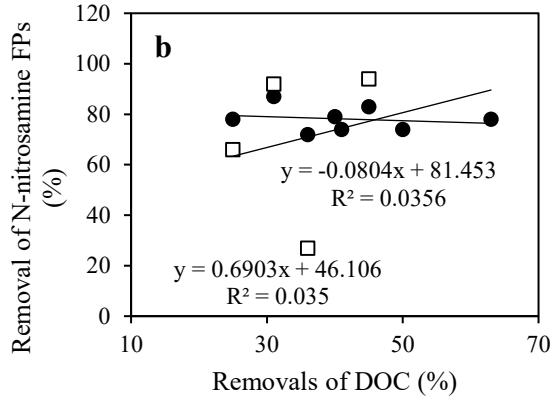

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144 **Fig. S9** Correlation between the reductions of NDMA (or NDEA) FPs and the removal of (a) NH_3-

145 N, and (b) DOC from wastewater samples (WW1-WW4) during treatment with the rural domestic

146 AS.

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