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Estrogen Receptor Agonist Fate during Wastewater and Biosolids Treatment Processes: A Mass Balance Analysis

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The estrogen receptor agonist fate of hexane extracts from various locations and phases (liquid and solid) within one pilot-scale and two full-scale wastewater treatment facilities were examined by use of the receptor-binding yeast estrogen screen (YES assay). Estrogenic activity was found in samples that contained a high concentration of biological solids and was particularly high in the suspended solid fraction from biosolids treatment facilities. Mass balances revealed that the estrogenic activity associated with the processed biosolids constituted between 5 and 10% of the influent estrogenic activity, while the treated liquid effluent prior to disinfection contained between 26 and 43%. Overall, this suggests that between 51 and 67% of the estrogenic activity contained in the influent wastewater was either biodegraded during the wastewater or biosolids treatment processes or was unavailable to the extraction/detection procedure. In both aerobic and anaerobic digestion, mass balances revealed an increase in estrogenic activity as treatment progressed and biosolids destruction occurred. The estrogenic activity associated with the solid phase decreased during mesophilic aerobic digestion. A correlation was observed between the estrogenicity of mixed liquor suspended solids and aerobic sludge age and suggests that wastewater treatment facilities can be designed and operated to enhance the sorption and removal of estrogenic compounds from the liquid phase.

Introduction

The scientific community and general public have expressed great interest in understanding the relationship between chemical exposure and potential alterations in the function of endocrine systems in humans and wildlife. These compounds, commonly referred to as endocrine disrupting compounds (EDCs), are defined by their ability to mimic or interfere with the mechanisms that govern the biosynthesis, transport or availability, and metabolism of hormones (*1*). While the impact of these compounds in the natural environment on human health remains a debated issue (*2*−*4*), a number of studies have correlated specific synthetic compounds to adverse effects in wildlife and fish (*5*−*11*).

The vast majority of literature available on the fate and transport of EDCs during wastewater treatment has focused on estrogen receptor agonists (*12*), which are compounds that amplify the expression of the estrogen receptor gene (*13*−*25*). The target compounds fall primarily into one of two groups: steroid estrogens, including estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2); and alkylphenol polyethoxylates (APEO) and their associated metabolites, including nonylphenol (NP) and octylphenol (OP). Several investigators have used analytical techniques in an attempt to quantify the concentrations of these compounds in treated effluents. Reported effluent concentrations for the steroid estrogens range from 3 to 9 ng/L for E1 (*16, 17*), 0.1 to 5 ng/L for E2 (*20, 22*), 1 to 8 ng/L for E3 (17), and 0.1 to 9 ng/L for EE2 (*16, 17, 26*). NP and OP have been detected in municipal biosolids at levels ranging from 5 to 887 mg/kg and <0.5 to 12 mg/kg, respectively (*27*). The broad range of reported effluent and biosolids concentrations is undoubtedly due, in part, to use of different analytical procedures as well as different treatment process conditions (e.g., hydraulic retention time (HRT), temperature, and solids retention time (SRT)).

Bench scale degradation experiments have also been performed on both groups of EDCs. Ternes et al. (*28*) reported that E2 was readily oxidized to E1 by municipal mixed liquor suspended solids (MLSS), whereas EE2 was fairly recalcitrant. These results are in agreement with the ^{14}C-labeled experiments conducted by Layton et al. (*29*) who reported rapid mineralization of ^{14}C-E2 but a significant reduction in the mineralization rate of ^{14}C-EE2. The degradation pathways of APEO and the persistence of APEO metabolites have been well documented (*12, 30*−*32*). During biological wastewater treatment, APEOs are biotransformed to more persistent, hydrophobic and estrogenic compounds such as alkylphenols, alkylphenol mono- and diethoxylates (AP$_n$EO), and alkylphenoxy carboxylic acids (NPEC) (*12*). Both the position and branching of the alkyl group have been shown to affect estrogenicity (*14*).

Of these two groups, it appears that the steroid estrogens represent the predominant form of estrogenic activity in wastewater effluents. Using a fractionalization method, Snyder et al. (*22*) concluded that 88−99.5% of the 17β-estradiol equivalence (E2-Eq) was due to E2 and EE2 with a very small portion (0.5%) attributed to alkylphenols. Similarly, Körner et al. (*21*) reported that alkylphenols were a small percentage of total E2-Eq.

Surprisingly, little attention has been directed to the total estrogenic load present in the solid phases discharged from biological treatment facilities. The vast majority of studies have focused on monitoring EDC concentrations contained in the liquid phase of sewage and effluents (*19, 23, 26, 33*−*35*). This research focus is warranted, considering the growing importance of water supply and reuse issues around the world. However, the biological suspended solids in activated sludge (mixed liquor suspended solids, MLSS) and digestion (biosolids) processes may represent a significant sink for estrogenic compounds. Based on the octanol−water partition coefficient (K_{ow}) of steroidal estrogens (values from 3 to 5 are typical), a relatively high percentage of these compounds are expected to partition into MLSS and biosolids before appreciable degradation occurs (*20, 23, 29*). Although high concentrations of NP and OP have been reported in biosolids (*27, 36*), we found only two studies that measured estrogenic activity in waste activated sludge (WAS) from full-scale wastewater treatment facilities (*21, 37*). Similarly, there have been no reports on the estrogenic activity of biosolids following aerobic and/or anaerobic digestion.

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TABLE 1. Overview of Pilot- and Full-Scale Facility Operating Conditions

Facility	A	B	C
		Liquid Treatment	
configuration	membrane	conventional	conventional
wastewater source	domestic	domestic	domestic
SRT (days)	20 to 25	20 to 25	8 to 12
HRT (hours)	8.5	10	14
influent flow (m^3/day)	76	12 245	22 248
chemical addition	alum	ferric chloride	none
polishing	activated carbon	none	none
		Solids Treatment	
configuration	NA	anaerobic	thermophilic/mesophilic aerobic
number of units	NA	2	3 thermophilc/2 mesophilic
HRT/unit (days)	NA	10	3 (thermophilic), 20 & 10 (mesophilic)
sludge type	NA	primary/WAS/chemical	WAS
dewatering	NA	no	yes

This paper reports on the estrogenic activity associated with the liquid and suspended solid phases of biological wastewater treatment and digestion processes. The concentration of estradiol-equivalent EDCs are determined using the YES biological screening method, and these concentrations are tracked through one pilot- and two full-scale treatment facilities that incorporate different wastewater and biosolids treatment and operational strategies. Mass balances of estradiol equivalents are performed on the three facilities, and a comparison is made between the results from this study and those reported in the literature by others.

Materials and Methods

Facility Overview. Samples were collected at various stages of the wastewater and biosolids treatment processes at one pilot-scale (Facility A) and two full-scale (Facilities B and C) activated sludge treatment plants. Facility A was a membrane bioreactor pilot plant that treated approximately 20 000 gallons per day and had no biosolids treatment process. Features of the three facilities are summarized in Table 1. All three facilities are required to nitrify in order to comply with effluent ammonia requirements. Chemical precipitation is used at Facilities A and B to reduce effluent phosphorus levels. Facilities B and C use secondary clarifiers for liquid−solid separation in the activated sludge process. The mixed liquor temperature for all of the facilities ranged from 12 to 18° C during sampling. Facility B uses anaerobic digestion for solids handling and maintains an operating temperature in the mesophilic range (30−40° C). Facility C uses thermophilic followed by mesophilic aerobic digestion processes, which maintain operating temperatures of 40−58° C and 30−35° C, respectively.

Sample Collection, Preparation, and Extraction. Three separate grab samples from Facilities A, B, and C were collected between March and May 2001. Separate samples of sewage, MLSS, and biosolids were collected for each sampling event. Estrogenic activity, as measured by the YES assay, was determined in all these samples and yielded E2-Eq concentrations, which were subsequently used to conduct a mass balance on each facility. Single MLSS samples from two additional conventional activated sludge facilities that receive a blend of domestic and industrial wastewater and are operated at SRTs ranging from 12 to 15 days were also sampled and analyzed by the YES assay. All samples to be analyzed by the YES assay were collected in EPA-approved, precleaned VOC vials with Teflon septa (Wheaton Science Products, Milliville, NJ) to minimize contamination with artifact organics. After sample collection, the VOC vials were placed on ice and shipped overnight to the laboratory for testing. Samples were also collected in polyethylene bottles for routine MLSS and mixed liquor volatile suspended solids (MLVSS) analysis according to Standard Methods (*38*). Regardless of site location, sample processing was initiated within 24 h of collection.

A number of different solvents have been used to extract synthetic organic chemicals from sewage and biosolids samples (*12*, *39*, *40*). Comparative experiments were conducted with dichloromethane, ethyl acetate, and hexane to identify the solvent that provided the best recovery of E2-Eq and minimal background interference, and hexane was selected as the preferred solvent for this study (data not shown). EDCs were extracted from unfiltered samples by blending known volumes of hexane (HPLC grade, Fisher Scientific, Pittsburgh, PA) and sample in aluminum foil-capped borosilicate glass tubes, vortex mixing for 30 s, and then shaking for 24 h. Sample volumes varied from 5 to 50 mL for sewage, 40 to 100 mL for effluents, and 1 to 4 g TSS for biosolids. All hexane volumes were adjusted to 2 mL, and, when necessary, hexane was concentrated by a gentle flow of nitrogen gas. Aluminum foil was used to prevent sample contamination and limit sample loss due to sorption on the Teflon top (*41*). Prior to liquid−liquid extraction, the glass tubes were heated to 550° C for 8 h to remove residual organic compounds. The shaker table was covered with aluminum foil to minimize photodegradation. Some samples had to be diluted with Milli-Q water to obtain a well-mixed slurry prior to extraction. Ten microliter aliquots of the solvent layer from extractions were transferred to 96-well microtiter plates (Linbro/Titertek, ICN Biomedicals, Aurora, OH) for use in the YES assay.

The efficiency of the liquid−liquid extraction using hexane was evaluated, and percent recoveries and standard deviations for the method at 1000 pM E2 in liquid samples (including raw influents, primary effluents, secondary effluents, and permeates) was 74% (±12) ($n = 6$). All E2-Eq concentrations presented in this paper are reported according to the original sample volume.

Recombinant Yeast Estrogen Screen (YES) Assay. Estrogenic activity was determined using the YES assay as previously described by Routledge and Sumpter (*42*), except for modifications as described below. A recombinant yeast strain (*Saccharomyces cerevisiase*) containing the human estrogen receptor gene was obtained from Dr. Sumpter of Brunel University (Middlesex, UK). The strain also contains an expression plasmid carrying the *lac-Z* reporter gene. When the cells are incubated in the presence of estrogenic compounds, the *lac-Z* product, β-galactosidase, is secreted into the medium and causes the chromogenic substrate, chlorophenol red-β-D-galactopyranoside (CPRG) (Roche Diagnostics, Indianapolis, IN), to turn red. This color change can be quantified by measuring absorbance (Spectracount Microplate Photometer BS-10,000, Packard, Meriden, CT).

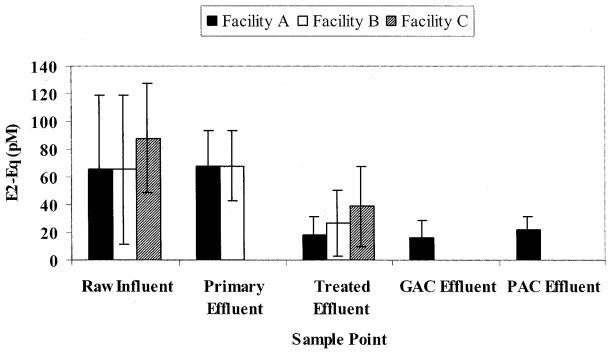

FIGURE 1. Estrogenic activity of liquid samples as treatment progressed in Facilities A, B, and C. The error bars represent 95% confidence intervals. Abbreviations: PAC is powdered activated carbon; GAC is granular activated carbon.

In the 96-well microtiter plates, solvent-extracted samples were allowed to evaporate to dryness in air. Then, 200 μL of growth medium containing yeast cell (grown to an absorbance of 1 at 600 nm) was added to each well. The plates were incubated at 32° C for 3 days and then moved to room temperature for 3 days, as suggested by Beresford et al. (*43*). After the total incubation period, absorbance readings were taken at 520 and 640 nm. All extracts were tested in sixteen separate wells in a 96-well microtiter plate.

A plate containing standards and control samples was included for every YES assay performed and included the following: 3 rows of serially diluted 17 β-estradiol (>98% purity, Sigma Chemical Company, St. Louis, MO) from which a standard curve was developed; a row containing negative control samples (Milli-Q water subjected to the hexane extraction procedure); and a row containing blank samples (hexane only). The concentration for the 17 β-E2 serially diluted wells ranged from 12 to 25 000 pM. The standard curve used to determine E2-Eq in molar units was determined from the arithmetic mean of the 3 wells containing the same 17β-E2 concentrations. The linear portion of the E2 standard curve was used to calculate E2-Eq concentrations and typically fell between 15 and 80 ng/L (60−320 pM). The negative control and hexane blank samples were never statistically different from each other ($p < 0.05$, $n = 22$) on an E2-Eq basis and were below detection levels in all cases.

Mass Balance Calculations. Mass balances were performed by multiplying E2-Eq concentrations by average daily flow rates that were recorded on the days when samples were taken. E2-Eq activity in the solid phase of biosolids samples was calculated by subtracting the E2-Eq in the centrate from a centrifuged sample (10 min at $10^3 \times$ g) from the E2-Eq activity in the total sample.

Results and Discussion

E2-Eq Concentrations through Treatment Processes. Treatment yielded a general reduction in E2-Eq concentration through three different biological wastewater treatment processes (Figure 1). The influent wastewater E2-Eq concentrations ranged from 65 (± 54) to 88 (± 40) pM, while the effluent E2-Eq concentrations ranged from 18 (± 13) to 39 (± 29) pM. Although the membrane bioreactor (Facility A) reduced the E2-Eq concentration more on average than the conventional treatment system that received the same influent wastewater (Facility B), the difference was not statistically significant ($p > 0.05$, $n = 3$). Interestingly, the estrogenic activity in the liquid-phase leaving the membrane bioreactor system was not reduced further by carbon polishing with either granular (GAC) or powdered (PAC) activated carbon, although other investigators have shown estrogenic activity removal through similar processes (*24*).

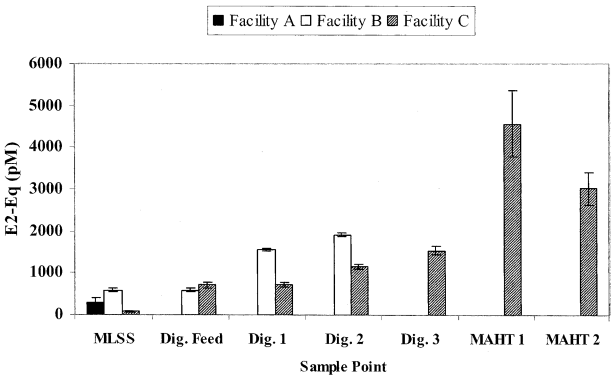

FIGURE 2. Estrogenic activity of biosolids samples from various points along the treatment process in Facilities A, B, and C. The error bars represents 95% confidence intervals. Abbreviations: MLSS is mixed liquor suspended solids; Dig. Feed is biosolids influent; Dig 1, 2, and 3 are biosolids from anaerobic digesters 1 and 2 (Facility B) and thermophilic aerobic digesters 1, 2, and 3 (Facility C); MAHT 1 and 2 are mesophilic aerobic holding tanks 1 and 2 (Facility C).

Conversely, the E2-Eq concentrations generally increased during the biosolids treatment processes (Figure 2). Additionally, the E2-Eq concentrations were significantly higher in the biosolids than in the liquid phase. The mass of E2-Eq per unit mass of TSS (specific estrogenic activity) and the TSS concentrations are reported for the biosolids handling phases of treatment in Table 2. TSS concentrations are much higher during biosolids stabilization (digestion) processes because thickened solids are added to reactor volumes that are smaller than the activated sludge system reactors. During the digestion process, a slow but steady decrease in TSS was observed and is due to the hydrolysis and degradation of biosolids. A more rapid increase in E2-Eq concentration was observed as treatment progressed, and specific estrogenic activity increased concomitantly. The reason for this increase is unclear but can be further clarified by performing mass balances on both the wastewater and biosolids treatment processes at each facility.

Mass Balance on the Activated Sludge Processes. Mass balances on E2-Eq for Facilities A, B, and C revealed that more estrogenic activity was found in the liquid effluent than in the waste activated sludge (WAS), which was ultimately directed toward the biosolids handling process (Table 3). Between 26 and 43% of the E2-Eq contained in the raw influent was found in the treated effluent (prior to disinfection), whereas between 2 and 14% of the influent E2-Eq mass was removed from the activated sludge process in the form of WAS. Overall, 33−49% of the E2-Eq activity was recovered by the mass balance across the activated sludge bioreactors, suggesting that a large fraction of the initial activity was either degraded or unavailable to the extraction/detection procedure. Initially, these results may appear to contrast with theoretical predictions put forth by others (*20*), where K_{OW} values were used to predict that most of the EDCs would be sequestered into the biosolids and not the liquid effluent. However, since 51−67% of the E2-Eq activity was not recovered by the protocol used in this study, it is possible that a large percentage of this unaccounted fraction is associated with the biosolids but remains in a nonextractable form. Radiolabeled studies are needed to fully elucidate the true fate of E2-Eq activity during wastewater and biosolids treatment processes.

The fate of E2-Eq can be directly compared in Facility A (membrane filtration) and Facility B (secondary clarifiers) since they received the same primary effluent and were operated at the same SRT. Most notably, the specific estrogenic activity of the solid phase of WAS (total WAS

TABLE 2. Specific Estrogenic Activity (SEA, μg E2-Eq/kg TSS) and TSS Concentrations (g/m^3) for Biosolids Processes

	digester feed[a]		digester 1		digester 2		digester 3		MAHT 1[b]		MAHT 2[b]	
Facility	SEA	TSS	SEA	TSS	SEA	TSS	SEA	TSS	SEA	TSS	SEA	TSS
A	NA		NA		NA		NA		NA		NA	
B	20 ($\pm$ 3.1)	13 100	23 ($\pm$ 5.6)	16 840	57 ($\pm$ 8.5)	8900	NA		NA		NA	
C	3.3 ($\pm$ 0.6)	43 660	4.2 ($\pm$ 0.7)	39 540	7.7 ($\pm$ 1.0)	37 140	11 ($\pm$ 1.5)	36 020	38 ($\pm$ 5.3)	28 580	24 ($\pm$ 4.7)	27 180

[a] Digester feed was collected as biosolids were pumped into digesters and includes a blend of WAS and primary sludge (Facility B) or WAS only (Facility C). [b] MAHT 1 and 2 are mesophilic aerobic holding tanks, which functioned as digesters.

TABLE 3. Results of Mass Balance on E2-Eq for Facilities A, B, and C[a]

Facility	raw influent, μg E2-Eq/day	primary effluent, μg E2-Eq/day	secondary effluent, μg E2-Eq/day	WAS, μg E2-Eq/day	digestion product, μg E2-Eq/day
A		1410	370 (26)	100 (7)	NA[b]
B	218 600	226 600 (104)	77 400 (35)	31 100 (14)	11 100 (5)
C	531 700	N/A[b]	229 800 (43)	11 800 (2)	55 120 (10)

[a] Parenthetic values represent percentage change from raw influent. Facility A was a pilot plant that used primary effluent from Facility B for its influent. Therefore, all calculated percentage change values for Facility A refer back to the primary effluent. [b] NA means not applicable to that Facility.

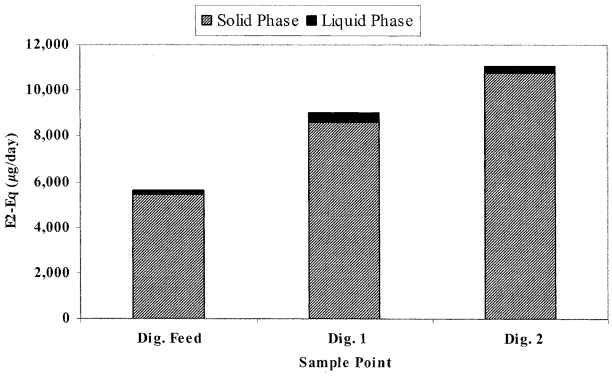

FIGURE 3. Mass balance results for the anaerobic digestion process in Facility B.

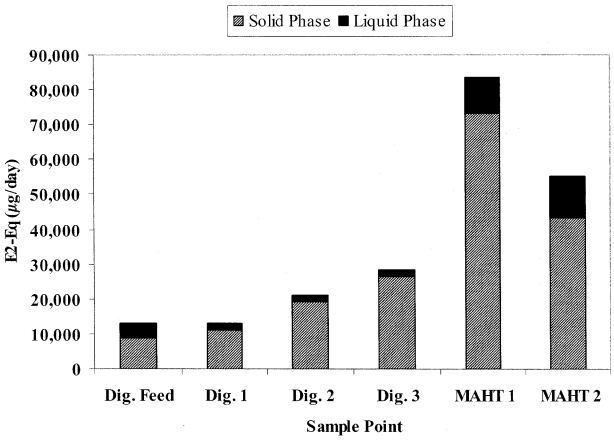

FIGURE 4. Mass balance results for the thermophilic/mesophilic aerobic digestion process in Facility C.

minus centrate) in Facility A was substantially lower than that in Facility B (7.9 μg E2-Eq/kg TSS compared to 35 μg E2-Eq/kg TSS). The membrane system enhances the capture and retainage of organic colloids greater than 0.1 μm. These organic colloids may have a significant impact on increasing the apparent aqueous solubility of estrogenic compounds, as similar phenomena have been reported from experiments utilizing compounds with similar K_{ow} values (*44*−*46*).

Mass Balance on the Biosolids Treatment Processes. The mass balances on both mesophilic anaerobic (Facility B) and thermophilic/mesophilic aerobic (Facility C) biosolids treatment processes (Figures 3 and 4, respectively) showed that the mass of E2-Eq activity increased as treatment progressed and is consistent with the trends revealed in Figure 2. All biosolids samples were centrifuged to distinguish between the E2-Eq activity associated with the liquid (centrate) and solid (total minus centrate) phases, to reveal the potential for E2-Eq activity to be recycled to the activated sludge system by dewatering processes. The results suggest that the different biosolids stabilization processes monitored during this study impact the E2-Eq quality of dewatering recycle water differently.

In Facility B (Figure 3), most of the estrogenic activity detected in the biosolids was associated with the suspended solids rather than the liquid phase. Additionally, the average total E2-Eq in digestor 2 was higher than digestor 1 ($p < 0.05$, $n = 3$). However, the E2-Eq of the liquid fraction did not change as treatment progressed, suggesting that the recycle streams from anaerobically digested biosolids will contribute relatively small amounts of E2-Eq to the wastewater treatment process relative to the sewage. The persistence of APEO and metabolites (NP and OP) have been reported in anaerobically treated biosolids and sediments (*12*, *27*, *47*). Based on measurements reported by LaGuardia et al. (*27*), anaerobically digested biosolids had average OP and NP concentrations of 10.4 and 754 mg/kg TSS, respectively. Using E2-Eq factors of 0.0015 (27) and 0.0001 (25) for OP and NP, respectively, the summed specific estrogenic activity is approximately 91 μg E2-Eq/kg. Although this value is higher than our measured values (Table 4), both studies suggest that anaerobically digested biosolids will contain appreciable levels of specific estrogenic activity. Although APEOs are not reported to be a significant fraction of the estrogenic activity in wastewater effluents (*21*, *22*), the results of La Guardia et al. (*27*) suggest that they can be fairly significant in anaerobically digested biosolids. To our knowledge, the fate of steroidal estrogens after anaerobic digestion and/or land application has not yet been reported.

For Facility B, we measured a substantial decrease in E2-Eq activity from 31 087 μg/day in the WAS to 5596 μg/day in the digester feed. This discrepancy may be explained by the fact that Facility B utilizes cosettling of WAS in their primary clarifier to facilitate solids handling, which complicates the mass balance. Facility C does not use primary clarification but, instead, digests WAS directly. Consequently, the mass loads of estrogenic activity for the WAS and digester feed for

TABLE 4. Comparison of E2-Eq Loadings (Influent/Effluent) from Various Wastewater Treatment Facilities

site	influent E2-Eq (μg/1000 m^3)	effluent E2-Eq (μg/1000 m^3)	E2-Eq removal—liquid phase (%)	sampling protocol[a]	E2-Eq method	ref
Southend, England	N/A	46 700	N/A	G	calculated[b,e]	(*16*)
Harpenden, England	N/A	5900	N/A	G	calculated[b,e]	(*16*)
Rye Meads, England	N/A	500	N/A	G	calculated[b,e]	(*16*)
Deephams, England	N/A	400	N/A	G	calculated[b,e]	(*16*)
Naburn, England	N/A	6600	N/A	G	calculated[b,e]	(*16*)
Horsham, England	N/A	3000	N/A	G	calculated[b,e]	(*16*)
Billing, England	N/A	1000	N/A	G	calculated[b,e]	(*16*)
Penha, Rio de Janeiro	32 000	2500	92	C−G	calculated[b]	(*19*)
Frankfurt/Main, Germany	17 400	10 500	40	C−FP	calculated[b]	(*19*)
Cobis, Italy	28 300	2900	90	C−FP	calculated[b,e]	(*17*)
Ostia, Italy	25 400	7500	71	C−FP	calculated[b,e]	(*17*)
Roma Sud, Italy	15 900	5500	65	C−FP	calculated[b,e]	(*17*)
Steinhaule, Germany (March)	58 000	6400	89	C−FP	measured[c]	(*21*)
Steinhaule, Germany (June)	56 000	5600	90	C−FP	measured[c]	(*21*)
WTP, Netherlands	31 800	3100	90	C−G	measured[d]	(*37*)
Facility A, U.S.A.	18 500	5000	73	G	measured[d]	this study
Facility B, U.S.A.	17 800	7200	60	G	measured[d]	this study
Facility C, U.S.A.	23 900	10 600	56	G	measured[d]	this study

[a] G = grab. C−G = composite from grab samples. C−FP = composite from flow proportioned samples. [b] Ex-Eq is calculated per Johnson and Sumpter (*23*) using estradiol equivalency factors for specific estrogenic compounds from Murk et al. (*37*). [c] E2-Eq is determined from MCF-7 breast cancer cells. [d] E2-Eq is determined from YES assay. [e] Concentrations reported as non-detectable for EE2 were assumed to be 10% of the detection limit for calculating E2-Eq concentrations.

Facility C were in good agreement (11 778 μg/day compared to 13 012 μg/day).

The mass balance for the biosolids treatment portion of Facility C revealed that estrogenic activity more than doubled from the raw feed through the third thermophilic digester, then tripled in the first mesophilic reactor (MAHT 1), and finally decreased by 34% in the second mesophilic digester (Figure 4). The thermophilic digesters were operated at 48−58 °C and included both aspirating aerators and high TSS concentrations (around 3.8%). Under these conditions, it is common to find microaerobic or anaerobic environments (*48*). The statistically relevant ($p < 0.05$, $n = 3$) yet subtle increase in E2-Eq mass through the thermophilic aerobic digesters is similar to the trend seen in the mesophilic anaerobic digesters at Facility B. The subsequent increase, then decrease in E2-Eq activity through the mesophilic digesters is dramatic. The significant reduction in suspended solids that occurred between Digester 3 and MAHT 1 (see Table 2) occurs concomitant with a significant increase in E2-Eq activity. Therefore, the increase in E2-Eq may be due to the release of estrogenic compounds that were previously nonextractable by the hexane liquid−liquid extraction method used in this study. It is also conceivable that estrogenic metabolites that are measured by the YES assay were generated during degradation of the parent estrogenic compounds during aerobic mesophilic digestion. This explanation is supported by the work of Knudsen et al. (*36*), who reported that mesophilic aerobic digestion following mesophilic anaerobic digestion reduced the concentration of organic micropollutants. The results shown in Figure 4 may support the notion that EDCs are biodegraded under mesophilic aerobic digestion conditions, but that there is a minimum aerobic processing time necessary to establish this biodegradation. There was also an increase in the E2-Eq activity associated with the liquid phase, suggesting that recycle streams from aerobic digesters may possess a significant concentration of estrogenic compounds, but the overall mass contribution is still small relative to the E2-Eq contained in the sewage.

The E2-Eq mass in the digested biosolids remain a small percentage of the influent E2-Eq (Table 3). However, the trends between anaerobic and aerobic digestion were somewhat different. The overall net percentage of E2-Eq associated with the biosolids decreased at Facility B (although there was a slight increase during the digestion process), while this same percentage increased at Facility C. The overall mass balances for Facilities B and C show that 40 and 53% of the influent E2-Eq were detected and are discharged from the plant as liquids or solids.

Comparison with Other Studies. Table 4 provides a comparison of the E2-Eq concentrations in influent and effluent wastewaters from this study and other published reports. When specific measurements from bioassays (e.g. YES or MCF-7 breast cancer cells) were not available, the E2-Eq was calculated by the method described by Johnson and Sumpter (*23*). Briefly, estrogen receptor agonist concentrations were multiplied by their respective E2-Eq equivalency factors (estrogenic potency relative to E2). The E2-Eq equivalency factors for E1, E2, and EE2 were 0.1, 1, and 1.2 (*37*), respectively, and for E3 was 0.005 (*23*). Only the estrogen steroids (E1, E2, E3, and EE2) were included in this comparison since they appear to cause the majority of in vitro responses in effluent wastewater (*21*, *22*). The influent E2-Eq from these studies averaged 33 000 μg/1000 m^3 with a maximum of 58 000 and a minimum of 15 900. The influent E2-Eq results reported here ranged from 17 800 to 23 900 μg/1000 m^3 and are lower on average than the other studies included in Table 4. The difference in the values from our study could be due to higher average levels of community water use in the United States relative to other developed countries or a high incidence of dilution due to inflow/infiltration in the collection system.

The percent removal from the liquid phase from previous studies averaged 74% with a maximum and minimum of 92 and 40%, respectively (Table 3). Ternes et al. (*19*) observed these low percent removals during the winter when unusually cold wastewater conditions existed. During this study, the average removal efficiency of E2-Eq from the liquid phase was 63% and is slightly lower than the average from all other facilities for which a removal was determined.

The results from Table 4 suggest that there is enough variability in removal efficiencies between treatment facilities and differences in sampling protocols to warrant standardization of the monitoring protocol. Many of the sample protocols used by others include filtration prior to a concentration step, such as solid-phase extraction (e.g., refs *17* and *19*). This filtration step can remove particles, which may have contained EDCs. Additionally, dissolved chemicals

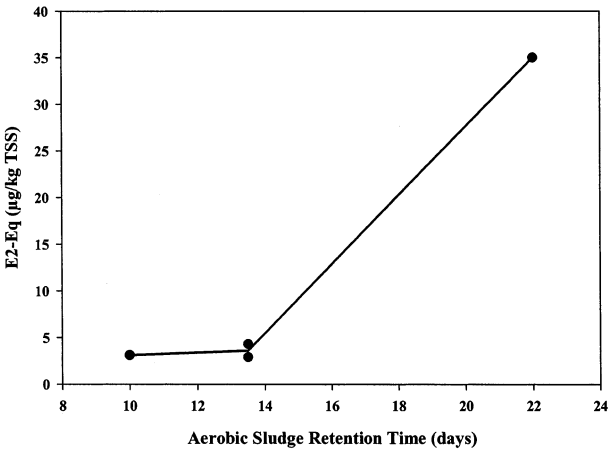

FIGURE 5. Relationship between SRT and specific equivalent estradiol activity (μg E2-Eq/kg TSS) for facilities utilizing secondary clarification.

may sorb to the filter material. Capangpangan et al. (*49*) reported a large variation (5−50%) in sorption of polyaromatic hydrocarbons to different filter materials for compounds with K_{ow} values that are similar to those for estrogenic steroids. Protocols have been employed to offset this effect and include post-washing the filter with a solvent (*17*) or preequilibrating the filter prior to analyte collection (*20*). The solvent extraction protocol used for the results reported here did not employ filtration and may help explain why the treatment process removal efficiencies reported in this study were lower than what was reported by others who employed filtration.

Impact of Activated Sludge Solids Retention Time. The data in Figure 5 suggest that there is a relationship between the estrogenic activity of the biological solids and operating aerobic SRT for facilities that use conventional treatment (Facilities B and C and two other activated sludge processes). Layton et al. (*29*) used ^{14}C-E2 to track the fate of the model estrogenic compound in biomass collected from a range of biological wastewater treatment plants. Although not explicitly stated, their data show that the radiolabeled EDC sorbed the least to biomass from a low SRT (3 days) facility. The correlation in Figure 5 suggests that the sorbent characteristics of MLSS may vary with SRT and that SRT is an important parameter to consider when studying the removal of estrogenic compounds from wastewater treatment processes. Furthermore, since SRT is a primary design and operating parameter, engineers and operators have the ability to influence the sorption potential of the biological solids by adjusting the SRT. These results are in agreement with Gulyas et al. (*50*), who reported changes in the sorption behavior of 2,6-dimethylphenol with respect to sludge retention time. Several investigators have reported a difference in sorption behavior of organic compounds with different organic matrices; partitioning behavior has been reported to be a function of polar-to-nonpolar group ratio (*51*), aromatic content (*52*), organic carbon content (*53*), and soft-to-hard carbon ratio (*54*, *55*). It is important to note that the above references were dealing exclusively with soils and natural organic matter that may be somewhat different from the biological material derived from activated sludge facilities. However, they offer solid experimental protocols that can be adapted to the activated sludge matrix.

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