

The effectiveness of anaerobic digestion in removing estrogens and nonylphenoethoxylates

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Abstract

The fate and behaviour of two groups of endocrine disrupting chemicals, steroid estrogens and nonylphenol ethoxylates, have been evaluated during the anaerobic digestion of primary and mixed sewage sludge under mesophilic and thermophilic conditions. Digestion occurred over six retention times, in laboratory scale reactors, treating sludges collected from a sewage treatment works in the United Kingdom. It has been established that sludge concentrations of both groups of compounds demonstrated temporal variations and that concentrations in mixed sludge were influenced by the presence of waste activated sludge as a result of transformations during aerobic treatment. The biodegradation of total steroid estrogens was >50% during primary sludge digestion with lower removals observed for mixed sludge, which reflected bulk organic solids removal efficiencies. The removal of nonylphenol ethoxylates was greater in mixed sludge digestion (>58%) compared with primary sludge digestion and did not reflect bulk organic removal efficiencies. It is apparent that anaerobic digestion reduces the concentrations of these compounds, and would therefore be expected to confer a degree of protection against exposure and transfer of both groups of compounds to the receiving/re-use environment.

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1. Introduction

Numerous organic micropollutants are present in crude sewage and these have been the cause of concern for many years [1]. Recently those organic micropollutants with endocrine disrupting ability have been the subject of major research investigations [2–5]. Endocrine disruption in fish has been predominantly attributed to the presences of free natural estrogens such as estrone (E1) and 17 β -estradiol (E2) together with the synthetic estrogen 17 α -ethinylestradiol (EE2) as well as nonylphenols (NP) and nonylphenol ethoxylates (NPEOs). Whilst conventional wastewater treatment plants are able to breakdown endocrine disrupting chemicals (EDCs) [6] as presently operated this capability is limited [3,5]. Once in the receiving waters, these compounds are likely to undergo biotransformation and EDCs have the potential to bio-concentrate [7,8] and accumulate in organisms. Such complex behaviour leads to uncertainty in determining the significance of their occurrence in the environment [10].

Those compounds which have not biodegraded or have only partially biodegraded during wastewater treatment may be adsorbed to the sewage solids [2,3] and will be subject to further treatment prior to reuse. A common form of sewage sludge treatment is anaerobic digestion (AD). Studies of the fate and behaviour of EDCs during anaerobic digestion are limited [11]. Undoubtedly the analytical difficulties of determining these compounds in such complex matrices at environmental concentrations (ng g⁻¹) have been a major constraint on such research [12,13]. However, some studies have been undertaken. One of these was by Carballa et al. [14] who examined pharmaceutical and personal care products. The behaviour of E1, E2 and EE2 in laboratory scale anaerobic sludge digesters under mesophilic conditions (37°C) with a sludge retention time (SRT) of 30 d and under thermophilic conditions (55°C) with an SRT of 20 d was examined. Removal of EE2 was reported to be initially 60% rising to 90% with time. These workers also reported the reduction of E1 to E2 and the subsequent removal of E2.

In anaerobic batch studies of four different sludge types [15] the transformation of E1 to E2 was observed, but no overall reduction in the combined concentrations of E1 and E2 was detected indicative of no further breakdown of E2. This observation was true for all four sludge types examined: digested pig manure; granular up-flow anaerobic sludge blanket (UASB) sludge from a paper mill; flocculent sludge from a pilot-scale UASB septic tank; and activated sludge from a full-scale oxidation ditch. The removal of EE2 was not observed for any sludge type. It was recognized by de Mes et al. [15] that information on the role of conjugation in determining the fate of estrogens in all forms of wastewater treatment

including anaerobic sludge digestion was lacking. However, it is known that sulphate conjugates are more stable than gluconarides [16]. Reports of the degradation of alkylphenol ethoxylates (APEOs) in anaerobic conditions are more scarce in comparison to steroid estrogens. In anaerobic, as well as aerobic conditions, the ethoxylate chain of higher ethoxylate NPEOs is shortened until persistent short-chained NP1–2EOs and NP are formed. This breakdown proceeds by the stepwise removal mechanism of one ethylene glycol unit [2,17]. It appears that NP, a major product of degradation, is frequently reported as not undergoing further transformation [18,19]. However, NP degradation under anaerobic conditions has been recently demonstrated [19–21].

The objective of this study was to determine the fate and behaviour of estrogens and NPEOs, already present in primary and mixed sludge (primary plus waste activated sludge, WAS) under both mesophilic and thermophilic conditions at environmental concentrations. This means that the complex interactions between indigenous compounds and sludge types will reflect reality when evaluating the impact of different sludge treatment approaches on degradation. Eggen and Majcherczyk [22] previously demonstrated that spiking experiments, as frequently undertaken, do not represent reality because added compounds behave differently to “aged” compounds, which are strongly linked to the matrix and therefore require more energy to be biodegraded [23].

2. Material and methods

2.1. Sludge types and collection

The sludges were collected under dry weather conditions on two occasions (April 2007 and April 2008) from a UK sewage treatment works (STWs) for the mesophilic and thermophilic trials respectively. Therefore, variations in sewage sludge composition within each trial were eliminated as an experimental variable. It was necessary to collect sludge on two occasions due to storage capacity limitations which resulted in solid and nonylphenoxy acetic acid(NP1–3EC) loading variations between the trials. The STWs was an activated sludge plant with a population equivalent (PE) of 155,000 and a trade input of c. 10%. Sludge stabilization at the STWs was carried out by two continuous mesophilic (35 °C) anaerobic digesters(450 m³d⁻¹) with 28 d nominal solid retention time (SRT). The digesters were fed every hour for 20 min continuously with mixed sludge (primary and waste activated sludge at a ratio of 60% (v/v) primary and 40% (v/v) WAS) from a balancing tank. The sludge types used in this study were primary sludge and thickened mixed sludge.

Primary sludge was collected directly from the pumped outlet of the primary sedimentation tank. Mixed sludge (primary and WAS) was sampled directly from a balancing tank. The inoculum sludge (10 l digested sludge) was collected directly from the mesophilic anaerobic digesters. Sludge processing commenced within 6 h of sampling. Sludges were sieved through a 2 mm mesh (Alana Ecology Ltd., Bishop's Castle, UK) and transferred to 1 l acid washed polypropylene containers (Fisher Scientific, Loughborough, UK) for storage at -25°C until needed.

2.2. Laboratory scale anaerobic digesters

Laboratory scale anaerobic digesters (1.5 l working volume) were operated in duplicate at each digestion temperature [11]. Each digester comprised a borosilicate glass bowl and lid containing five access ports. The digesters were kept in a water bath at 35°C (mesophilic) or 55°C (thermophilic) and stirred automatically for 10 min in every hour at approximately 100 rpm. For the mesophilic trial the digesters were initially filled with the digested seed sludge from the selected STW. The seed sludge for the thermophilic digesters was obtained by operating an additional 5 l laboratory digester at mesophilic temperature and increasing the temperature by 0.3°C d⁻¹ until the digester was operating successfully at 55°C. Then two batches of 1.5 l thermophilic seed sludge were removed to start the thermophilic trial digesters. This was required as no thermophilic sludge digesters were operating in the UK from which an inoculum could be sourced. The digesters were stabilized for two retention times and were then operated for a further six solid retention times which was 180 d for the mesophilic trial and 90 d for the thermophilic trial.

To feed the digesters, frozen feed sludge was thawed overnight and warmed to 35°C or 55°C. The hydraulic retention time (HRT) was 30 d at 35°C (mesophilic) or 15 d at 55°C (thermophilic). Gas was collected by the displacement of acidified water [11] and measured at atmospheric pressure by the use of a balancing reservoir. The methane (CH₄) content was analysed using a Servomex 1440D infra-red analyser (Servomex Group Ltd., Crowborough, UK) modified for CH₄ analysis. Redox potential and pH were monitored using portable meters (Ion Meter 3340, Jenway Ltd., Essex, UK and VWR pH meter-100 (VWR International Limited, Lutterworth UK). Temperature was measured using a mercury and glass thermometer. On a weekly basis total solids (TS), volatile solids (VS) and alkalinity were determined using standard methods [11]. Volatile fatty acids (VFAs) (acetic acid, butyric acid, propionic acid and valeric acid) were separated and quantified using HPLC (VP Series, Shimadzu, Milton Keynes, UK) fitted with an Aminex® fermentation column

(150mm × 7.8mm, 5µm; Bio-Rad Laboratories, Hemel Hempstead, UK) with detection at 208 nm.

2.3. Analytical procedure for the determination of estrogens and nonylphenol ethoxylates

The standards, reagents and analytical method used in the determination of estrogens (estrone (E1), 17β-estradiol (E2), estriol (E3), sulfate conjugate of estrone (E1-3S) and 17α-ethinylestradiol (EE2) and alkylphenolic compounds (nonylphenols (NP), nonylphenol polyethoxylates (NPEO), and nonylphenol ethoxycarboxylates (NPEC)) have been reported previously [24,25].

In summary, steroid estrogens and nonylphenolic compounds were solvent extracted separately from freeze-dried sludge. Therefore, the results reported here are for total concentrations in the sludge (solid and liquid phases). A Multi-Reax system (Heidolph Instruments, Schwabach, Germany) was applied for solvent extraction using 10 ml ethyl acetate for steroid estrogens and 10 ml MeOH/acetone (1:1) for the alkylphenolics in 25 ml Teflon tubes with mechanical shaking for 1 h followed by centrifugation at 1500 × g for 10 min. The extraction was repeated twice and combined supernatants were evaporated to approximately 0.2 ml then made to 2 ml with hexane. The separate steroid estrogen and alkylphenolic solutions were subjected to clean-up bypassing through a 500 mg/3 ml silica solid phase extraction (SPE) cartridge (Waters Ltd., Watford, UK). The alkylphenolic eluates were then quantified whilst the combined eluates for steroid estrogens were evaporated to dryness on a rotary evaporator then re-constituted in 2 ml of DCM/MeOH (90:10). This purified sample was then subjected to further clean-up by gel permeation chromatography (GPC), anion-exchange SPE and finally quantification by liquid chromatography tandem mass spectrometry in ESI mode (LC/ESI/MS/MS) using an HPLC (Waters Alliance HPLC system 2695) coupled to a Waters Quattro Premier XE mass spectrometer with a Z-Spray ESI source (Micromass, Manchester, UK) as described previously [24]. Further method detail is available in Chiu et al. [26] and Koh et al. [27]. The limit of detection in sewage sludge matrices for NP, NPEOs and NPECs ranged from 6 to 60 µg kg⁻¹ [27] whilst the limit of detection for estrogens (E1, E1-3S, E2, E3 and EE2) were 2.1–5.3 ng g⁻¹ [26].

3. Results and discussion

The overall performance of the reactors in terms of gas volume, percentage methane, VFAs and solids reduction are presented in Table 1. Mean pH values, VFA contents and alkalinity lie within the typical range observed for anaerobic digestion [28] which is indicative of a well established methanogenic population. Redox potential (ORP) values, in all types of digesters, were always slower than -320 mV which favoured the survival of anaerobes and the enzymatic activity of methane-forming bacteria during the entire digestion periods. The solids loading rates for the digesters were 0.8–1.6 kg VS m⁻³d⁻¹ and 1.9–3.0 kg VS m⁻³d⁻¹ for mesophilic and thermophilic digesters. These values are in the range of those reported for digested sludge [28]. The gas production rate of the thermophilic process (0.7–1.2 m³m⁻³d⁻¹) was higher than the mesophilic process (0.5 m³m⁻³d⁻¹). However, the average methane content of the biogas was similar in both digesters (73–75%).

3.1. Effect of sludge type on steroid estrogen removal

Storage capacity necessitated collection of sludge on two occasions, once for the mesophilic experiments in April 2007 and once for the thermophilic experiments in April 2008. Thus influent sludge concentrations reflected not only differences in sludge type (primary or mixed), but also temporal variation (Table 1). The concentration of E1 varied by a factor of 2.5 for the primary sludge and 2.8 for the mixed sludge between the sampling periods. However, although the concentration of E1 varied between the sampling periods, the proportion of E1 remained consistent at 69–78% of the total estrogens. Primary sludge concentrations for E1 were 158 and 64.3 µg kg⁻¹dw for the April 2007 and 2008 periods respectively which were higher than those estimated by Andersen et al. [6] of c. 30 µg kg⁻¹dw (for E1 + E2). Variations for the estrogens E2, E3 and the conjugate E1-3S for both sludge types were not as marked between the sample periods with the influent concentrations varying by 3–3.6 µg kg⁻¹dw. However, the synthetic estrogen (EE2) exhibited a variation factor of two, between 9 and 18 µg kg⁻¹dw for the primary sludge. The mixed sludge EE2 concentrations were equivalent at 10 µg kg⁻¹dw which was equivalent to Muller et al. [29]. It is postulated that variation in sewage treatment works operating conditions, for example flow to works or presence of return liquors, may have contributed to the temporal variations observed in this study.

Table 1 Influent sludge characteristics, operational conditions and digester performance at mesophilic and thermophilic temperatures after six retention times.

	Mesophilic 2007 sampling		Thermophilic 2008 sampling	
	Primary sludge	Mixed sludge	Primary sludge	Mixed sludge
<i>Influent sludges</i>				
TS (g l ⁻¹)	51.1±3.7	57.1±4.3	39.5±0.1	49.7±0.1
VS (g l ⁻¹)	36.5±2.6	44.0±3.0	29.2±0.1	38.1±0.1
VFA (mg acetic acid l ⁻¹)	1314±68	1592±44	1168±98	1470±52
Estrone (E1) µg kg ⁻¹ dw	158	90	64.3	32.3
17β-estradiol (E2)	9	6	6	3
Estriol (E3)	9	8	6	
Estrone-3-sulfate (E1-3S)	7.6	7	4	4
17α-ethinyl estradiol (EE2)	18	10	9	10
4-nonylphenol (NP) mg kg ⁻¹ dw	0.3	0.23	0.23	0.1
Nonylphenoxy acetic acids (NP ₁₋₃ EC) mg kg ⁻¹ dw	26.5	241.5	0.1	0.08
Nonylphenol monoethoxylate and diethoxylate (NP ₁₋₂ EO) mg kg ⁻¹ dw	2.1	1.7	15	90
Nonylphenol polyethoxylates (NP ₃₋₁₂ EO) mg kg ⁻¹ dw	1.5	0.7	1.3	0.7
<i>Operational conditions</i>				
T (°C)	35±0.2	35±0.2	55±0.2	55±0.2
SRT (d)	30	30	15	15
OLR (kg VS m ⁻³ d ⁻¹)	1.3±0.1	1.5±0.1	1.9±0.0	2.5±0.0
TS (g l ⁻¹)	26.7±2.3	38.5±1.3	22.7±1.8	33.9±1.3
VS (g l ⁻¹)	19.5±1.6	23.9±2.0	11.5±4.5	22.0±2.2
pH	7.1±0.1	7.5±0.1	7.2±0.0	7.6±0.1
ORP (mV)	-320.8±12.8	-380.6±29.8	-411.6±36.9	-419.0±34.9
VFA (mg acetic acid l ⁻¹)	176.4±7.3	132.9±17.3	1098.5±189.6	829.3±145.9
Total alkalinity (mg l ⁻¹)	2399±37	5362±63	4000±453	4770±85
<i>Biogas</i>				
Daily production (l d ⁻¹)	0.8±0.0	0.8±0.1	1.0±0.1	1.6±0.1
GRP (m ³ m ⁻³ d ⁻¹)	0.51±0.0	0.52±0.0	0.67±0.0	1.08±0.0
SGP (m ³ CH ₄ kg ⁻¹ VS _{removed})	0.7±0.1	0.6±0.1	0.4±0.1	0.7±0.1
Biogas yield (m ³ kg ⁻¹ VS _{removed})	0.95±0.2	0.80±0.1	0.60±0.1	1.02±0.1
<i>Removal efficiencies (%)</i>				
VS	53.5±6.9	40.1±2.1	43.2±3.0	32.4±1.0
TS	47.3±8.5	33.7±4.6	37.0±4.4	29.8±2.6
g VS _{removed} d ⁻¹	1.07±0.1	0.98±0.1	2.24±0.2	1.84±0.1

Key: TS=Total Solids; VS=Volatile Solids; VFA=Volatile Fatty Acids; T= temperature; SRT=Solid Retention Time; OLR=Organic Loading Rate; ORP=Oxidation/Reduction Potential; GRP=Gas Production Rate; SGP=Specific Gas Production

Sludge type had an impact on the initial concentrations of steroid estrogens. In the primary sludge feed the concentration order was $E1 > EE2 > E2 \sim E3 > E1-3S$ whilst in the mixed sludge feed the concentrations were in the order of $E1 > EE2 > E3 > E13S \sim E2$. This was indicative of biodegradation/biotransformation in the activated sludge plant, which produced the WAS component of the mixed sludge as there was a higher proportion of E3 which was a degradation product of E1 in the activated sludge process.

During digestion, E1 was reduced to E2 in all sludge types, which is consistent with the observations of Carballa et al. [14,30], de Meset al. [15] and Hospido et al. [31]. Oxidation of E2 to E1 has also been reported in anaerobic sediments spiked with E2 (5 mg l^{-1}) [32]. It is apparent that the transformations of E1 and E2 are effected by their initial concentrations. If higher concentrations of E2 dominate then E2 oxidation occurs as observed by [32].

However, in this study higher starting concentrations of E1 were present which were reduced to E2. Overall there is evidence that equilibrium between E1 and E2 was achieved. Nevertheless, the different sludge types had an effect on the transformation rate of E1 and E2. An examination of Fig. 1 demonstrates that for primary sludge a 50% reduction of E1 to E2 occurred after two retention times (60 d) in comparison to the first retention time (30 d) for the mixed sludge at the mesophilic temperatures with the rate of production of E2 faster in the first retention period for mixed sludge ($0.006 \mu\text{g d}^{-1}$) as opposed to primary sludge ($0.001 \mu\text{g d}^{-1}$). A comparable trend occurred at the thermophilic temperature although this was less pronounced. The implication was that the mixed sludge biomass, which at start up had previously been acclimated to steroid estrogens during the activated sludge process, could adapt to anaerobic conditions and continue to function effectively [33]. The overall removal of steroid estrogens in the primary sludge was 53% and 51% for ΣEST (sum of endocrine steroid estrogens E1, E2, E3, E1-3S and EE2) at mesophilic and thermophilic temperatures respectively compared with lower overall removals for mixed sludge of 39% and 12% at mesophilic and thermophilic temperatures respectively (Fig. 2). The overall percentage removals therefore reflected the bulk organic transformations in the anaerobic digesters (Table 1), with the highest steroid estrogen removal occurring in mesophilic digestion of primary sludge and the lowest in the thermophilic digestion of mixed sludge. However, the presence of nitrate in mixed sludge (up to $28 \text{ NO}_3\text{-N mg l}^{-1}$), which is not present in the primary sludges, could also influence steroid estrogen transformations with the final steady state concentration of E2 depending on the electron-accepting condition. Czajka and Londry [32] have demonstrated that nitrate, as an electron acceptor, played a role in the bio-transformations between E1 and E2.

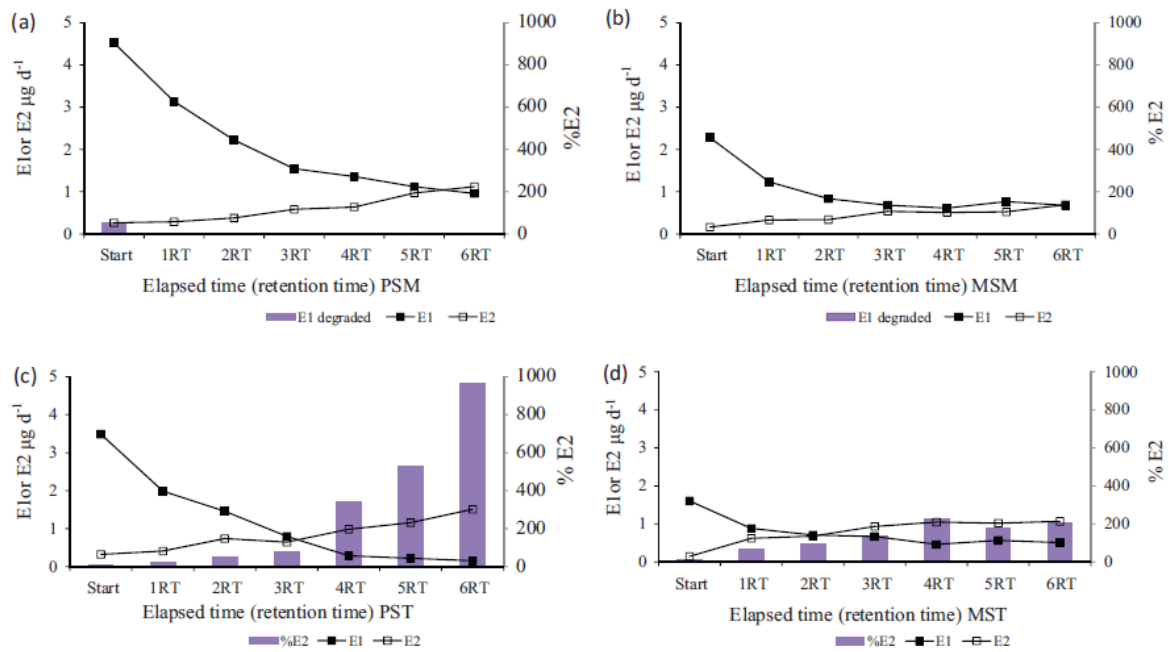


Fig. 1. The mass ($\mu\text{g d}^{-1}$) of E2 and E1 and the %E2 of E1 at each retention period for (a) mesophilic digestion of primary sludge (PSM), (b) mesophilic digestion of mixed sludge (MSM), (c) thermophilic digestion of primary sludge and (d) thermophilic digestion of mixed sludge.

Table 2 Mass flux removal/accumulation percentages and first order rate constant for steroid estrogens and nonylphenol ethoxylates during digestion of primary and mixed sludge(at the 6th retention time) under mesophilic and thermophilic conditions.

	Mesophilic				Thermophilic			
	Primary Sludge		Mixed Sludge		Primary Sludge		Mixed Sludge	
	%	k (d^{-1})	%	k (d^{-1})	%	k (d^{-1})	%	k (d^{-1})
EE2	34%	0.013	4%	0.0052	43%	0.052	14%	0.075
E2	-324%	-0.026	-325%	-0.026	-367%	-0.015	-621%	-0.051
E1	79 %	0.117	70%	0.081	96%	0.042	68%	0.046
E3	45%	0.025	43%	0.023	17%	0.021	4%	0.020
E1-3S	36%	0.017	21%	0.011	30%	0.021	28%	0.024
NP	0%	0.025	100%	0.030	50%	0.091	100%	0.350
NPEC	-215	n/c	0.0035	n/c	>-1000	n/c	-5800%	n/c
NP ₁₋₂ EO	88%	n/c	-0.0274	n/c	2.5%	n/c	100%	n/c
NP ₃₋₁₂ EO	66%	n/c	67%	n/c	73%	n/c	83%	n/c

First order rate constant k (d^{-1}) was calculated for the 6th SRT according to mass flux = $(S_0 - S_e) \times (\frac{V}{R})$

Where, S_0 = volumetric cumulative influent substrate concentration ($\mu\text{g m}^{-3} \text{d}^{-1}$), S_e = volumetric cumulative effluent substrate concentration ($\mu\text{g m}^{-3} \text{d}^{-1}$), V = reactor volume (m^3), R = solid retention time (days).
n/c = not calculated

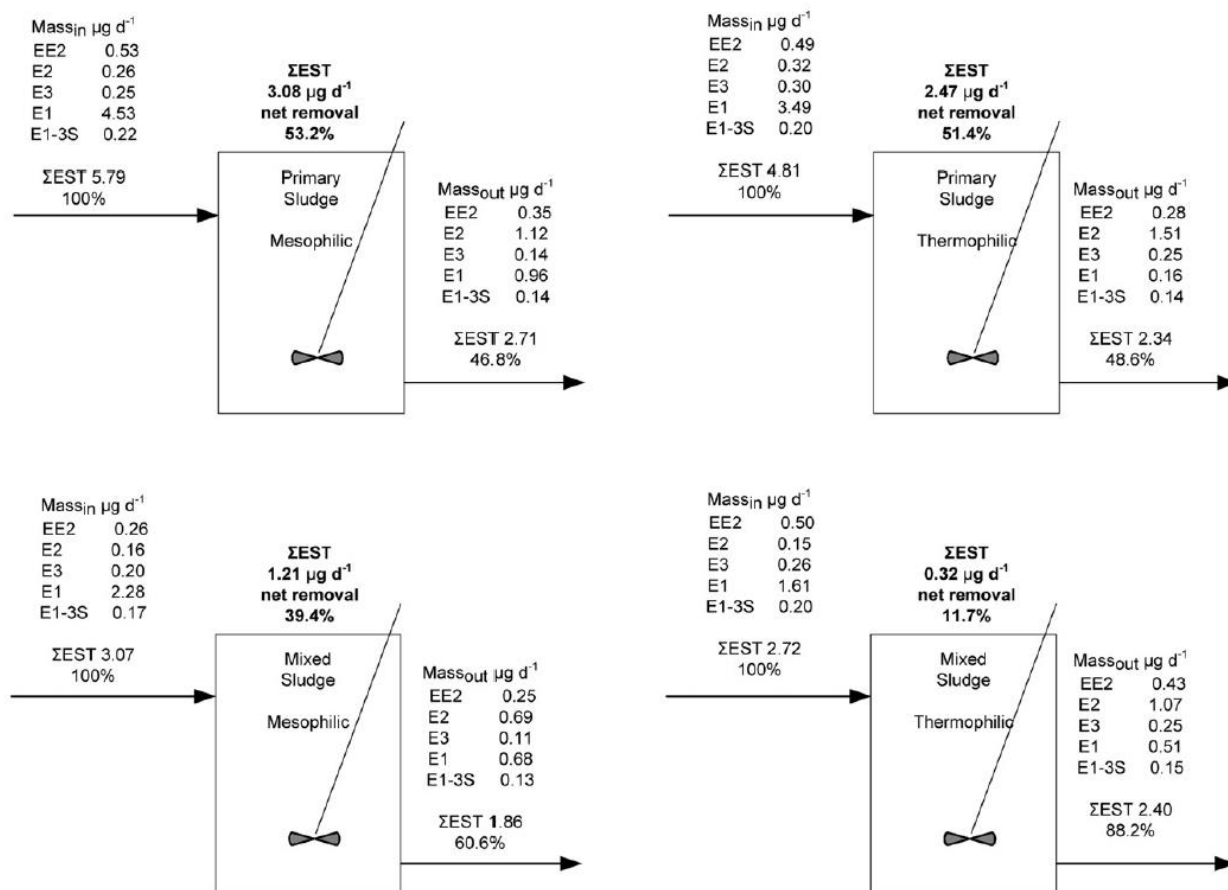


Fig. 2. Mass flux ($\mu\text{g d}^{-1}$) for steroid estrogens at the start and at the end of the anaerobic mesophilic and thermophilic digestion trials for both sludge types.

The behaviour of E1-3S and E3 did not appear to exhibit any differences between the two sludge types. However, EE2 removal appeared higher in the primary sludge reactors with 34% and 43% removal in the mesophilic and thermophilic digesters respectively compared to the mixed sludge digesters at 4% and 14% for mesophilic and thermophilic temperatures respectively (Table 2). The range of removal efficiencies for EE2 under mesophilic and thermophilic conditions is consistent with the observations of Carballa et al. [14] who reported about 40% removals under both conditions.

3.2. Effect of temperature on the removal of steroid estrogens

Under thermophilic conditions greater formation of E2 occurred for both primary and mixed sludge with the highest formation achieved in mixed sludge (-621%) compared to -367% for primary sludge (Table 2). The first order rate constants are also approximately double at 0.05 d^{-1} under thermophilic conditions compared to 0.026 d^{-1} under mesophilic conditions

(Table 2). Therefore, as expected this biochemical reaction was strongly influenced by temperature proceeding faster under thermophilic conditions.

In addition a positive effect of loading was observed on the biotransformation of steroid estrogens. Overall greater percentage removals occurred under thermophilic conditions in comparison to mesophilic conditions (Table 2). However, the mesophilic digesters were more highly loaded than the thermophilic digesters with Σ EST loadings differing by 2.4 times in comparison to the thermophilic digesters (Table 1). As a result mesophilic digestion removed 2.5–7.6 times more Σ EST than the thermophilic digestion of primary and mixed sludge respectively over the six retention times. This was mirrored by the rate of Σ EST removal in terms of VS destroyed under mesophilic conditions which was 4.8–14 times higher than the rate of removal observed under thermophilic conditions for primary and mixed sludge respectively. The exception to this was for EE2 where the rate of removal was higher under thermophilic conditions.

This study and that of Carballa et al. [30] both observed biodegradation, which does not concur with the conclusion of de Mes et al. [15], who reported that the most likely removal mechanism of steroid estrogens during anaerobic digestion was adsorption. However, if adsorption was the only removal mechanism this would be expected to potentially decrease with temperature [34]. Carballa et al. [30] observed removal percentages of (Σ E1 + E2) of $85 \pm 10\%$ during mesophilic and thermophilic sludge digestion.

In this study lower removals were observed with up to half of Σ E1 + E2 biodegraded. In primary sludge at mesophilic and thermophilic temperatures 57% removal was observed and 43% and 10% removal was observed for mixed sludge at mesophilic and thermophilic temperatures respectively (Fig. 2). This would be contrary to what would be expected if physico-chemical adsorption alone were responsible for the changes observed. However, further experimental work would be required to fully clarify the possible impact of adsorption capacity. No transformations of E1 and E2 were observed by Czajaka and Londry [32] in sterile sediments. However, transformations of E2 to E1 occurred in samples which had not been sterilized and this was attributed to biological inter-conversion under anaerobic conditions possibly catalysed by other biological conversions. Higher levels of E1 were observed in the sludge entering an anaerobic digester than leaving in the treated sludge suggesting the conversion of E1 to E2 [35] providing further potential evidence of biotransformation. The de-conjugation of E1-3S, and thus its conversion to E1, appeared to be unaffected by temperature as the percent removal range has a maximum of 7% between thermophilic and mesophilic temperatures for the equivalent sludge types (Table 2). In the

study by de Mes [15] with anaerobic treatment of swine manure in an UASB it was concluded that de-conjugation was not occurring to a substantial degree (70% of E1 was in a conjugated form) in this study only 10% of the influent E1 was conjugated as E1-3S. Nevertheless, although temperature did not appear to have an effect on de-conjugation there would appear to be a small effect on the removal of EE2 with an increase of 11% between the mesophilic and thermophilic temperatures for both sludge types.

3.3. Effect of retention time on the removal of steroid estrogens

Under thermophilic conditions over 80% of the E1 was transformed within 45 d (3 SRT) for both primary and mixed sludge. After this period biotransformations of E1 and E2 reached equilibrium (Fig. 1). Similarly, under mesophilic conditions biotransformation changes were again greater over the first three retention periods.

Thus, although the impact of retention time cannot be isolated from the impact of temperature, retention time has a significant effect on the reduction of E1 to E2 with the bulk of the transformation being observed within 45 d under thermophilic conditions and 90 d for mesophilic conditions.

This reduction of E1 to E2 could be mediated by hydrogen or other organic electron donors as is the case for some aromatic and chlorinated organic compounds [36,37]. The oxidation of E2 to E1 under anaerobic conditions is unlikely to provide energy to the cells involved and therefore E2 is used instead as an electron acceptor [32]. However, there appears to be evidence that more E1 is biodegraded than is reduced to E2 in primary sludge under thermophilic digestion conditions. Detailed examination of Fig. 1 indicates that after 60 d E2 is over 300% of the mass of E1. This trend was also observed for the mixed thermophilic sludge. In addition these digesters had the lowest percentage removal of E3 observed (Table 2) where only 17% or 4% was removed in comparison to >43% at mesophilic conditions which implies that E1 had also been converted to E3.

3.3.4. Effect of sludge type on the removal of nonyl phenoethoxylates.

The differences in concentration between the two sampling periods (April 2007 and 2008) for the sum of the NPEOs appears consistent with the steroid estrogens with overall higher concentrations (by a factor of 1.8) detected for the 2007 sampling period (mesophilic) compared to the 2008 sampling period (thermophilic) for primary sludge and a factor of 2.7 for the mixed sludge. The most striking difference, however, is in the distribution of the breakdown products of NP3–12EO between 2007 and 2008. In the 2007 primary sludge the breakdown products were overwhelmingly the carboxylated species at 26.5 mg kg⁻¹dw compared to 0.06 mg kg⁻¹dw in 2008. By

comparison the concentration of NP1–2EO in the primary sludge was 2.1 mg kg⁻¹dw in 2007 and 15 mg kg⁻¹dw in 2008. This trend was comparable for the mixed sludge with the carboxylated species at 241.5 mg kg⁻¹dw in 2007 and 0.1 mg kg⁻¹dw in 2008.

Whilst the concentration of NP1–2EO in 2007 was 1.6 mg kg⁻¹dw for the mixed sludge and 90 mg kg⁻¹dw in 2008 implying that the breakdown to the NPECs had not occurred in 2008 possibly due to some operational alterations at the works affecting the solids and/or hydraulic retention times or food to micro-organism ratio in the activated sludge plant which can effect NPEO breakdown[38].

Total NP1–2EO concentrations in the mixed sludge were higher than the primary sludge, this was due to the polar nature of these compounds and their poor removal during primary sedimentation [27]. However, the breakdown products produced during biological treatment are more hydrophobic and are therefore much better removed than the parent compounds leading to higher concentrations in the biomass and hence in the mixed sludge [2,24]. Therefore, mixed sludge NP1–2EO concentrations reflect the breakdown which has occurred during the biological waste water treatment producing the WAS component of the mixed sludge.

The influent concentrations of NP were higher in the primary sludge at 0.5 mg kg⁻¹dw in 2007 and 2.2 mg kg⁻¹dw in 2008 in comparison with the mixed sludge at 0.2 mg kg⁻¹dw in 2007 and 0.15 mg kg⁻¹dw in 2008. This was slightly lower than values reported by Minamiyama et al. [39]. Primary sludge had higher concentrations by c. 35% of the NPEOs compared to the mixed sludge. However, the degradation products e.g. the NPECs were higher in the mixed sludge which was consistent with the biodegradation of the parent compound in the activated sludge process and the incorporation of these breakdown products into the WAS a component of the mixed sludge. The higher loading of the NPECs in the mesophilic mixed sludge were also reflected in higher removals of NPECs (6 mg d⁻¹) over the six retention periods (Fig. 3). Overall, greater removal of ΣNPEOs was observed for the mixed sludge >50% in comparison to primary sludge. This reflected results of Barret et al. [40] who observed greater metabolism of polycyclic aromatic hydrocarbons (PAHs), 7 polychlorobiphenyls (PCBs) and NP in secondary sludge digestion. In addition Patureau et al. [41] also observed the removal of NP1–2EO and NP in a mixed sludge under mesophilic anaerobic conditions.

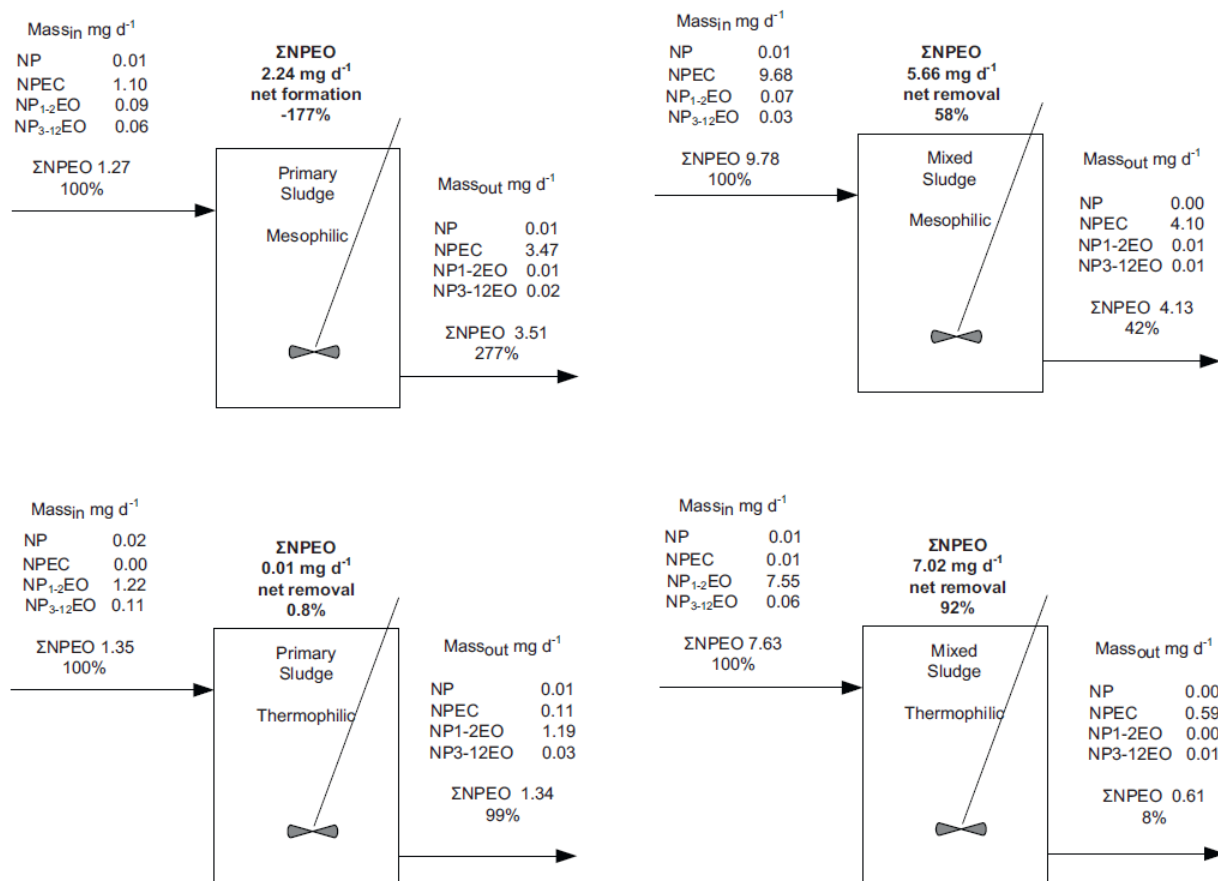


Fig. 3. Mass flux (mg d⁻¹) for alkylphenol ethoxylates at the start and at the end of the anaerobic mesophilic and thermophilic digestion trials for both primary and mixed sludge types.

3.5. Effect of temperature on the removal of NPEOs

The removal efficiency of nonylphenolics (ΣNPEOs) in the mixed sludge was 92% for thermophilic digestion compared to 58% for mesophilic digestion. Although primary sludge also exhibited higher removal efficiencies under thermophilic conditions this was less pronounced due to the build up of the carboxylated and NP1–2EO compounds (Table 2). In comparison a reduction in carboxylated concentrations was observed for mixed sludge under thermophilic conditions. A reduction was also observed for the mixed sludge carboxylated compounds under mesophilic conditions even though high starting concentrations (241.5 mg kg⁻¹ dw) were recorded.

The effect of temperature on the biodegradation of the longer chain NP3–12EO in the primary sludge was negligible with equivalent concentrations of 0.4 mg kg⁻¹ dw remaining in the digester effluent for both mesophilic and thermophilic digesters at the sixth retention period. A moderately faster rate of removal was observed at the thermophilic temperature

with NP3–12EO concentrations $<0.8 \text{ mg kg}^{-1}\text{dw}$ being observed after one retention time (15 d).

Nonylphenol only accumulated in the mixed sludge thermophilic digesters during the first three retention times. Then from day 60 through to day 90 NP was removed. Overall removal of NP was observed at all temperatures with treated sludge concentrations of $0.2 \text{ mg kg}^{-1}\text{dw}$ and $0.1 \text{ mg kg}^{-1}\text{dw}$ observed for primary sludge under mesophilic and thermophilic conditions respectively whilst for mixed sludge the final NP concentrations were $0.08 \text{ mg kg}^{-1}\text{dw}$ and $0.03 \text{ mg kg}^{-1}\text{dw}$ respectively for mesophilic and thermophilic conditions. The NP1–3ECs initially accumulated in the primary sludge under both mesophilic and thermophilic conditions and no overall removal of NP1–3EC appeared to occur under thermophilic conditions (Fig. 3). However, in the mixed sludge no accumulation of NP1–3ECs was observed at either temperature and overall removal of NP1–3ECs was observed for the mixed sludge mesophilic digestion (Fig. 3). Short-chained nonylphenolics in primary sludge did not accumulate under mesophilic conditions, however some persistence of NP1EO was observed in the thermophilic digesters.

The NP1EO persistence could be partially explained by the higher NP3–12EOs loadings in these digesters, which resulted in metabolic products like NP1–2EOs in anaerobic conditions [42].

4. Conclusions

1. Anaerobic digestion affords protection of the re-use environment from contamination by steroid estrogens and NPEOs. The two sludge types studied (primary and mixed sludge) exhibited different behaviour with primary sludge showing superior removal for steroid estrogens and mixed sludge higher removal for NPEOs.

2. In both types of untreated sludges E1 predominated, constituting 69–78% of the total estrogens. In the mixed sludge, containing WAS capable of biodegrading E1, higher concentrations of E3 were observed which was the ultimate breakdown product of E1 and E2.

3. Overall removal of ΣEST was $>50\%$ for primary sludge under both mesophilic and thermophilic conditions whilst for mixed sludge overall removals were lower at 39% and 12% respectively reflecting bulk organic solids removal during digestion.

4. In the mixed sludge NP1–2EO's were higher than in the primary sludge, reflecting the breakdown which had occurred during secondary aerobic (activated sludge) treatment.

Overall removals were higher in the digestion of mixed sludge in comparison to primary sludge digestion and in comparisons to steroid estrogen removals were not influenced by the efficiency of solid removal in anaerobic digestion.

5. There is evidence to suggest that the introduction of WAS already aerobically acclimated to estrogens was able to function in the anaerobic environment and enhanced the conversion of E1 to E2.

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