Occurrence and Fate of Nitrosamines and Their Precursors in Municipal Sludge and Anaerobic Digestion Systems

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · JUNE 2009

Impact Factor: 5.33 · DOI: 10.1021/es803067p · Source: PubMed

5 AUTHORS, INCLUDING:

Lokesh P. Padhye
University of Auckland
21 PUBLICATIONS **177** CITATIONS
SEE PROFILE

Ulas Tezel
Bogazici University
54 PUBLICATIONS **659** CITATIONS
SEE PROFILE

Ching-Hua Huang
Georgia Institute of Technology
78 PUBLICATIONS **2,077** CITATIONS
SEE PROFILE

Occurrence and Fate of Nitrosamines and Their Precursors in Municipal Sludge and Anaerobic Digestion Systems

LOKESH PADHYE,[†] ULAS TEZEL,[†] WILLIAM A. MITCH,[‡] SPYROS G. PAVLOSTATHIS,*[,†] AND CHING-HUA HUANG*[,†]

School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, and Department of Chemical Engineering, Yale University, 9 Hillhouse Avenue, New Haven, Connecticut 06520

Received October 30, 2008. Revised manuscript received February 9, 2009. Accepted February 11, 2009.

The fate of six nitrosamines and their secondary amine precursors and total precursors in three municipal wastewater treatment plants' primary sludge (PS), waste-activated sludge (WAS), and anaerobic digester mixed liquor (ADML) was investigated. *N*-Nitrosodimethylamine was detected at significant concentrations, with mean concentrations at 678 ± 302, 394 ± 322, and 271 ± 100 ng/L in PS, WAS, and ADML samples, respectively. *N*-Nitrosopyrrolidine was the other nitrosamine detected in sludge samples but at about an order of magnitude lower concentrations. PS samples also contained the highest concentrations of secondary amines (mostly dimethylamine (DMA) and pyrrolidine) followed by WAS and ADML samples, with mean DMA concentrations at 1280 ± 689, 210 ± 266, and 6.2 ± 3.9 μg/L, respectively. Secondary amines in ADML and some WAS samples accounted for only 20−30% of total nitrosamine precursors underlining the significance of as of yet uncharacterized precursors. Overall, anaerobic sludge digestion was a sink for nitrosamines and secondary amines on the basis of the decreasing trends of these compounds from PS to WAS to ADML after taking mass balances into account. An anaerobic bioassay conducted with ADML showed complete degradation of secondary amines even without additional carbon sources, while nitrosamine removal required carbon addition and was directly related to the chemical oxygen demand consumption.

Introduction

Nitrosamines are probable human carcinogens, with water concentrations as low as 0.2 ng/L associated with a 10^{-6} lifetime cancer risk (*1*). Currently, six nitrosamines are included in the Unregulated Contaminant Monitoring Regulation (UCMR 2) (*2*) and listed in the recently proposed Contaminant Candidate List 3 (CCL 3) (*3*) by the U.S. EPA. The six nitrosamines are *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), and *N*-nitrosodi-*n*-butylamine (NDBA). Industrial applications of nitrosamines have largely been curtailed; however, research in recent years has shown that nitrosamines, particularly NDMA, can be generated in water and wastewater treatment systems by chlorine-based disinfection processes, making them an important emerging group of potentially hazardous disinfection byproducts (DBPs) (*4*−*7*). Several members of nitrosamines have been detected at concentrations 1 or 2 orders of magnitude higher than their cancer risk levels in the effluents of water and wastewater treatment plants (WWTPs) throughout the United States and Canada (*8, 9*).

Increases in world population as well as the paucity of water resources have made reusing treated municipal wastewater as a source of potable water a need for the coming decades (*10*). Studying the fate of contaminants during wastewater treatment is of importance as more and more municipal water treatment plants are engaged in the practice of intentional or unintentional reuse of the treated wastewater. A study by Swayne et al. (*11*) estimated approximately 4 million of the 62 million people served by surface water supplies in the United States were using wastewater-impacted water supplies. The persistence of nitrosamines and nitrosamine precursors in surface waters impacted by wastewater is of concern for communities exploiting such waters, particularly if their drinking water plants practice chloramination. In a study carried out to understand the fate of nitrosamines and their precursors in wastewater-impacted surface waters (*12*), it was observed that the half-life of NDMA precursors originating from wastewater effluents was sufficiently long to negatively impact downstream communities. In addition, NDMA was detected in river water downstream of known effluent discharge locations that received NDMA-containing wastewater effluent from one of the treatment plants.

To date, few studies have investigated the occurrence and fate of nitrosamines and their precursors in primary and waste-activated sludges and in anaerobic digestion systems. Dimethylamine-based cationic treatment polymers (*13*), which are used in primary and waste-activated sludge thickening processes, are important nitrosamine precursors in sludge systems; other secondary, tertiary, and quaternary amines may also be potent precursors. These precursors may (i) be released during sludge dewatering or digestion and recycled back to the main wastewater stream that later becomes a part of the source water for the potable supply, (ii) undergo biodegradation within the clarifiers, or (iii) undergo *N*-nitrosation reactions to produce nitrosamines (*14*) during sludge thickening in the secondary clarifier. In the latter case, nitrite may be present in the clarifier from the reduction of nitrate or oxidation of ammonium during nitrification−denitrification processes. *N*-Nitrosation is most favorable at acidic pH, but can be catalyzed by carbonyl compounds such as formaldehyde, which is abundantly detected in industrial and municipal wastewater (*15, 16*) at milligram per liter levels, at near neutral pH conditions (*17*). Furthermore, particle-associated nitrosamine precursors as well as residual nitrosamines and nitrite are transferred to anaerobic digesters. Anaerobic biotransformation of primary and waste-activated sludge, commonly practiced as a means of sludge stabilization, may either deactivate precursors or form additional ones from larger (bio)molecules. *N*-Nitrosation of precursors in anaerobic digesters may be particularly important due to the formation by fermentation of carbonyl

* Address correspondence to either author. Phone: (404) 894-7694 (C.-H.H.); (404) 894-9367 (S.G.P.). Fax: (404) 385-7087 (C.-H.H.); (404) 894-8266 (S.G.P.). E-mail: ching-hua.huang@ce.gatech.edu (C.-H.H.); spyros.pavlostathis@ce.gatech.edu (S.G.P.).
[†] Georgia Institute of Technology.
[‡] Yale University.

10.1021/es803067p CCC: $40.75
Published on Web 03/25/2009

TABLE 1. Nitrosamine and Secondary Amine Concentrations in Primary Sludge, Waste-Activated Sludge, and Anaerobic Digester Mixed Liquor Samples of Three WWTPs

sample	[NDMA] (ng/L)	[NPYR] (ng/L)	[DMA] (μg/L)	[PYR] (μg/L)	[DEA] (μg/L)	[DBA] (μg/L)
Plant A						
PS supernatant	834 ± 48	(<50)	2159 ± 14	477 ± 13	(<1)	39 ± 1
TWAS[a]	805 ± 8[b]	(<50)	542 ± 106	27 ± 8	(<1)	(<1)
ADML	383 ± 1	(<25)	11.2 ± 0.7	(<0.1)	7.6 ± 0.7	5.3 ± 0.6
Plant B						
PS supernatant	994 ± 0	59 ± 0	883 ± 126	71 ± 18	(<1)	6 ± 0
WAS	139 ± 34	57 ± 3	58 ± 3	100 ± 1		
ADML	273 ± 4	(<25)	3.0 ± 0.2	(<0.1)	(<0.1)	5.6 ± 0.5
Plant C						
PS supernatant	363 ± 84	(<50)	799 ± 89	292 ± 25	(<1)	14 ± 1
TWAS	240 ± 30	(<50)	30 ± 1	10 ± 0	(<1)	(<1)
ADML	159 ± 2	(<25)	4.5 ± 0.3	(<0.1)	2.2 ± 0.3	5.0 ± 0.5

[a] TWAS = thickened waste-activated sludge. [b] Mean ± standard deviation (n = 2).

catalysts and possible low pH conditions in not well mixed digesters. On the other hand, nitrosamines may also undergo biodegradation during sludge digestion. Recent studies have shown that nitrosamines, such as NDMA, can be degraded under aerobic as well as anaerobic conditions (*18*–*21*). Clearly, more research is needed to address these information gaps.

In the current study, we have specifically (i) investigated the occurrence and fate of the above-mentioned six nitrosamines and their secondary amine precursors (dimethylamine (DMA), methylethylamine (MEA), pyrrolidine (PYR), diethylamine (DEA), di-*n*-propylamine (DPA), and di-*n*-butylamine (DBA)) and total nitrosamine precursors in the primary sludge (PS), waste-activated sludge (WAS), and anaerobic digester mixed liquor (ADML) at three municipal WWTPs to evaluate the overall role of sludge digestion processes in the fate of these compounds and (ii) evaluated the biotransformation potential of these nitrosamines and secondary amines in anaerobic digestion systems. The possibility of the *N*-nitrosation pathway for nitrosamine formation during sludge digestion was assessed in selected experiments. Results of this study can contribute to the development of new source control strategies to reduce nitrosamine formation during municipal wastewater treatment.

Materials and Methods

Chemicals. Sources of chemical standards and reagents are described in Supporting Information Text S1.

Municipal Sludge and Anaerobic Digester Samples. Single PS, WAS, and ADML samples were collected at three municipal WWTPs in the southeastern United States (referred to as plants A, B, and C) as grab samples from sludge draw-off lines. Plants were selected to provide variability in terms of geographical location, size, biological treatment system configuration, and industrial discharge contribution. Composite samples were not available due to unavailability of the samplers. These plants treated an average of 20, 42, and 25 million gal/day of municipal wastewater, respectively. The relative contribution of industrial discharge for each plant was <5%, 25%, and 5–10%, respectively. All three plants used the activated sludge process as the secondary treatment. In addition, plants A and C used biological N and P removal. At all three plants, the sludge collected in the continuous-flow primary and secondary clarifiers was combined in the range of 0.15–0.35 PS/WAS ratio (total solids basis) and digested in mesophilic (35–37 °C) digesters. Plants A and C did not use any polymers for PS, WAS, or the combined PS and WAS mixture fed to the digester. However, plant A employed cationic polyacrylamide (PAM) and plant C employed an unknown polymer for dewatering digested sludge. Plant B used cationic PAM for dewatering digested sludge as well as for thickening of the PS and WAS mixture fed to the digesters. After dewatering, the supernatant was sent back to the head of the plants. The portion of polymers remaining in the supernatant can wind up in the wastewater stream and may have an effect on the nitrosamine concentrations in sludge samples because of their role in nitrosamine formation as discussed previously. The average sludge total solids (TS) loading rate and solids retention time (SRT) of the anaerobic digesters were 0.8–3.2 kg of TS/(m^3 day) and 15–25 days, respectively. After arriving in the laboratory, the PS and WAS samples were stored at 4 °C whereas the ADML samples were transferred to glass reactors flushed with helium and incubated at 35 °C with continuous mixing. The following analyses were conducted on the sludge samples according to standard methods (*22*): pH, TS, volatile solids (VS), total and soluble chemical oxygen demand (COD), dissolved organic carbon (DOC), ammonia, and volatile fatty acids (VFAs) (Text S2, Table S1, Supporting Information). All sludge samples were analyzed in duplicate.

Nitrosamine and Secondary Amine Analyses. Sludge filtrates were analyzed for six nitrosamines, six secondary amines, methylamine, ethylamine, and ammonia. Nitrosamines were extracted from filtrates using the solid-phase extraction method described by Taguchi et al. (*23*) and analyzed by gas chromatography/tandem mass spectrometry (GC/MS/MS) in chemical ionization (CI) mode or by gas chromatography/mass spectrometry (GC/MS) in electron impact ionization (EI) mode using selected ion monitoring (SIM). Details of the methods are described in Text S3 and Table S2 in the Supporting Information. Detection of nitrosamines in some PS filtrates could not be achieved due to the complexity of the sample matrix; in those cases, a new extraction protocol (Text S4) was employed to specifically quantify NDMA, which was the most abundant nitrosamine detected in this study. Secondary amines were analyzed by a method adapted from Sacher et al. (*24*) which involves derivatization with benzenesulfonyl chloride and then analysis by GC/MS in SIM mode (Text S3 and Table S2).

Total Nitrosamine Precursor Analysis. In addition to secondary amines, dissolved and particle-associated uncharacterized nitrosamine precursors can persist at various stages in wastewater treatment plants (*13, 25*). Since monitoring of all nitrosamine precursors is not feasible, an indirect measurement of these precursors can be achieved by chloraminating wastewater to transform all the precursors to corresponding nitrosamines. On the basis of the protocol developed by Mitch et al. (*25*), 200 mL of sludge filtrate was chloraminated for 3 days by 2 mM monochloramine (140 mg of Cl_2/L) from a freshly prepared monochloramine stock.

The reaction was quenched with excess ascorbic acid (6 mM), and the nitrosamines formed were quantified by GC/MS or GC/MS/MS as described above. Secondary amines were also quantified before and after chloramination to evaluate their contribution to nitrosamine formation.

Biodegradability Assays. Three digester sludge reactors were set up with about 4 L ADML samples from the three WWTPs in 9 L glass reactors preflushed with helium for 30 min. Incubation was carried out for 170 days at 35 °C with continuous mixing without any external carbon amendment. At the beginning and the end of the incubation period, TS, total COD, residual nitrosamines, and secondary amines in the reactors were measured to evaluate the biodegradation potential of pre-existing nitrosamines and secondary amines in the unfed digester sludge reactors.

Subsequently, batch assays were performed to investigate the biotransformation potential of six nitrosamines and six secondary amines. A sample (123 mL) from the plant A digester sludge reactor was taken at the end of the 170 day incubation period and anaerobically transferred to two sets of 160 mL serum bottles. Nitrosamines and secondary amines were added into each of the two sets of bottles to achieve initial concentrations of 16 μM NDMA, NDEA, NMEA, and NPYR, 12 μM NDPA,and 7 μM NDBA for nitrosamines and 16 μM DMA, DEA, MEA, and PYR, 10 μM DPA, and 8 μM DBA for secondary amines. The above concentrations were used for accurate quantification of target compounds over time on the basis of the availability of the sample volume for periodic analysis. The biomass concentration in the bottles was 8.5 ± 0.1 g of VS/L. The bottles were analyzed for pH, TS, VS, COD, DOC, total gas production, gas composition, and VFAs (Text S2, Supporting Information). Sample aliquots of approximately 2 mL were taken for nitrosamine and secondary amine analyses (see Text S5 for details). All bioassay samples were analyzed in triplicate, except the controls, which were analyzed in duplicate.

Results and Discussion

Nitrosamines and Secondary Amines in Sludge Samples. NDMA and NPYR were the only nitrosamines detected in sludge samples (Table 1). Note that the concentrations measured were in sludge sample filtrates. However, on the basis of experiments in this study to assess the adsorption of nitrosamines to biosolids and inorganic particles, it was observed that lower molecular weight nitrosamines, NDMA, NMEA, and NPYR, had negligible affinity for sludge biosolids (Table S3, Supporting Information), and hence, the detected concentrations were attributed to the sludge. The mean NDMA concentrations at the three plants were 678 ± 302, 394 ± 322, and 271 ± 100 ng/L in PS, WAS, and ADML samples, respectively. Because sampling occurred only once, temporal wastewater and process variations in the treatment plants may contribute to the observed NDMA variability. No previous data are available on the concentrations of nitrosamines in sludge samples. Since the detectable nitrosamines adsorb negligibly to biosolids, we compared our results to the reported nitrosamine concentrations in municipal primary effluents. Notably, the NDMA concentrations in PS ranged from 280 to 1000 ng/L, about an order of magnitude higher than the median NDMA concentration (73 ng/L) reported previously in primary wastewater effluents (*26*). This difference may be related to (i) the different local sources of NDMA as industrial waste composition varies locally, (ii) the temporal variability associated with sampling, and (iii) the potential formation of nitrosamines through *N*-nitrosation.

The NDMA concentration in WAS ranged from 100 to 800 ng/L. The secondary biological treatment was effective in reducing the NDMA concentration for plants B and C by 86% and 34%, respectively, but the plant A WAS sample showed little reduction in NDMA concentration compared to the PS sample. These results are in agreement with the study by Sedlak et al. (*26*), which showed high variability in the removal of NDMA by secondary biological treatment.

Relatively low concentrations of NDMA, from 160 to 385 ng/L, were detected in ADML samples. To compare the concentrations of NDMA in the ADML samples, it was necessary to perform a mass balance to account for the NDMA contribution from PS and WAS, since the anaerobic digesters in the three plants were fed with a mixture of PS and WAS. Although the sampling procedures in this study did not address temporal variations, daily fluctuations are unlikely to result in large concentration differences given the relatively long retention times (15−25 days) in the anaerobic digesters. Thus, the mass balances calculated are appropriate. Expected minimum and maximum steady-state NDMA concentrations in the mixed liquor of the three digesters were calculated by using measured NDMA concentrations in PS and WAS samples and the TS fraction of PS and WAS in the combined sludge assuming no biodegradation or adsorption of NDMA to sludge biosolids, as follows:

$$C_{\mathrm{NDMA,ADML}} = [C_{\mathrm{NDMA,PS}} f_{\mathrm{PS}} l_{\mathrm{PS}} + C_{\mathrm{NDMA,WAS}} f_{\mathrm{WAS}} l_{\mathrm{WAS}}] \frac{1}{l_{\mathrm{composite}}} \quad (1)$$

where C_{NDMA} is the NDMA concentration in the sludge samples (ng/L), f is the volumetric fraction of PS or WAS in the combined sludge, and l is the liquid fraction of these sludge samples.

Plants A and C had about 52% and 45%, respectively, less NDMA in ADML than expected on the basis of the contribution from PS and WAS, whereas plant B's ADML NDMA concentration was close to that expected (Table S4, Supporting Information). Since NDMA has a low affinity for biosolids (*25*), the loss due to adsorption is expected to be insignificant. As a result, the measured lower NDMA concentrations in ADML at plants A and C may be attributed to microbial degradation.

The mean DMA concentrations at the three plants were 1280 ± 689, 210 ± 266, and 6.2 ± 3.9 μg/L in PS, WAS, and ADML samples, respectively (Table 1). The DMA concentrations in PS ranged from 700 to 2100 μg/L. Similar to NDMA, the DMA concentrations were an order of magnitude higher for PS samples compared to DMA concentrations in primary effluent samples reported in the literature (*13, 26*). Probable reasons might be similar to those for NDMA as explained above. DMA concentrations in WAS ranged from 30 to 650 μg/L. The secondary biological treatment effectively removed DMA by an average of 88 ± 12%. DMA concentrations in ADML samples ranged from 2.8 to 12 μg/L. Similar to NDMA, the apparent decrease in DMA concentrations in ADML may be attributed to the biodegradation of DMA in the anaerobic digesters.

The mean PYR concentrations were 280 ± 183, 45 ± 43, and <0.1 (MDL) μg/L for PS, WAS, and ADML samples, respectively. This trend is analogous to DMA concentrations at the different stages of wastewater treatment. Note that the corresponding nitrosamine, NPYR, was the only other nitrosamine detected in PS and WAS samples of plant B, with a significant concentration of PYR in PS and WAS samples, at concentrations of 58 ± 0 and 56 ± 4 ng/L, respectively. This finding, along with the fact that the plant A samples had higher concentrations of DMA than the samples of the other two WWTPs and it also had the highest concentration of NDMA for WAS and ADML samples, implies a possible relationship between residual secondary amine concentrations and the level of nitrosamines detected. Such a relationship is consistent with the hypothesis of formation of nitrosamines in sludge samples via *N*-nitrosation. The potential of nitrosamine formation via *N*-nitrosation in these sludge samples was further evaluated and is discussed later.

TABLE 2. Nitrosamine and Secondary Amine Concentrations in Primary Sludge, Waste-Activated Sludge, and Anaerobic Digester Mixed Liquor Samples of Three WWTPs after the Nitrosamine Formation Potential Test[a]

sample	[NDMA] (ng/L)	[NPYR] (ng/L)	[DMA] (μg/L)	[PYR] (μg/L)	[DEA] (μg/L)	[DBA] (μg/L)
Plant A						
PS supernatant	NA[c]	NA	447 ± 4	208 ± 5	(<1)	(<1)
TWAS[b]	1907 ± 190[d]	(<50)	514 ± 22	26 ± 2	(<1)	(<1)
ADML	587 ± 42	(<25)	8.3 ± 1.2	(<0.1)	5.0 ± 0.6	4.7 ± 0.6
Plant B						
PS supernatant	NA	NA	490 ± 39	65 ± 0	(<1)	(<1)
WAS	4278 ± 693	291 ± 11	191 ± 11	12 ± 2	(<1)	(<1)
ADML	418 ± 34	(<25)	4.1 ± 0.1	(<0.1)	(<0.1)	6.0 ± 0.0
Plant C						
PS supernatant	NA	NA	614 ± 26	160 ± 1	(<1)	3 ± 0
TWAS	532 ± 5	101 ± 3	34 ± 2	8 ± 0	(<1)	(<1)
ADML	1778 ± 79	(<25)	5.6 ± 0.4	(<0.1)	1.6 ± 0.2	2.8 ± 0.3

[a] With 140 mg/L monochloramine for 3 days. [b] TWAS = thickened waste-activated sludge. [c] NA = not available (the concentration could not be obtained due to strong interference from the sample matrix). [d] Mean ± standard deviation (n = 2).

DBA was detected in all PS (20 ± 15 μg/L) and ADML (5.3 ± 0.6 μg/L) samples from the three plants, while plant A (7.6 ± 1.0 μg/L) and plant C (2.2 ± 0.3 μg/L) also had detectable DEA in ADML samples. The concentrations of DMA and PYR were the lowest in ADML samples compared to PS and TWAS samples for all plants, implying significant degradation under anaerobic conditions. From bioassay experiments conducted in our laboratory with secondary amines, the lower molecular weight secondary amines had the highest rate of biodegradation under anaerobic conditions and, hence, are expected to be removed relatively fast.

Previous studies have reported the formation of NDMA from DMA and nitrite in sewage samples (*27*−*29*). *N*-Nitrosation is most favorable at acidic pH, but can be catalyzed by carbonyl compounds such as formaldehyde or formic acid at near neutral pH conditions (*17*). It has been proposed that formaldehyde catalyzes *N*-nitrosation by interacting with a secondary amine to form an adduct that is highly reactive toward nucleophilic attack by nitrite (*17*). Formaldehyde is a common industrial chemical and has been reported at up to 2 g/L levels in wastewater (*30*). Other naturally occurring organic compounds have also been linked to an increased rate of nitrosation at near neutral pH (*31*). Significant nitrite levels in plants practicing nitrification−denitrification for N removal may occur under conditions of high N loading and suboptimal pH and temperature values as well as in the presence of toxicants. The PS and WAS samples in this study had significant levels of VFAs, which indicates productive fermentation (Table S1, Supporting Information). Under fermentative conditions, nitrite and formaldehyde are transient species and thus are difficult to analyze. Nevertheless, the concentrations of these two compounds may be sufficient for *N*-nitrosation of DMA to occur in the wastewater. To test the potential of NDMA formation via *N*-nitrosation in wastewater sludge, batch experiments were conducted with plant A PS, WAS, and ADML samples by spiking nitrite and formaldehyde or formic acid into the samples. Separate experiments conducted with deionized water verified the enhancing effect of formaldehyde and formic acid on the formation of NDMA from *N*-nitrosation of DMA with nitrite at pH 6.8 (Table S5). When nitrite and formaldehyde or nitrite and formic acid were both spiked into the sludge samples (pH 5.1−7.4) at high concentrations (250 mg/L) and allowed to react for 6 h, the NDMA concentration increased by more than an order of magnitude compared to the initial NDMA concentrations (Figure S1). PS samples had the highest level of NDMA formed followed by WAS and then ADML samples. The above results verified the possibility of NDMA formation via *N*-nitrosation in the sludge samples provided that sufficient nitrite and catalyzing agents are present. The formation of nitrosamines may contribute to the higher levels of NDMA detected in PS than those found in primary wastewater effluent and may explain the relatively high levels of residual NDMA detected in sludge samples despite favorable conditions for nitrosamine biodegradation (results shown later).

Total Nitrosamine Precursors in Sludge Samples. To estimate the total nitrosamine precursors, sludge samples were subjected to extended chloramination. Residual monochloramine was measured at intermediate time intervals and at the end of the 3 day reaction for plant A sludge samples. A 2 mM concentration of monochloramine was exhausted in the PS samples, while WAS and ADML had detectable residual monochloramine at the end of the 3 day reaction.

Total nitrosamine precursors could not be quantified for PS samples because of significant background noise in GC/MS and GC/MS/MS analyses due to the complexity of the sample matrix. The mean total NDMA precursor concentrations were 2239 ± 1755 ng/L for WAS and 928 ± 666 ng/L for ADML samples (Table 2). The plant B WAS sample had the highest level of total NDMA precursors at approximately 5 μg/L. On the basis of the DMA concentration detected at the plant B WAS sample and assuming an NDMA yield of approximately 2% from DMA for a 3 day reaction period with monochloramine (*13*), only 30% of the total NDMA precursors could be accounted for, indicating the significance of other unidentified NDMA precursors. In contrast, the 2% NDMA yield from residual DMA was able to account for nearly all NDMA precursors for the other two plants. Using a similar method, the ADML samples from all three plants were estimated to have 80 ± 17% of unknown NDMA precursors. Assuming a comparable NPYR yield (also 2%) upon chloramination of PYR, the detected PYR concentration alone also could not explain the levels of residual NPYR detected in the WAS and ADML samples, again suggesting that other NPYR precursors were present in the sludge samples.

The total NDMA precursor concentrations were found to decrease for plant A and B ADML samples by 70% and 90%, respectively, compared to WAS samples, implying that anaerobic digestion is acting as a sink for total nitrosamine precursors along with secondary amines. In contrast, the total nitrosamine precursor concentration in plant C ADML samples showed a 3-fold increase compared to WAS samples, underlining the complexity of biological processes involved in sludge digestion and differences in sludge components among the three plants. These results, combined with measurements of secondary amines before (Table 1) and

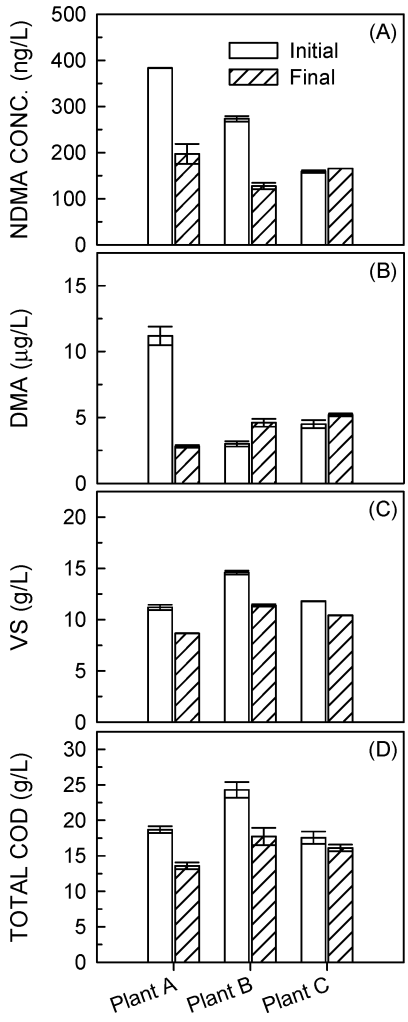

FIGURE 1. Initial and final *N*-nitrosodimethylamine (A), dimethylamine (B), volatile solids (C), and total chemical oxygen demand (D) concentrations in plant A, B, and C anaerobic digester mixed liquor before and after 170 days of incubation (error bars represent 1 standard deviation of the means).

after (Table 2) reaction with monochloramine, demonstrate the important contribution of uncharacterized NDMA precursors in municipal wastewater sludge toward the formation of nitrosamines.

Anaerobic Biodegradation Potential of Nitrosamines and Secondary Amines. The plant A, B, and C digester mixed liquors, which had initial NDMA concentrations of 383 ± 1, 273 ± 6, and 159 ± 3 ng/L, respectively, were anaerobically incubated for 170 days without any external carbon or energy source addition. The NDMA concentration in the plant A and B reactors decreased to 197 ± 22 and 127 ± 7 ng/L, respectively, whereas the NDMA concentration change in the plant C reactor was statistically insignificant ($p = 0.192$) (Figure 1). The DMA concentration in the plant A digester sludge decreased 75%, whereas it increased slightly in the plant B and C digester sludges (Figure 1B) after 170 days of incubation. These results show the variability associated with the complex biotic processes occurring in sludge samples. DMA can be biodegraded, but can also be a product from breakdown of organic molecules, including NDMA, present in sludge samples. The total COD processed and VS destruction in the reactors that achieved about 50% NDMA removal were higher than those in the reactor that had no NDMA removal (Figure 2), suggesting that NDMA removal may be dependent on the biological activity and thus the extent of COD destruction.

To test the above hypothesis, a sludge bioassay was set up using the plant A ADML as the inoculum and fortified with the six nitrosamines. The assay bottles were not amended with any external carbon and energy source initially. At the end of the 48 day incubation, the nitrosamine concentrations did not decrease and there was little COD destruction, reflected by the low methane production (Figure 2). A PS/WAS (36%:64% TS basis) mixture was then added as the carbon and energy source to each bottle and incubation continued for another 54 days. The nitrosamine and secondary amine concentrations were adjusted to their initial values to correct for dilution. The VS and total COD of the combined sludge were 32 and 57 g/L, respectively. The solids concentration in the bottles after the feeding was 4.4 ± 0.1 g of VS/L. The total volume of methane produced and the amount of COD processed during the incubation period in the nitrosamine-amended bottles were 568 mL and 1.44 g, respectively. All nitrosamines, except NDPA and NDBA, were partially removed. The concentration of NDMA, NPYR, NMEA, and NDEA decreased from 16.7, 17.6, 17.4, and 16.9 μM to 7.8, 2.6, 6.6, and 7.0 μM, respectively, during the incubation. A good correlation ($r^2 = 0.95$) was obtained between the nitrosamine removal and COD destruction (Figure 3), which shows that nitrosamines may be utilized as electron acceptors or transformed cometabolically during the degradation of complex organics under anaerobic conditions.

The initial biomass-VS normalized NDMA, NPYR, NMEA, and NDEA removal rates were 1.49, 2.30, 1.41, and 1.59 μmol/(g of COD processed-g of VS), respectively. In contrast, the NDPA and NDBA removal was statistically insignificant ($p = 0.052$ for NDPA and $p = 0.800$ for NDBA). The lower removal rates of NDPA and NDBA are attributed to their higher hydrophobicity, lower bioavailability, and steric hindrance resulting from the longer chain, bulkier alkyl groups present in these molecules.

A recent study by Chung et al. (*20*) showed that NDMA can be used as an electron acceptor and reduced to ammonia and DMA by microorganisms using H_2 as the electron donor in a hydrogen-based membrane biofilm reactor and concluded that NDMA reduction kinetics is controlled by electron donor availability and NDMA reduction competes directly with nitrate reduction. Moreover, the NDMA biotransformation pathway may involve the reduction of the nitroso group and subsequent N−N cleavage, which results in the formation of DMA and ammonia. A similar pathway may be involved in the biotransformation of nitrosamines in the anaerobic digestion systems used in our study. In fact, not only H_2 but also fermentation products of complex organics, such as long-chain or short-chain fatty acids and alcohols, may serve as an electron donor in nitrosamine biotransformation during the anaerobic sludge digestion process. However, utilization of the available electron donors via fermentation (acid and alcohol fermentation) and methanogenesis (acetoclastic and hydrogenotrophic methanogenesis) may compete with their use for nitrosamine biotransformation, which results in partial removal of nitrosamines in a mixed microbial system such as the one used in the present study. The effect of process interactions and the type of electron donor on nitrosamine biotransformation as well as the biotransformation pathway are the focus of an ongoing study using a well-characterized enriched mixed methanogenic culture, and the results will be reported later.

The bioassay testing the biotransformation potential of six secondary amines lasted 102 days. All secondary amines were degraded completely before the addition of the PS and WAS mixture in the secondary amine-amended bottles (Figure 2). As a result, secondary amine degradation was not related to the COD destruction, and these compounds may have been utilized directly, serving as electron donors.

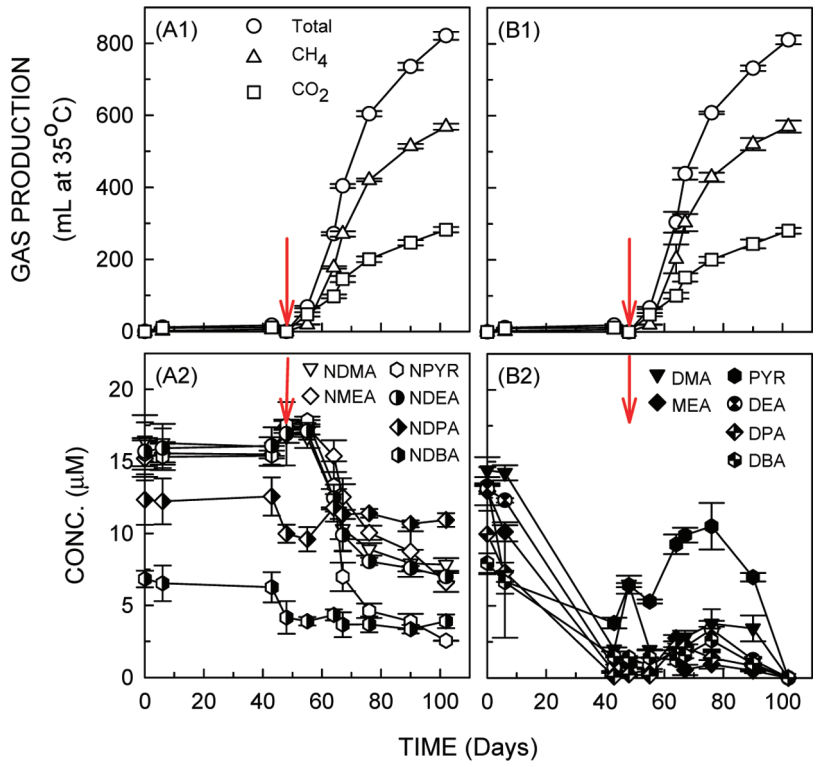

FIGURE 2. Time course of (1) total gas, methane, and carbon dioxide production and (2) nitrosamine or secondary amine consumption in the plant A anaerobic digester mixed liquor amended with (A) 16 μM NDMA, NDEA, NMEA, and NPYR, 12 μM NDPA, and 7 μM NDBA and (B) 16 μM DMA, DEA, MEA, and PYR, 10 μM DPA, and 8 μM DBA. Error bars represent 1 standard deviation of the means. Arrows indicate the addition of combined PS and WAS sludge on the 48th day of incubation.

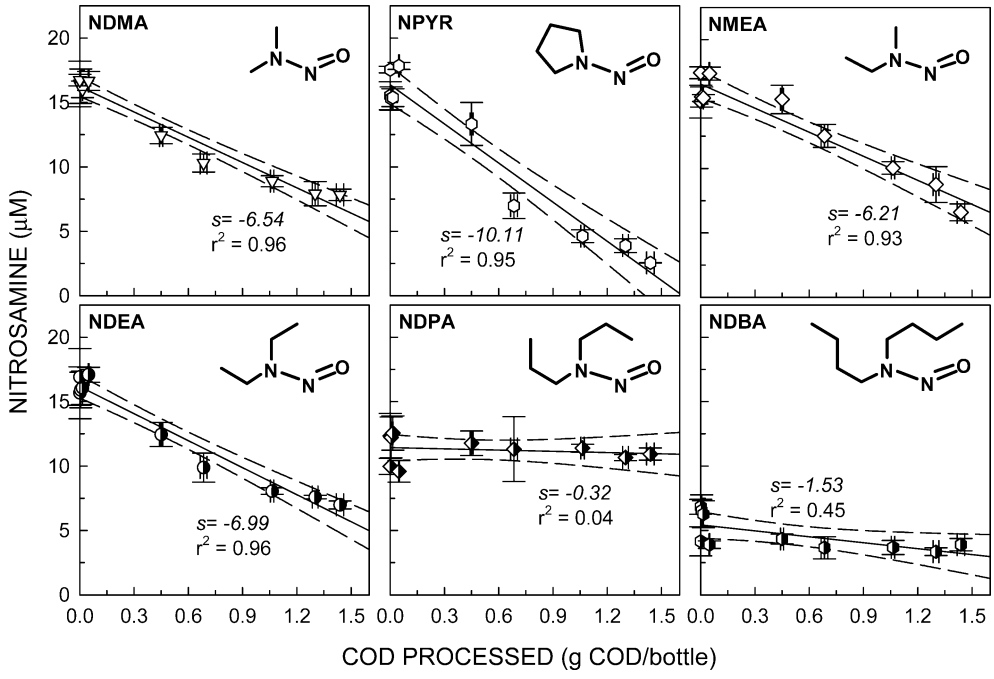

FIGURE 3. Trend of removal of different nitrosamines with respect to the total amount of COD processed in plant A anaerobic digester mixed liquor amended with 16 μM NDMA, NDEA, NMEA, and NPYR, 12 μM NDPA, and 7 μM NDBA (error bars represent 1 standard deviation of the means; s = slope of the regression line).

Previous studies have reported that *Methanosarcina barkeri* utilizes *N*-methyl compounds, including low molecular weight secondary amines, for growth and methane formation by using methyl transferases (*32*−*35*).

Environmental Significance. The overall trend of an NDMA concentration decrease from PS to WAS to ADML at three municipal WWTPs indicates the greater role as an NDMA sink of the sludge digestion process, even though *N*-nitrosation reactions to form nitrosamines in sludges are possible. This is one of the first studies to report nitrosamine and their precursor concentrations in municipal wastewater sludges. Despite the decrease in NDMA concentration, persistent levels of NDMA still existed in the digested sludge samples without addition of external electron donors after a prolonged incubation (170 days) that is considerably longer than typical digester SRT values. Since the rate of nitrosamine

biodegradation in anaerobic assays was found to be related to COD consumption, one strategy to reduce NDMA levels in anaerobic digesters can be addition of external electron donor sources to accelerate the NDMA removal rate (e.g., codigestion of sludge and fat, oil, and grease). Another strategy to reduce nitrosamine levels is to control the NDMA precursors participating in *N*-nitrosation reactions. Anaerobic digestion is effective in reducing the DMA levels and may reduce the other NDMA precursors as observed at two of the WWTPs. Nitrification−denitrification operations can produce nitrite; any strategy to reduce nitrite levels may also be effective in reducing NDMA concentrations in municipal WWTP sludge. Finally, on the basis of the weak adsorption of low molecular weight nitrosamines to sludge, biosolids are not expected to have significant levels of accumulated NDMA.

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant BES-0610358. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Supporting Information Available

Text S1−S5, Tables S1−S5, and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) United States Environmental Protection Agency IRIS Database. http://www.epa.gov/ncea/iris/.
(2) United States Environmental Protection Agency Unregulated Contaminant Monitoring Regulation for Public Water Systems. http://www.epa.gov/fedrgstr/EPA-WATER/2007/January/Day-04/w22123.htm.
(3) United States Environmental Protection Agency Contaminant Candidate List 3. http://www.epa.gov/OGWDW/ccl/ccl3.html.
(4) Choi, J.; Valentine, R. L. Formation of N-nitrosodimethylamine (NDMA) from reaction of monochloramine: A new disinfection by-product. *Water Res.* **2002**, *36* (4), 817–824.
(5) Mitch, W. A.; Sedlak, D. L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ. Sci. Technol.* **2002**, *36* (4), 588–595.
(6) Schreiber, I. M.; Mitch, W. A. Influence of the order of reagent addition on NDMA formation during chloramination. *Environ. Sci. Technol.* **2005**, *39* (10), 3811–3818.
(7) Schreiber, I. M.; Mitch, W. A. Nitrosamine formation pathway revisited: The importance of chloramine speciation and dissolved oxygen. *Environ. Sci. Technol.* **2006**, *40* (19), 6007–6014.
(8) Mitch, W. A.; Sharp, J. O.; Trussell, R. R.; Valentine, R. L.; Alvarez-Cohen, L.; Sedlak, D. L. N-nitrosodimethylamine (NDMA) as a drinking water contaminant: A review. *Environ. Eng. Sci.* **2003**, *20* (5), 389–404.
(9) Krauss, M.; Hollender, J. Analysis of nitrosamines in wastewater: Exploring the trace level quantification capabilities of a hybrid linear ion trap/orbitrap mass spectrometer. *Anal. Chem.* **2008**, *80* (3), 834–842.
(10) Shannon, M. A.; Bohn, P. W.; Elimelech, M.; Georgiadis, J. G.; Marinas, B. J.; Mayes, A. M. Science and technology for water purification in the coming decades. *Nature (London, U.K.)* **2008**, *452* (7185), 301–310.
(11) Swayne, M. D.; Boone, C. H.; Bauer, D.; Lee, J. S. *Wastewater in Receiving Waters at Water Supply Abstraction Points*; EPA-600/2-80-044; United States Environmental Protection Agency: Washington, DC, 1980.
(12) Schreiber, I. M.; Mitch, W. A. Occurrence and fate of nitrosamines and nitrosamine precursors in wastewater-impacted surface waters using boron as a conservative tracer. *Environ. Sci. Technol.* **2006**, *40* (10), 3203–3210.
(13) Mitch, W. A.; Sedlak, D. L. Characterization and fate of N-nitrosodimethylamine precursors in municipal wastewater treatment plants. *Environ. Sci. Technol.* **2004**, *38* (5), 1445–1454.
(14) Mirvish, S. S. Formation of N-nitroso compounds—chemistry, kinetics, and in vivo occurrence. *Toxicol. Appl. Pharmacol.* **1975**, *31* (3), 325–351.
(15) Lotfy, H. R.; Rashed, I. G. A method for treating wastewater containing formaldehyde. *Water Res.* **2002**, *36* (3), 633–637.
(16) Eiroa, M.; Kennes, C.; Veiga, M. C. Formaldehyde and urea removal in a denitrifying granular sludge blanket reactor. *Water Res.* **2004**, *38* (16), 3495–3502.
(17) Keefer, L. K.; Roller, P. P. *N*-Nitrosation by nitrite ion in neutral and basic medium. *Science* **1973**, *181* (4106), 1245–1247.
(18) Kaplan, D. L.; Kaplan, A. M. Biodegradation of N-nitrosodimethylamine in aqueous and soil systems. *Appl. Environ. Microbiol.* **1985**, *50* (4), 1077–1086.
(19) Sharp, J. O.; Sales, C. M.; LeBlanc, J. C.; Liu, J.; Wood, T. K.; Eltis, L. D.; Mohn, W. W.; Alvarez-Cohen, L. An inducible propane monooxygenase is responsible for N-nitrosodimethylamine degradation by *Rhodococcus* sp strain RHA1. *Appl. Environ. Microbiol.* **2007**, *73* (21), 6930–6938.
(20) Chung, J.; Ahn, C. H.; Chen, Z.; Rittmann, B. E. Bio-reduction of N-nitrosodimethylamine (NDMA) using a hydrogen-based membrane biofilm reactor. *Chemosphere* **2008**, *70* (3), 516–520.
(21) Bradley, P. M.; Carr, S. A.; Baird, R. B.; Chapelle, F. H. Biodegradation of N-nitrosodimethylamine in soil from a water reclamation facility. *Biorem. J.* **2005**, *9* (2), 115–120.
(22) Eaton, A. D., Clesceri, L. S., Greenberg, A. E., Eds. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association, American Water Works Association, Water Environment Federation Publishers: Washington, DC, 1998.
(23) Taguchi, V.; Jenkins, S. D. W.; Wang, D. T.; Palmentier, J.; Reiner, E. J. Determination of N-nitrosodimethylamine by isotope-dilution, high-resolution mass-spectrometry. *Can. J. Appl. Spectrosc.* **1994**, *39* (3), 87–93.
(24) Sacher, F.; Lenz, S.; Brauch, H. J. Analysis of primary and secondary aliphatic amines in waste water and surface water by gas chromatography mass spectrometry after derivatization with 2,4-dinitrofluorobenzene or benzenesulfonyl chloride. *J. Chromatogr., A* **1997**, *764* (1), 85–93.
(25) Mitch, W. A.; Gerecke, A. C.; Sedlak, D. L. A N-nitrosodimethylamine (NDMA) precursor analysis for chlorination of water and wastewater. *Water Res.* **2003**, *37* (15), 3733–3741.
(26) Sedlak, D. L.; Deeb, R. A.; Hawley, E. L.; Mitch, W. A.; Durbin, T. D.; Mowbray, S.; Carr, S. Sources and fate of nitrosodimethylamine and its precursors in municipal wastewater treatment plants. *Water Environ. Res.* **2005**, *77* (1), 32–39.
(27) Ayanaba, A.; Alexander, M. Transformations of methylamines and formation of a hazardous product, dimethylnitrosamine, in samples of treated sewage and lake water. *J. Environ. Qual.* **1974**, *3* (1), 83–89.
(28) Mills, A. L.; Alexander, M. N-nitrosamine formation by cultures of several microorganisms. *Appl. Environ. Microbiol.* **1976**, *31* (6), 892–895.
(29) Mills, A. L.; Alexander, M. Factors affecting dimethylnitrosamine formation in samples of soil and water. *J. Environ. Qual.* **1976**, *5* (4), 437–440.
(30) Eiroa, M.; Vilar, A.; Kennes, C.; Veiga, M. C. Biological treatment of industrial wastewater containing formaldehyde and formic acid. *Water SA* **2006**, *32* (1), 115–118.
(31) Weerasooriya, S. V. R.; Dissanayake, C. B. Modeling the nitrosation kinetics in simulated natural environmental conditions. *Toxicol. Environ. Chem.* **1992**, *36* (3−4), 131–137.
(32) Hippe, H.; Caspari, D.; Fiebig, K.; Gottschalk, G. Utilization of trimethylamine and other N-methyl compounds for growth and methane formation by *Methanosarcina barkeri*. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76* (1), 494–498.
(33) Ferguson, D. J.; Krzycki, J. A. Reconstitution of trimethylamine-dependent coenzyme M methylation with the trimethylamine corrinoid protein and the isozymes of methyltransferase II from *Methanosarcina barkeri*. *J. Bacteriol.* **1997**, *179* (3), 846–852.
(34) Burke, S. A.; Krzycki, J. A. Reconstitution of monomethylamine: Coenzyme M methyl transfer with a corrinoid protein and two methyltransferases purified from *Methanosarcina barkeri*. *J. Biol. Chem.* **1997**, *272* (26), 16570–16577.
(35) Ferguson, D. J.; Gorlatova, N.; Grahame, D. A.; Krzycki, J. A. Reconstitution of dimethylamine: Coenzyme M methyl transfer with a discrete corrinoid protein and two methyltransferases purified from *Methanosarcina barkeri*. *J. Biol. Chem.* **2000**, *275* (37), 29053–29060.

ES803067P