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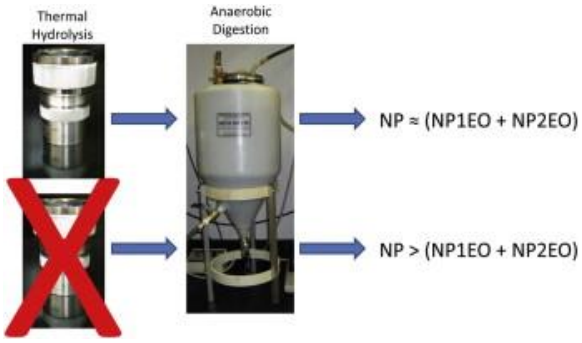
Abstract

The presence of micropollutants can be a concern for land application of biosolids. Of particular interest are nonylphenol diethoxylate (NP_2EO), nonylphenol monoethoxylate (NP_1EO), and nonylphenol (NP), collectively referred to as NPE, which accumulate in anaerobically digested biosolids and are subject to regulation based on the environmental risks associated with them. Because biosolids are a valuable nutrient resource, it is essential that we understand how various treatment processes impact the fate of NPE in biosolids. Thermal hydrolysis (TH) coupled with mesophilic anaerobic digestion (MAD) is an advanced digestion process that destroys pathogens in biosolids and increases methane yields and volatile solids destruction. We investigated the impact of thermal hydrolysis pretreatment on the subsequent biodegradation of NPE in digested biosolids. Biosolids were treated with TH, anaerobic digestion, and aerobic digestion in laboratory-scale reactors, and NPE were analyzed in the influent and effluent of the digesters. NP_2EO and NP_1EO have been observed to degrade to the more estrogenic NP under anaerobic conditions; therefore, changes in the ratio of NP:NPE were of interest. The increase in NP:NPE following MAD was 56%; the average increase of this ratio in four sets of TH-MAD samples, however, was only 24.6 ± 3.1%. In addition, TH experiments performed in pure water verified that, during TH, the high temperature and pressure alone did not directly destroy NPE; TH experiments with NP added to sludge also showed that NP was not destroyed by the high temperature and pressure of TH when in a more complex sludge matrix. The post-aerobic digestion phases removed NPE, regardless of whether TH pretreatment occurred. This research indicates that changes in biosolids processing can have impacts beyond just gas production and solids destruction.

Graphical abstract



Keywords

Anaerobic digestion, Thermal hydrolysis, Nonylphenol, Nonylphenol ethoxylates, Biosolids, Estrogenicity

1. Introduction

Anaerobic digestion has been used worldwide for over 100 years to stabilize wastewater solids (Van Lier et al., 2001). As global energy demands continue to increase, it is important to make this process as efficient as possible and to effectively utilize all byproducts of wastewater treatment, including biosolids. A recent study indicated that anaerobic digesters could emerge as an important and useful renewable energy source for electricity generation in the face of new greenhouse gas emission limits (Zaks et al., 2011). Approximately half of the biosolids produced in the European Union (EU) and the United States (US) are used as fertilizer through land application (European Commission, 2008, U.S. Environmental Protection Agency, 2002). According to a life-cycle assessment on the use of biosolids, land application can greatly reduce the carbon footprint of solids handling when compared to the landfilling of biosolids (Peters and Rowley, 2009). Additionally, agricultural phosphorus demand is reduced through the land application of biosolids, helping to alleviate a growing global problem of diminishing phosphorus reserves (Weikard and Seyhan, 2009). Land application of biosolids is not without risks,

however, including potential problems associated with the presence of micropollutants, particularly estrogenic compounds, in biosolids.

Of the micropollutants found in wastewater solids, nonylphenol monoethoxylate (NP_1EO), nonylphenol diethoxylate (NP_2EO; these two ethoxylates collectively referred to as NPEO) and NP (all three compounds collectively referred to as NPE) are of particular interest as a result of their endocrine disrupting capabilities (Masuyama et al., 2000) and estrogenicity (Jobling and Sumpter, 1993). Nonylphenol has been detected at levels greater than 1000 mg/kg in biosolids used for land application (Kinney et al., 2006), and was shown to bioaccumulate in earthworms inhabiting a field fertilized with biosolids (Kinney et al., 2008). In 2003 the EU passed a directive that restricted the sale or use of NPE (European Union, 2003). In the US the Environmental Protection Agency has started a "Safer Detergents Stewardship Initiative" to encourage use of surfactants other than NPE. A report that updated the 2003 working document on land application of biosolids has suggested that NPE should not exceed 450 mg/kg of dry solids (Salado et al., 2010, Commission of European Communities, 2003); nevertheless, NPE in biosolids have exceeded this level in both the EU (Santos et al., 2007) and the US (Kinney et al., 2006). It is reasonable to expect that the presence of NP in biosolids could result in additional bans or restrictions on the use of NP and NPE within the EU or elsewhere. Therefore, it is critical that we understand how both conventional biosolids handling technologies and emerging technologies impact the fate of NPE in biosolids.

Anaerobic sludge stabilization often results in an accumulation of NP in biosolids (Giger et al., 1984), likely because the anaerobic degradation of NP is very slow compared to its formation from longer-chain ethoxylates (Chang et al., 2008), either NP_nEO (n = 3–20) or NP_1EO plus NP_2EO. Anaerobic biodegradation of longer chain NP_nEOs (n = 3–20) to short-chain NPEO has been demonstrated in the laboratory when fed at very high concentrations (100 g/kg) (Lu et al., 2008); nevertheless, other research has found NP_nEO to be more recalcitrant under anaerobic conditions, with half-lives greater than 100 days in sediments (Ferguson and Brownawell, 2003). The biodegradation of NPEO to NP under anaerobic conditions, on the other hand, is well documented in full-scale systems (Giger et al., 1984, Ahel et al., 1994, Patureau et al., 2008) and is often the immediate precursor to NP in digesters.

Because NP is more estrogenic than NPEO (Routledge and Sumpter, 1996) anaerobic digestion can increase the estrogenicity of biosolids (Holbrook et al., 2002). This production of estrogenicity is of particular concern to wastewater utilities because anaerobic digestion is widely considered a beneficial technology for solids stabilization, producing methane gas and recoverable soluble nutrients, and effectively reducing the number of pathogens and pathogenic indicators in biosolids prior to land application.

An emerging process for solids handling is the advanced pretreatment process of thermal hydrolysis (TH). This process of TH followed by mesophilic anaerobic digestion (TH-MAD) has been implemented in plants worldwide because of benefits such as increased volatile solids reduction, improved sludge dewaterability, and minimization of digester volume requirements (Kepp et al., 2000, Pickworth et al., 2006, Wilson et al., 2011). During TH, sludge is heated to 150 °C for 30 min at a pressure of 5–8 bar; under these conditions particulate organic matter is hydrolyzed into soluble organic matter. Because the hydrolytic pretreatment step substantially alters the chemical and physical properties of the sludge, the biodegradation of NPEO to NP during the subsequent digestion stage could also be altered relative to a conventional digestion process with no pretreatment. Carballa et al. (2006) found that autoclaving sludge at 130 °C for 60 min prior to MAD did not improve removal of estrone, 17β-estradiol, or 17α-ethinylestradiol and Barret et al. (2010a) found that thermal treatment of primary sludge did not improve removal of NP. Research is still needed on how TH pretreatment of blended primary and secondary sludge affects the fate of NPE during subsequent anaerobic digestion.

The objectives of this research were to determine the direct impact of TH on NPE, and the impact of TH-MAD, relative to conventional MAD and aerobic digestion, on the biodegradation of NPEO to NP. Laboratory digester studies were conducted to determine if sludge pretreatment alters the efficiency of NPEO and NP biodegradation during downstream anaerobic and aerobic digestion. Batch thermal hydrolysis experiments were conducted to determine if the TH pretreatment step directly destroyed NPE.

2. Materials and methods

2.1. Chemicals

Triclosan (sold as Irgasan, ≥97%), 17β-estradiol (E2) (≥98%), nonylphenol (NP, technical grade mixture), bisphenol A (BPA) (97%), NP_1EO and NP_2EO technical analytical standards, and IGEPAL CO – 210 (a mixture of NP_1EO and NP_2EO) were purchased from Sigma–Aldrich (St. Louis, MO). Non-labeled p-n-NP_1EO and p-n-NP_2EO, and labeled $^{13}C_6$-p-n-NP (99%), were purchased from Cambridge Isotope Laboratories (Andover, MA). Cyclohexane, dichloromethane (DCM), acetone, and methanol were all HPLC grade or higher.

2.2. Experimental setup

2.2.1. Thermal hydrolysis of micropollutants in water or sludge

Thermal hydrolysis experiments were performed in a general chemical digestion bomb (No. 4745, Parr Instrument Co., Moline IL) to determine if pertinent micropollutants were degraded from the high temperature and pressure conditions of TH. Bisphenol-A, triclosan, NP, NP_1EO, and NP_2EO were dissolved into methanol and were mixed with Milli-Q water to achieve a final methanol concentration of 0.4%; chemical concentrations were approximately 10 μM. Experiments were also performed with neat NP, NP_1EO + NP_2EO, or triclosan (no methanol) mixed with Milli-Q water. Ten mL of water/methanol or water were placed in the bomb, which was then heated for two hours at 150 °C in an oven at a pressure of 5.1 bar. Samples were removed from the bomb the following day to ensure that they had cooled sufficiently to avoid volatile loss of compound upon opening the bombs. Additional experiments were performed with NP_1EO + NP_2EO in which the sample was exposed to two consecutive thermal hydrolysis treatments (1 per day) to determine if degradation occurred after multiple thermal treatments. Controls were run in an identical manner except that they were not heated. Initial and final concentrations of the analytes were determined by liquid chromatography–mass spectrometry (LC/MS). Replicate bomb experiments were performed for all compounds tested.

Experiments were also performed on NP added to sludge and subjected to TH or NP added to sludge and allowed to sit at room temperature (room temperature control). Additional details on these TH experiments in sludge are available in the Supporting Information (SI Text 1).

2.2.2. Thermal hydrolysis of wastewater sludge followed by anaerobic digestion

Lab-scale experiments were also performed in which raw sludge was subjected to TH followed by anaerobic digestion to determine if TH pretreatment altered the biodegradation of NPEO to NP. Three thermal hydrolysis-mesophilic anaerobic digestion (TH-MAD) reactors were operated. One reactor (TH150-MAD) received sludge that underwent TH at 150 °C followed by MAD with an SRT of 15 days; a second reactor (TH170-MAD) received sludge that underwent TH at 170 °C followed by MAD with an SRT of 15 days; a third reactor (TH150-MAD20) received sludge that underwent TH at 150 °C followed by MAD with an SRT of 20 days. A conventional mesophilic anaerobic digester (MAD) was fed untreated sludge and served as a control. Biosolids from full-scale MAD reactors at Pepper's Ferry Regional Wastewater Treatment Plant (Radford, VA) were used to seed the lab-scale MAD reactor, and biosolids from a full-scale TH-MAD reactor were used to seed the lab-scale TH-MAD reactors. The reactor operating conditions and general reactor performance are described in Table 1. The feed to either the MAD digester or the TH step was a 50/50 mixture (by mass) of primary and secondary (activated) municipal wastewater sludge obtained from the Blue Plains wastewater treatment plant (WWTP) in Washington

D.C. Feed for the control MAD reactor was shipped weekly on ice from Blue Plains WWTP to Virginia Tech overnight and stored at 4 °C until fed to the digester (less than two weeks). Sludge subjected to TH was shipped on ice overnight from Blue Plains WWTP to the TH pilot-scale plant (see below) where it was stored at 4 °C until treatment (less than two weeks).

Table 1. Average reactor operating conditions and performance.

Sample	SRT (days)	Temp (°C)	Total solids in samples (%)	Volatile solids reduction (%)	VFA (mg/L as HAc)	Ammonia (mg/L)	Duration of operation at sampling time (SRTs)	Days past steady-state at time of sampling
MAD Inf[a]			5.5			915		
MAD Eff[a]	15	37	3.1	50	230	1760	11.8	40
MAD-AER Eff[a]	6	34	2.5	62	36	282	35.4	40
TH150-MAD Inf[b]			9.8[d]; 12.8[e]		30,000	535		
TH170-MAD Inf[b]			9.8		34,950	550		
TH150-MAD Eff[c]	15	42	5.8[d]; 6.1[e]	59	6180	2510	5.5[d]; 21.3[e]	21[d]; 258[e]
TH170-MAD Eff[c]	15	42	5.8	58	4250	2470	5.5	33
TH150-MAD20 Eff[c]	20	42	6.7	60	3230	2130	7	87
TH-M-AER 20/20 Eff[b]	5	34	4.0	70	760	828	2.5	15[f]
TH-M-AER 12/12 Eff[b]	6	34	6.0	70	760	171	2	12[f]

[a]Banjade, 2008.

[b]Tanneru, 2009.

[c]Wilson et al., 2011.

[d]TH150-MADa.

[e]TH150-MADb.

[f]Days in operation with consistent aeration/anoxic cycling.

The TH treatment step took place at a pilot-scale system operated by RDP Technologies, Inc. in Norristown, PA. In the pilot system approximately 30–40 L of blended sludge were heated to either 150 °C or 170 °C under high pressure (5–8 bar) for 30 min. The reactor was vented to the atmosphere by a quick flash to reduce the pressure. This TH-treated sludge was then shipped to Virginia Tech overnight on ice where it was stored at 4 °C until used as the influent feed to the TH-MAD reactors. The lab-scale anaerobic digesters were Minibrew 6.5 gallon fermenters (Hobby Beverage Equipment Co.). The operational and performance parameters of each reactor are summarized in Table 1.

Influent and effluent samples from the TH170-MAD and TH150-MAD20 reactors were taken once, and influent and effluent from the TH150-MAD reactor were sampled twice, to generate an overall total of four sets of influent/effluent TH-MAD samples. The influent and effluent of the conventional MAD reactor was sampled once. While having one sample from the MAD reactor might not be representative of the conventional MAD process, much work is already available on the transformation of NPEO to NP in conventional MAD (Giger et al., 1984, Brunner et al., 1988), and thus it was deemed most important to operate and sample multiple TH-MAD reactors. The first set of TH150-MAD samples are labeled with an 'a' and the second set of TH150-MAD samples are denoted with a 'b'; these samples were taken 237 days apart. All influent and effluent digester samples were packed in a cooler with ice packs, stored in plastic bottles, and shipped to the University of Minnesota overnight; once at the University of Minnesota, the samples were stored at 4 °C until they were freeze dried for further cleanup and analysis (maximum storage time of any sample at 4 °C was 104 days). A test on the effect of storage time on NP showed that there was a 5.5% difference between NP in a sample analyzed immediately (Day 0) and a sample analyzed after 154 days of storage at 4 °C. Samples were normalized to amount of influent raw sludge present in the sample to account for variations in solids destruction during digestion.

The samples from the anaerobic digesters (MAD + TH-MAD digesters) were taken after at least one sludge retention time (SRT) following the initiation of steady-state (Table 1). Steady-state was defined as a time in operation in which the daily gas production differed by less than 10% from the average, and the pH varied by 0.1 or less from the average.

2.2.3. Aerobic digestion following anaerobic digestion

Aerobic digesters were set up to determine the benefit, with respect to NPE and total estrogenicity removal, of an aerobic digestion phase following TH-MAD or MAD. Following MAD, the MAD effluent served as the influent to an aerobic reactor (MAD-AER reactor); therefore, the MAD effluent was the same as the MAD-AER influent. The aerobic digester consisted of a 9.5-L glass reactor (Novak et al., 2011) that was aerated continuously. Dissolved oxygen levels were maintained at 2.5–3 mg/L (Banjade, 2008). The MAD-AER reactor was sampled after at least one sludge retention time (SRT) following the initiation of steady-state (Table 1).

Following TH-MAD, an aerobic/anoxic reactor was operated under two different conditions. In the first operational setup, aerobic and anoxic phases were alternated every 20 min. The influent and effluent to this reactor are called TH-M-AER 20/20 Inf and TH-M-AER 20/20 Eff, respectively. The influent to this reactor was a blend of the digested effluent from the TH150-MAD and TH170-MAD reactors. Under the second set of operational conditions the aerobic and anoxic phases were alternated every 12 min. The influent and effluent to this reactor are called TH-M-AER 12/12 Inf and TH-M-AER 12/12 Eff, respectively. The influent to this reactor was a blend of the digested effluents from the TH150-MAD and TH150-MAD20 reactors. The SRTs in these TH-M-AER digesters were held constant for more than nine SRTs, but the cycling scheme of the aerobic and anoxic phase was held constant for just two SRTs. We therefore did not consider the aerobic digesters following the TH-MAD treatments to be at steady-state.

2.3. Biosolids sample handling and processing for LC/MS and YES analysis

Samples were sent for freeze drying to either the National Lacustrine Core Repository (LacCore) or the Gortner Laboratory located at the University of Minnesota. A fraction of the dried solids was weighed (approximately 1 g) and underwent successive overnight Soxhlet extractions in methanol followed by DCM. The liquid extract was reduced via evaporation using a Rotovap (Rotovapor R-3000, BUCHI Laboratory Equipment). Dichloromethane completely evaporated and the remaining methanol extract was reduced to 10 mL. A quantitative aliquot (approximately 10% by volume) was removed and filtered through a 0.2 µm filter for yeast estrogen screen (YES) analysis. The remaining extract was solvent-exchanged into cyclohexane for further cleanup prior to LC/MS analysis. This fraction was loaded onto a normal phase cleanup column containing sodium sulfate, alumina, and silica, and extracted with cyclohexane, DCM, and acetone. The cyclohexane fraction was wasted, and the DCM and acetone fractions were pooled for further preparation. Samples from the TH170-MAD, TH150-MADa, and TH-M-AER 20/20 reactors were analyzed at this point, whereas samples from the conventional MAD, TH150-MADb, and TH-M-AER 12/12 reactors were loaded onto a gel permeation column (GPC) for further cleanup. The GPC had a 25 mm diameter and 100 cm length, and contained Bio-Beads S-X3 (Bio Rad Co., Hercules CA). The mobile phase, a mixture of cyclohexane/DCM (60/40 by volume), was pumped at a rate of 5 mL per min and the eluent collected from 50 min to 120 min was retained for LC/MS analysis. This final aliquot was exchanged into methanol, blown down under a nitrogen stream to approximately one mL, and filtered through 0.2 µm filters for analysis via LC/MS. The relative standard deviation (RSD) of the ratio of NP/NPE on duplicate samples extracted from the same freeze-dried aliquot was 11.8% after alumina/silica processing, and 2.8% after GPC processing. The extraction and cleanup method for the TH experiments in sludge were different and are described in detail in the Supporting Information (SI Text 1).

2.4. Analytical methods and binding assay

2.4.1. LC/MS operation

Analyte quantification was performed on an 1050 Agilent HPLC connected to an MS equipped with electrospray ionization (ESI). Chromatography was performed on a Synergi (150 × 2.0 mm, 4 µm) RP column that was protected by a guard column (4 × 2.0 mm) (Phenomenex, Inc.; Torrance, CA). Mobile phase was constantly pumped at a rate of 200 µL per min and consisted of an isocratic 23.5%/76.5% mixture of solvent A (90% water, 10% methanol, 2 mM ammonium acetate) and solvent B (100% methanol, 2 mM ammonium acetate). The MS was operated in selective ion monitoring (SIM) mode. The drying gas temperature was 350 °C. Nitrogen was the desolvation gas and the nebulizing gas. The MS capillary voltage was set to 4 kV. NP (analyzed in biosolids and clean water TH experiments), triclosan, and bisphenol-A (clean water only) were quantified in negative mode. NP_1EO and NP_2EO (analyzed in biosolids and clean water experiments) were quantified in positive mode.

Internal standards ($^{13}C_6$-NP for negative mode; p-n-NP_1EO and p-n-NP_2EO for positive mode) were used to account for matrix suppression in biosolids samples and variation in instrument injection volumes. A methanol blank and a standard were injected at least once every 12 samples to check for column contamination and consistency in MS response. The instrument detection limit (IDL; ng injected) for NP, NP_1EO, and NP_2EO were 0.4, 1.25, 0.23, respectively. The corresponding method quantification limits (based on analyte recovery, sample concentration factors during cleanup, and a ratio of 10/3 for quantification to detection limits) for NP, NP_1EO, and NP_2EO were 0.4, 1.3, and 0.2 mg/kg, respectively. NPE levels were typically 1–2 orders of magnitude greater than the MQL. Spike-recovery tests on samples to which analytes were added following lyophilization resulted in recoveries for NP, NP_1EO, and NP_2EO of 94%, 94%, and 112%, respectively. The relative standard deviation (RSD) on duplicate recovery tests for NP, NP_1EO, and NP_2EO were 1.3%, 4.1%, and 10.9%. Ten blanks were extracted along with samples and were also analyzed for NP, NP_1EO, and NP_2EO. Only one blank contained an analyte (NP) with a concentration greater than 10% of that detected in a sample; NP_1EO and NP_2EO were never detected above 10% of the sample concentration.

2.4.2. YES assay procedure

The YES assay was performed as described by Routledge and Sumpter (1996), with minor modifications. Yeast absorbance was measured at 540 nm and 620 nm by a microplate spectrophotometer (Molecular Devices, Inc.; Sunnyvale, CA), and readings were taken after incubation at 32 °C for 3 days or until a standard curve fully developed. Adjusted absorbance values used to quantify response were calculated as shown in Section 2.5. Plates were shaken for two minutes prior to incubation. YES assay data are generally assumed to be semi-quantitative (Van den Belt et al., 2004) because chemical analysis and the YES assay produce non-identical estrogenic values of the same order of magnitude (Rutishauser et al., 2004). Therefore, comparisons were made between samples that were tested on plates with a similar response in standard curves. Comparisons between samples were made by obtaining EC50 values from the modeled dose–response curve as described below in Section 2.5.

2.5. YES assay calculations

When a complete dose–response curve was generated, E2-equivalents (EEQ) were calculated from the ratio of EC50 values in the sample and the standard curve (Eq. (1)).

(1) $\mathrm{EEQ}\left(\frac{\mathrm{ng\_E2}}{\mathrm{g\_sample}}\right) = \frac{\mathrm{EC50_{std}(ngE2/L)}}{\mathrm{EC50_{sample}(g/L)}}$

Absorption data at 540 nm were corrected for background absorbance and turbidity using Eq. (2).

(2) $\mathrm{Adjustedabsorbance} = A_{540}\mathrm{total} - A_{540}\mathrm{matrix} - 1.07 * [A_{620}\mathrm{total} - A_{620}\mathrm{matrix}]$

Some sludge samples were toxic to the yeast at high concentrations and caused early die off during the YES assay, resulting in no development of a classic dose–response curve. Best-fit models were applied to the useable data in these toxic samples to complete a full curve, as described in detail elsewhere (Stanford and Weinberg, 2010). Briefly, data were fit to the dose–response equation (Eq. (3)), where *Y* is adjusted absorbance, *L* is minimum response, *H* is maximum response, slope is the Hillslope of the curve, and *X* is the concentration. *H* and *L* were manually selected based on response of the standard and the slope and EC50 were automatically generated by Graphpad Prism software (Graphpad Software, Inc.; La Jolla, CA).

(3) $Y = L + \frac{H-L}{(1+\mathrm{e}^{(\mathrm{slope}*[\log \mathrm{EC50} - \log X])})}$

Calculated estrogenic equivalents (CEEQ), reported in ng E2/g sample, were calculated from the LC/MS-generated NPE concentrations and the relative potencies (*i.e.*, estrogenicity of analyte relative to estrogenicity of E2) of the measured compound (Eq. (4)). Relative potencies for NP, NP_1EO, and NP_2EO were taken from Gabriel et al., 2008, Cespedes et al., 2004, and Routledge and Sumpter (1996), respectively. These CEEQ values were compared to the measured EEQ values obtained from the YES assay to estimate the fraction of estrogenicity that NPE accounted for in the biosolids samples.

(4) $\mathrm{CEEQ} = \left(\frac{\mathrm{ng}(X)}{\mathrm{g\_sample}}\right)\left(\frac{\mathrm{ngE2\_eq}}{\mathrm{ng}(X)}\right)$

3. Results and discussion

3.1. Thermal hydrolysis of micropollutants in a water or sludge matrix

Our initial experiments focused on whether the high temperature and pressure conditions used during TH could directly destroy micropollutants typically found in high concentrations in solid wastewater residuals, *i.e.*, NP, NP_1EO, NP_2EO, triclosan, and bisphenol A (Citulski and Farahbakhsh, 2010, McClellan and Halden, 2010, Staples et al., 2010). The high temperature and pressure during TH has been shown to transform lipids to volatile fatty acids by fragmentation of the hydrocarbon chain (Wilson and Novak, 2009), thus suggesting that compounds

with hydrocarbon chains such as NP might be degraded via TH. No destruction of NP nor of the other micropollutants tested was observed during TH (Table 2; Figure S2; Figure S3). In the sludge matrix there was no significant difference at the 95% confidence (Student's *t*-test) interval between the concentration of NP in the TH-treated and room temperature control systems, although the standard deviation between samples was high (Figure S3). Any effect that TH might have on the transformation of these micropollutants therefore would be a result of altered downstream biodegradation via secondary impacts (*e.g.*, changing bioavailability or soluble carbon), and not a result of direct TH-mediated transformation. Thus, we focus the remainder of our discussion on the effect of TH on downstream biological processes and what occurs during anaerobic and aerobic digestion following TH.

Table 2. Impact of TH on micropollutants in water.

Analyte	**Replicates**	**Solution**	***p*-Value**	**Removal efficiency (η)**
(C_{RT} v. C_{THP})[b]				
NP	3	Water	0.555	NS[a]
NP_1EO	3	Water	0.940	NS
NP_2EO	3	Water	0.516	NS
(C_o v. C_{THP})[c]				
Triclosan	3	Water	0.336	NS
BPA	3	0.4% MeOH	0.724	NS
($C_{DAY\,1}$ v. $C_{DAY\,2}$)[d]				
NP_1EO	3	Water	0.584	NS
NP_2EO	3	Water	0.898	NS

[a]NS = no significant difference at $p \leq 0.05$ between the two sets of samples.

[b]*t*-test on concentrations in room temperature samples vs. TH samples.

[c]*t*-test on concentrations in initial sample taken prior to TH vs. sample taken after TH.

[d]*t*-test on concentrations in samples taken after one TH experiment vs. samples taken after a consecutive second TH experiment.

3.2. Impact of TH pretreatment on NPE mass in digester effluent

The amount of NPE in the influent sludge to the TH-MAD reactors was consistently lower than the NPE in the influent sludge to the MAD reactor. This loss is not likely a result of chemical destruction, as demonstrated by our TH experiments in water (see Section 3.1; Table 2) and TH experiments in sludge (SI Figure S3, SI Text 1); rather, this loss is likely a result of volatilization during the flashing process at the pilot-scale plant. In a full-scale operation, the sludge cooling process occurs in a closed system, and thus NPE will not be removed via volatilization.

In all of the anaerobic reactors, with one exception (TH150-MAD20), the total masses of NPE in the influent and effluent were within 10% of each other (Fig. 1). This finding indicates that, in general, (1) NPEO was transformed to NP and (2) neither substantial loss of NP nor (3) long-chain NP_nEO (n = 3–20) degradation to NPE occurred during anaerobic digestion, regardless of TH pretreatment. This result was expected, as work has shown that NP_nEOs are long-lived, with half-lives greater than 100 days in anaerobic sediments (Ferguson and Brownawell, 2003), while the biodegradation of NP_2EO to NP_1EO and NP_1EO to NP readily occurs under anaerobic conditions (Giger et al., 1984, Soares et al., 2008). In addition, although NP degradation under anaerobic conditions is possible (Chang et al., 2005, Chang et al., 2008), the rate is slow, and under typical digester conditions with large quantities of carbon available, NP degradation is not thought to readily occur (Giger et al., 1984).

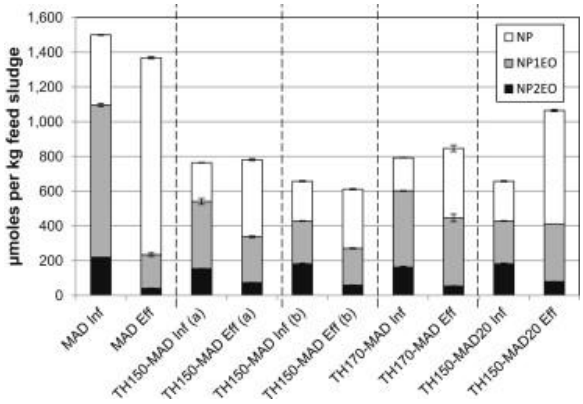


Fig. 1. Impact of TH pretreatment to anaerobic digestion on total NPE in biosolids. Each bar represents the total NPE in each sample, with the individual concentrations of NP_2EO, NP_1EO, and NP represented as labeled on the plot. Error bars refer to standard error of the mean between triplicate runs on the LC/MS, with the exception of the MAD Eff sample where the error bars represent standard error of the mean on duplicate extractions.

In the TH150-MAD20 reactor, however, the mass of NPE increased by 62% during digestion. Longer-chain ethoxylates (NP_3EO–$NP_{20}EO$, not analyzed in this study) are parent compounds of NPE. Although not observed in the MAD, TH150-MAD, and TH170-MAD reactors, the increase of total NPE seen in the TH150-MAD20 effluent suggests that higher chain ethoxylates can be broken down to NPE under these typical digester conditions. As a result, further investigation on the transformation of long-chain NP_nEOs during TH-MAD with an extended SRT is warranted.

3.3. Impact of TH pretreatment on the ratio of NP to NPE in digester effluent

The conventional MAD treatment process produced biosolids with a higher ratio of NP:NPE than the TH-MAD treatments (Fig. 2). The ratios of NP to NPE increased from influent to effluent in all anaerobic digesters as a result of the biodegradation of NPEO to NP (Fig. 2). The biodegradation efficiency of NPEO to NP varied, however, depending on whether TH preceded MAD. In conventional MAD the ratio of NP:NPE increased 56% following digestion, whereas in the three sets samples from TH-MAD reactors operated at 15 d SRT, the ratio of NP:NPE only increased 23.9 ± 3.4% (average ± standard deviation). The ratio of NP:NPE in the TH150-MAD20 reactor increased 26.6%. It is interesting that the conventional MAD treatment process was more efficient at biodegrading NPEO to NP than the advanced TH-MAD process, even though the TH-MAD reactors outperformed the conventional MAD reactor based on volatile solids reduction. Thus, digester performance based on classical criteria was not directly related to the biodegradation efficiency of NPEO to NP. These results, showing reduced NPEO biodegradation to NP following TH, are similar to other results that show PAH removal during anaerobic digestion decreased after TH of the feed sludge (Barret et al., 2010a).

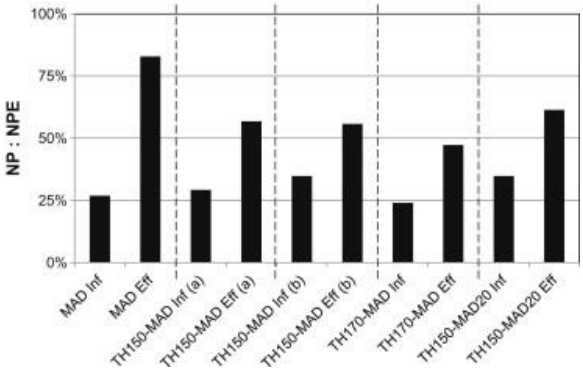


Fig. 2. Impact of TH pretreatment to anaerobic digestion on the ratio of NP to NPE.

Barret et al. (2010c) have suggested that PAH degradation during anaerobic digestion can be limited by bioavailability or cometabolism. The fact that the TH-MAD reactors had higher solids destruction, an indicator of microbial activity, but lower conversion of NPEO to NP, indicates that, either 1) cometabolism was not the limiting factor, or 2) overall metabolism in the reactor was not a relevant indictor of NPEO biodegradation. Indeed, the TH process generated much higher levels of volatile fatty acids, perhaps resulting in the inhibition of ethoxylate-degrading organisms or greater substrate competition (Russell, 1992, Chang et al., 2008). The TH process also resulted in higher ammonia levels from the rapid conversion of soluble proteins (Wilson and Novak, 2009), which could inhibit the degradation of NPEO to NP. Thermal hydrolysis also reduces the bioavailability of hydrophobic micropollutants (Barret et al., 2010b), which could explain the reduction of NPEO biodegradation seen in the TH-MAD reactors with a 15 day SRT.

This trend of less NPEO conversion to NP in the TH-MAD setups was also observed when comparing results from our research to that of published full-scale studies (Fig. 3, SI Table 1). The average ratio of NP:NPE in 36 full-scale conventional mesophilic anaerobic digesters was 82%; in this study the NP/NPE ratio was 83% for the MAD reactor. Because the conversion of NPEO to NP in a few of the full-scale conventional MAD reactors was lower than in the TH-MAD reactors in this study, it cannot be stated that TH will always hamper the biodegradation of NPEOs to NP in downstream digestion. Nevertheless, the difference between the NP:NPEO ratio in all of the 36 full-scale reactors depicted in Fig. 3 and the 4 sets of samples from the TH-MAD reactors in this study was statistically significant (p = 0.0006; Student's t-test).

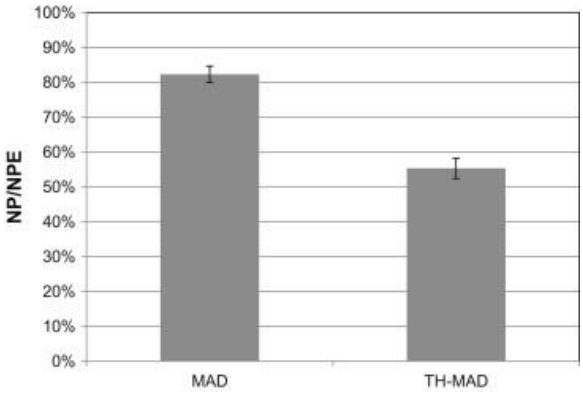


Fig. 3. Impact of TH-MAD on ratio of NP to NPE relative to full-scale MAD digesters. MAD samples (n = 36) taken from Gonzáles et al. (2010) and Brunner et al. (1988); TH-MAD samples (n = 4) taken from this study. Error bars depict standard error of the mean. Actual values of NPE concentrations in all samples are shown in Table S1 of supporting information.

3.4. Impact of a post-aerobic digestion phase on fate of NPE

The total NPE decreased in the additional aerobic phases that followed the MAD reactor and in the anoxic/aerobic phases that followed TH-MAD (Fig. 4). Under aerobic conditions, the total NPE decreased by nearly 70%, whereas in the post-digestion reactors (with anoxic/aerobic cycling) that followed TH-MAD, 30–45% of total NPE mass was removed. NP is mineralized under aerobic conditions (thus accounting for a decrease in total NPE), but nonylphenoxy carboxylates (NPECs) can also form during aerobic degradation of NPEO (Hesselsoe et al., 2001, Ahel et al., 1994). NPEC were not analyzed and might have accounted for some loss of NPE during aerobic digestion. The LC/MS results showing a decrease in total NPE following aerobic or anoxic/aerobic treatment were corroborated by an observed reduction in total estrogenicity as measured via the YES assay. Total estrogenicity was reduced by approximately 50% during aeration following conventional MAD (Figure S1). Similar results have been observed by Knudsen et al. (2000), who found that aerobic treatment following anaerobic digestion reduced NPE concentrations by over 75%. These results indicate that an additional

treatment phase that includes aeration of some fashion would decrease total NPE regardless of whether TH pretreatment was used.

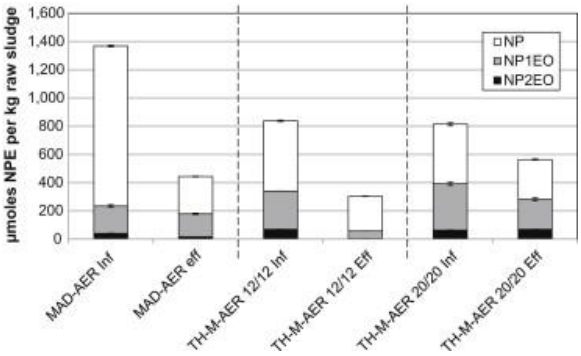


Fig. 4. Impact of post-aerobic digestion following anaerobic digestion on total NPE in biosolids. Error bars refer to standard error of the mean between triplicate runs on the LC/MS, with the exception of the MAD-AER Inf sample where the error bars represent standard error of the mean on duplicate extractions.

3.5. Contribution of NPE to total estrogenicity

The NPE are only weakly estrogenic (Routledge and Sumpter, 1996). As a result of the high concentrations of NPE that accumulate in biosolids, however, NPE may still represent a notable fraction of the total estrogenicity in biosolids. Indeed, this study found the contribution of estrogenicity from NPE in anaerobically and aerobically treated biosolids was 21 ± 27% and 32 ± 16%, respectively. These results indicate that NPE represent a marked fraction of the total estrogenicity in biosolids. The NPE were shown to account for less than 4% of total estrogenicity in sewage treatment plant liquid effluent in two different studies (Rutishauser et al., 2004, Zhang et al., 2011). Conversely, Holbrook et al. (2002) estimated that nonylphenol and octylphenol accounted for >100% of the estrogenicity in biosolids. While NPE might be less of a concern regarding impacts in liquid discharge, their contribution to the estrogenicity of biosolids must be considered when evaluating the environmental impacts of land application. Even though NPE have been shown to readily degrade in soil (Gomez-Rico et al., 2008), NP was detected at mg/kg levels in soils that consistently received biosolids (Xia et al., 2010). Certainly the concentration of NPE in soil will depend on when biosolids are land applied and the concentration of NPE in the biosolids.

It is likely that low concentrations of natural estrogens (estrone and 17β-estradiol) contributed to the total estrogenicity of the biosolids (Janex-Habibi et al., 2009). Estrone and 17β-estradiol are several orders of magnitude more potent than NP in terms of estrogenicity (Jobling and Sumpter, 1993, Matsui et al., 2000), so even low concentrations (ng/kg levels) of natural estrogens could greatly increase the estrogenicity of biosolids. Yet, whereas the production and discharge of natural estrogens cannot be controlled or decreased, NPE usage and discharge can be limited. Our work showed that additional aerobic digestion, or perhaps composting (Jones and Westmoreland, 1998), is needed to remove total NPE after MAD (with or without an advanced TH process). Either widespread additional treatment of biosolids, concomitant with greater energy use and a larger carbon footprint, or a ban on NPE might therefore be needed to mitigate the impacts of estrogenicity from NPE in biosolids.

4. Conclusions

- The high temperature and pressure conditions used for TH were not sufficient to degrade NP in sludge. Similarly, NPE were not degraded during TH in a water matrix (nor were other micropollutants tested, such as BPA and triclosan). While TH might indirectly impact the fate of NPE in digested biosolids by altering physico-chemical properties in sludge (*e.g.*, bioavailability), this process does not directly degrade NPE abiotically.

- The total NPE in the influent and effluent of the anaerobic digesters (15 day SRT) was approximately equal, regardless of whether TH pretreatment was used or not. Under anaerobic conditions, NPEO were biodegraded to NP more rapidly than NP was biodegraded; this resulted in a decrease in NPEO and an increase of NP in digester effluents, with similar amounts of total NPE in the influent and effluent.
- The NPE in the effluent of the TH-MAD-20d reactor increased relative to the influent. This increase in NPE suggests that longer chain NP_nEOs were broken down to NPE during this extended SRT (20 days).
- The biodegradation of NPEO to NP was greater in the conventional MAD reactor than in the TH-MAD reactors, as evidenced by the ratios of NP to NPE in the digester effluents. The ratio of NP to NPE increased 56% following conventional MAD, while the average increase of NP to NPE was only 23.9% in the three samples from TH-MAD reactors with a 15 day SRT. One possible explanation for this reduced biodegradation of NPEO to NP is that the TH process reduced the bioavailability of NPEO.
- The addition of a post-digestion treatment step that included either alternating aerobic/anoxic conditions or strictly aerobic conditions reduced NPE by 30–70%. This additional treatment step could be costly as a result of oxygen requirements, but would help reduce NPE in biosolids used for land application.
- The NPE accounted for 21 ± 27% and 32 ± 16% of the total estrogenicity in the biosolids after anaerobic and aerobic digestion, respectively.
- A ban on NPE would undoubtedly reduce NPE present in biosolids. Alternatively, additional wastewater treatment steps such as aerobic digestion or composting could be used to help mitigate the impact of NPE in biosolids.

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Appendix. Supplementary material

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Figure S1. Impact of a post-aeration digestion following anaerobic digestion on total estrogenicity in biosolids as depicted by YES assay results. A sample curve shifted to the left is more estrogenic than a sample curve on its right. The “MAD Eff” sample was the feed to the MAD-AER reactor.

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Figure S2. Effect of thermal hydrolysis treatment in a sludge matrix on the concentration of NP (all replicates shown). ‘Inf’ is influent, ‘TH’ is thermal hydrolysis treated sludge, ‘RT’ is room temperature. Numbers 1, 2, 3 refer to replicate experiments.

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Figure S3. Effect of thermal hydrolysis treatment in a sludge matrix on the concentration of NP (averages shown plus standard deviation). Sample “TH-1” was removed from analysis.

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