Critical Review

# Fate, Transport, and Biodegradation of Natural Estrogens in the Environment and Engineered Systems

SAMIR KUMAR KHANAL,*,† BIN XIE,†
MICHAEL L. THOMPSON,‡ SHIHWU SUNG,†
SAY-KEE ONG,† AND J. (HANS) VAN LEEUWEN†

*Departments of Civil, Construction and Environmental Engineering and Agronomy, Iowa State University, Ames, Iowa 50011*

Natural steroidal estrogen hormones, e.g., estrone (E1), 17$\beta$-estradiol (E2), estriol (E3), and 17$\alpha$-estradiol (17$\alpha$), are released by humans and livestock in the environment and are the most potent endocrine disrupters even at nanogram per liter levels. Published studies broadly conclude that conventional wastewater treatment is efficient in the removal of 17$\beta$-estradiol (85−99%), but estrone removal is relatively poor (25−80%). The removal occurs mainly through sorption by sludge and subsequent biodegradation. The long solids retention time in wastewater treatment systems enhances estrogen removal due to longer exposure and the presence of a diverse microbial community, particularly nitrifiers. In spite of the treatment, the effluent from conventional biological wastewater treatment systems still contains estrogenic compounds at a level that may cause disruption of endocrine systems in some species. Advanced wastewater treatment systems such as membrane processes remove the estrogen compounds mainly through physical straining of particle-bound estrogens. Another major source, which accounts for 90% of the estrogen load, is animal manure from concentrated animal-feeding operations (CAFOs). Manure is not required to be treated in the United States as long as it is not discharged directly into water bodies. Thus, there is an urgent need to study the fate of animal-borne estrogens from these facilities into the environment. A number of studies have reported the feminization of male aquatic species in water bodies receiving the effluents from wastewater treatment plants (WWTPs) or surface runoff from fields amended with livestock manure and municipal biosolids. Estrogenicity monitoring studies have been conducted in more than 30 countries, and abundant research articles are now available in refereed journals. This review paper focuses on estrogen contributions by wastewater and livestock manure, their removal rate and mechanisms in an engineered system, and their transport and ultimate fate in an engineered system and the environment. The review aims to advance our understanding of fate, transport, and biodegradation of estrogen compounds and outlines some directions for future research.

## 1. Introduction

Reports about steroidal estrogen hormones in municipal wastewater and their fate in wastewater treatment plants started to appear in the mid-1990s (*1*−*3*). These studies were primarily confined to quantification of human-derived estrogens in raw sewage and treated effluent and their biodegradability during conventional biological wastewater treatment. Humans excrete natural estrogen hormones, e.g., 17$\beta$-estradiol, at levels as high as 5 mg/day for pregnant women (*4*). The estrogen hormone (particularly 17$\beta$-estradiol, estrone, and estriol) concentrations in the effluent of a conventional biological wastewater treatment plant (WWTP) typically range from a few nanograms per liter (ng/L) to several $\mu$g/L. These micropollutants have properties of endocrine-disrupting chemicals (EDCs) and have been found to disrupt the endocrine system of many species, including humans even at ng/L (*5*, *6*). Human and animal waste-borne steroidal hormones, also called natural steroidal estrogens, belong to the group of endogenous steroidal EDC, which is characterized by extremely high estrogenic potency as compared to exogenous EDCs or synthetic chemicals such as organochlorine aromatic compounds (*7*). Hanselman et al. (*8*) reported that endogenous steroidal estrogens possess estrogenic potency 10 000−100 000 times higher than exogenous EDCs. Human and livestock excretions contain appreciable quantities of natural estrogens, namely, estrone (E1), 17$\beta$-estradiol (E2), estriol (E3), and 17$\alpha$-estradiol (17$\alpha$). A number of aquatic species, for example, turtles, trout, and minnows, may be sexually inhibited or reversed by the presence of natural estrogens, even at concentrations in the range of a few tens of ng/L (*9*−*13*). E2 at a concentration 5 ng/L induced the production of female specific proteins in male Japanese medaka (*11*). A recent report linked the feminization of Pacific Ocean coastal fish with wastewater effluent discharge from the city of Los Angeles (*13*).

The goal of this paper is to critically review the existing literature with respect to fate, transport, and removal of natural estrogenic compounds in engineered systems and in the natural environment. Relevant background information, such as chemical structures, properties, sources, and analytical techniques, is also briefly discussed.

## 2. Properties and Structures of Common Estrogenic Compounds

Natural steroidal estrogens (also known as the C18 steroidal group) share the same tetracyclic molecular framework,

* Corresponding author phone: (515)294-7089; fax: (515)294-8216; e-mail: samirk@iastate.edu. Corresponding author address: 394 Town Engineering Building, Iowa State University, Ames, IA 50011-3232.

† Department of Civil, Construction and Environmental Engineering.

‡ Department of Agronomy.

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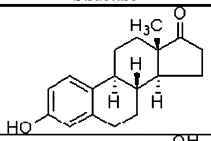
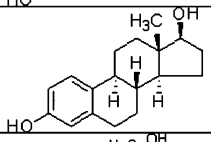
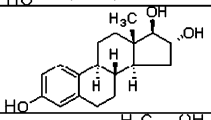
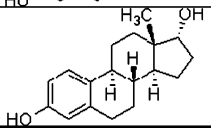
**TABLE 1. Structure and Property of Natural Steroidal Estrogen Hormones[a]**

| Estrogen Hormone | Acronyms | Chemical Structure | MW* (g/mole) | $S_w$# (mg/L) | Log $K_{ow}$ψ | VPφ (kPa) | E2 Equivalent | Reference |
|---|---|---|---|---|---|---|---|---|
| Estrone | E1 |  | 270.37 | 0.8 – 12.4 | 3.1 – 3.4 | $3\times10^{-8}$ | 0.1 – 0.2 | (1) |
| 17β-estradiol | E2 |  | 272.38 | 5.4 – 13.3 | 3.8 – 4.0 | $3\times10^{-8}$ | 1 | (14) |
| Estriol | E3 |  | 288.38 | 3.2 – 13.3 | 2.6 – 2.8 | $9\times10^{-13}$ | 0.02 | (14) |
| 17α-estradiol | 17α |  | 272.38 | 3.2 – 13.3 | 3.4 – 4.0 | $3\times10^{-8}$ | 1-2 | (15) |

[a] *MW: molecular weight; #$S_w$: solubility in water; ψ$K_{ow}$: octanol−water partition coefficient; φVP: vapor pressure.

which is composed of the four rings: a phenol, two cyclohexanes, and a cyclopentane (Table 1). The difference in the compounds within the C18 group lies in the configuration of the D-ring at positions C16 and C17. For example, estrone has a carbonyl group on C17, estradiol has a hydroxyl group on C17, and estriol has two alcohol groups on C16 and C17. The C17 hydroxyl group of the estradiol can either point downward of the molecular plane, forming the 17α compound, or upward, forming the E2 compound. E2 is used industrially for the synthesis of ethinyl estradiol (EE2), the commonly used active ingredient for oral contraceptive pills. Also included in Table 1 are the physical and chemical properties and the biological potency of free estrogens. As evident from the table, free estrogens, also known as unconjugated estrogens, are moderately hydrophobic and poorly soluble in water. Conjugated estrogens are formed by esterification of free estrogens by glucuronide (GLU) and/or sulfate (SUL) groups at the position(s) of C3 and/or C17. The conjugated estrogens can be written as the following: E1-3-SUL, E1-3-GLU, E2-3-SUL, E2-3,17-GLU, etc. Conjugated estrogens do not possess biological activity and dissolve in aqueous solution at a much larger quantity than their unconjugated counterparts. This is due to the substitution of the original hydroxyl group by polar SUL/GLU functional groups with a large molecular weight. The esterification of SUL/GLU group into free estrogens is a common biological mechanism to partially detoxify the free estrogens and transform them into a more readily excretable form.

## 3. Analysis and Quantification of Estrogens

Sample preparation for estrogen analyses is accomplished by different approaches based on the type of samples. Liquid samples (e.g., municipal sewage, river, lake, ocean, etc.) are relatively free of particulates and do not require comprehensive cleanup steps (*16*−*22*). On the other hand, solid samples require disintegration (e.g., by sonication) and extraction of estrogens into the aqueous phase by an organic solvent, such as alcohol (*21, 24*) or ethyl acetate. Impurities in solid matrices, e.g., sludge, sediment, manure, and manure-amended soils, can be reduced by precleanup using gel permeation chromatography (GPC) and/or separation by high-performance liquid chromatography (HPLC) (*23*−*25*). Samples in the aqueous phase then go through pretreatment procedures, such as solid-phase extraction (SPE) and/or HPLC, so that estrogens can be separated from impurities and preconcentrated. A good SPE recovery rate or a well-separated HPLC pretreatment will contribute to achieve the best limit of quantification (LOQ). The pretreated samples are either derivatized and reconstituted in a nonpolar phase for GC analysis or reconstituted in a mobile phase for LC or immuno-assay based analysis. Analytical methods can be physicochemically based, such as gas/liquid chromatography coupled with tandem mass spectrometry (GC/MS or LC/MS), or immuno-assay based, such as radio-immunoassay (RIA) and enzyme-linked-immunosorbent assay (ELISA). The former has gained popularity because of its higher selectivity (*16*−*21*, *24*, *25*). Total sample treatment and analysis follow the toxicity identification and evaluation (TIE) methodology (Figure S1, Supporting Information). Literature data about the determination of estrogen compounds in liquid and solid phases are summarized in Table S1 (Supporting Information).

## 4. Sources and Occurrence of Steroidal Estrogens in the Environment

Natural steroidal estrogens are contributed predominantly by humans and livestock through feces and urine. E2 is the primary metabolite with the highest potency, whereas 17α and E1 are the secondary metabolites with reduced potency. E3 is considered to be the final metabolite with the least potency. Dairy cows excrete 17α, E2, E1, and E3, while other animal species mainly excrete E2, E1, and E3. Human excretion of E2 can reach as high as 5 mg/day in the case of pregnant women (*4*). A nonpregnant dairy cow excretes approximately 0.8−1.2 mg/day of 17α, and a nonpregnant sow excretes approximately 0.6−1.4 mg/day of E1; whereas a pregnant dairy cow could excrete up to 11.4 mg/day of 17α, and a pregnant sow excretes up to 10.8 mg/day of E1 (in terms of unit live animal weight of 1000 kg) (*26, 27*). Considerable amounts of steroidal estrogen hormones are associated with solids such as municipal sludge and livestock manure. Estrogenic activity has been detected in a range of municipal biosolids and livestock (e.g. swine and beef and dairy cattle) wastes using hormone receptor binding assays. As high as 6 mg estrogenicity as 17$\beta$-estradiol equivalent (EEQ)/per kg dry wt has been found in swine manure (*28*). A review by Lange et al. (*29*) reported a total estrogenic activity of 48.5 metric ton EEQ per year contributed by livestock excretion in the United States. The respective annual

contributions by cattle, swine, and chicken manure were 45, 0.8, and 2.7 metric ton. E2 and E1 account for more than 95% of the total estrogenic potency of natural steroidal hormone excretion by human beings and livestock. The excretion rates and types of estrogens differ in different species, for example, cattle wastes contain a large amount of 17α over E2, while 17α is rarely found in swine and poultry manures (*30*). The origin of estrogen hormones and their amounts are listed in Table S2 (see Supporting Information).

A survey of 139 streams from 30 states conducted by the United States Geological Survey (USGS) in 1999−2000 revealed that these water bodies contain EDCs, including estrogen compounds, with E2 and E1 concentrations as high as 200 and 112 ng/L, respectively (*31*). Confined animal-feeding operations (CAFOs) do not require treatment of manure as long as it is not disposed directly into water bodies (*32*). However, there are reports that surface runoff and subsurface tiles could carry contaminants to receiving surface and groundwaters. Land applications of manure based on agronomic rates showed that the total estrogenic compounds had a positive correlation with total Kjeldahl nitrogen (TKN), and runoff collected from research plots had E2 concentrations ranging from 2.1 to 41 ng/L (*33*). Finlay-Moore et al. (*34*) reported estrogenic activity up to 675 ng/kg dry weight as E2 equivalent in soil amended with broiler litter, and the surface runoff contained estrogenic activity ranging from 50 to 2300 ng/kg dry weight waste as E2 equivalent. Furthermore, the groundwater adjacent to the field was impacted with estrogen ranging from 6 to 66 ng E2 equivalent/kg dry weight waste. Shore et al. (*35*) observed that the runoff from agricultural land amended with poultry manure had a significant impact on the environment, resulting in not only an elevated level of E2 in adjacent streams but also an elevated level of phytoestrogen in the alfalfa (*lucerne*) crops grown on the manure-amended land.

## 5. Estrogenicity and Biotransformation of Estrogens

**5.1. Hydrolysis of Conjugated Estrogens into Free Estrogens.** Conjugated estrogens are hydrolyzed into free estrogens and glucuronide/sulfuric acid (HGLU/HSUL) in the presence of fecal bacteria - *Eschericia coli* (*36, 37*)

$$\mathrm{E-GLU/SUL} + \mathrm{H_2O} \xrightarrow{K_{hd}} \mathrm{E} + \mathrm{HGLU/HSUL} \qquad (1)$$

where $K_{hd}$ is the hydrolysis equilibrium constant.

The final concentration of free estrogen at equilibrium can be calculated as

$$C_e = \frac{-k + \sqrt{k^2 + 4kC_0}}{2} \qquad (2)$$

where $k = K_{hd}{}^*[\mathrm{H_2O}]$.

The hydrolysis of conjugated estrogen to free estrogen is always considered thermodynamically irreversible and complete with conjugated estrogen at concentrations of $C_0 = 10^{-12}$−$10^{-3}$ mol/L, once the reaction proceeds under the right enzymatic conditions. The enzymes required for the hydrolysis are called glucuronidase and sulfatase, respectively, for $E_i$-3, (17)-GLU/SUL (where $i = 1, 2, 3$). The hydrolysis of $E_i$-3, (17)-SUL is much more difficult than that of $E_i$-3, (17)-GUL. Therefore, $E_i$-3, (17)-SUL is more stable in the environment than $E_i$-3, (17)-GUL, which is consistent with the fact that detection of $E_i$-3, (17)-SUL has been reported in municipal wastewater, WWTP, and sediment, while no report of $E_i$-3, (17)-GUL existence could be found (*1, 37*). As conjugated estrogens do not exhibit estrogenicity, the hydrolysis of conjugated estrogen into free estrogen results in an increase in total estrogenic potency.

**5.2. Bioaccumulation of Estrogens through Microorganisms.** Liebig et al. (*38*) reported that the aquatic oligochaete *Lumbriculus variegatus* was able to take up radioactively labeled 17α-ethinylestradiol ($^{14}$C-EE2) from the environment during an observation period of 35 days. The authors further found that *L. variegatus* with accumulated EE2 excreted 50% of the EE2 into the estrogen-free environment 10 days after the bioaccumulation reached its peak. It can be inferred that the secondary predators of *L. variegatus*, mainly benthivorous fish species, would probably be considered feminization victims of estrogens partially due to the bioaccumulation of estrogens. This is consistent with the induction of vitellogenin observed in rainbow trout (*Oncorhynchus mykiss*) (*5, 40*), zebrafish (*Danio rerio*) (*39*), and fathead minnow (*Pimephales promelas*) (*14*). No current bioaccumulation data are available for natural estrogens, but it is likely that natural estrogens might also be accumulated through the same route.

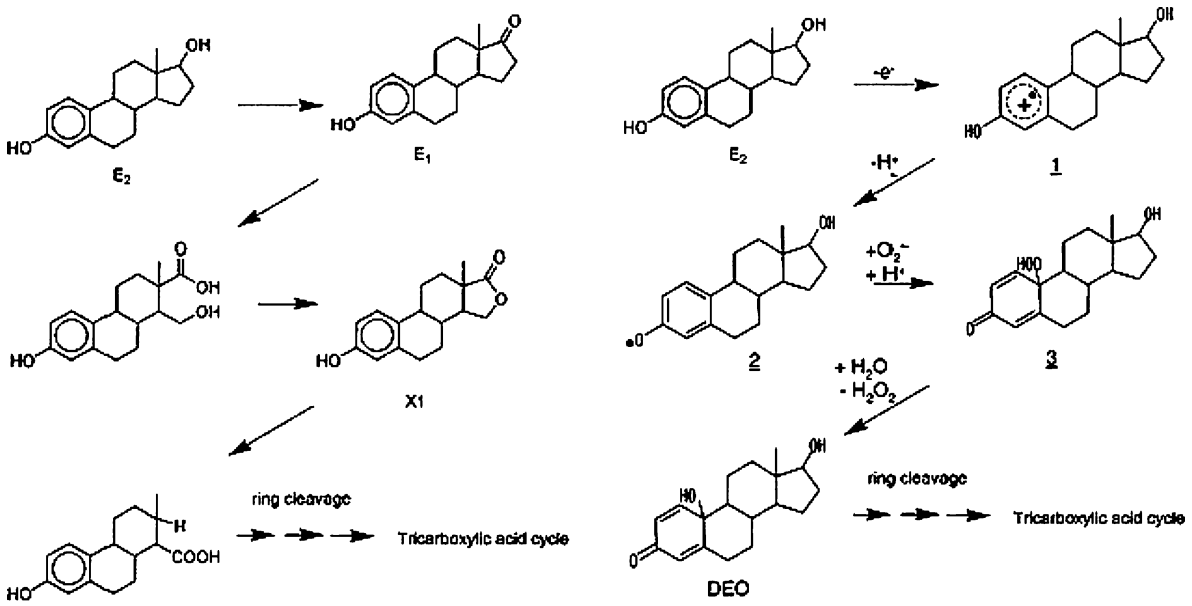
## 6. Removal of Free Estrogen Compounds in the Environment

**6.1. Removal of Free Estrogens from the Aqueous Phase.** Removal of free estrogen compounds from the aqueous phase could be achieved through three major pathways (*41*): volatilization from the liquid phase into the gas phase; biotic and abiotic degradation; and sorption onto solids.

*Volatilization.* The loss of natural estrogens through volatilization could be judged best by their Henry's law constants. As indicated in Table 1, free natural estrogens have low vapor pressures. They are likely to have very small Henry's law constants. Thus natural estrogens are not easily volatilized under normal temperature and pressure conditions, and their loss from the aqueous phase through volatilization is likely to be quite limited.

*Biotic and Abiotic Degradation.* A column study indicated that degradation of free estrogens was achieved mainly through a biotic route, whereas under abiotic conditions, the estrogen level remained fairly constant at an initial estrogen level of 500 ng/L as E2 equivalent (*42*). The presence of common waterborne algae—*Chlorella vulgaris* reduced the oxidation half-life from 48 h to only 3 h, at standard temperature and pressure under daylight (*43*). Aquatic bacteria are also capable of enhancing E2 oxidation into E1. Matsuoka et al. (*44*) reported that E2 concentration in the Tokyo River declined from an initial level of 4−10 nM to below detection limit in 5 days in summer and 7 days in winter. In three English rivers, the Aire, Calder, and Thames, the biotransformation of E2 into E1 followed a first-order decay with half-lives of 0.2−9 days, whereas the photolysis of E2 into E1 under ideal conditions had a half-life of at least 10 days (*45*). The oxidation of E2 into E1 is considered incomplete in terms of estrogenicity removal, because E1 still retains estrogenicity level at 0.1−0.2 of E2 equivalent (*46*).

Bacteria present in the wastewater have been found capable of completely degrading estrogenic compounds into harmless products (*47*). Microbes extracted from sewage sludge were identified and further tested to examine their estrogen degrading capacity (*48*, *49*). Gram-negative bacteria isolated from activated sludge samples in a Tokyo WWTP degraded E2 at an initial level of 30 mg/L with pseudo-zero-order kinetics (*48*). The gram-negative sewage bacteria were identified as *Rhodococcus zopfii* and *Rhodococcus equi*, which were capable of degrading E2 from an initial concentration of 100 mg/L to only 1 mg/L during a 24-h period (*49*). Microbial groups in municipal WWTPs have shown a much better biodegradation capacity than that in industrial WWTPs. The mineralization of E2 was found to be 84% within 24 h by municipal WWTP sludge, whereas for industrial WWTP sludge, the mineralization was just about 4% (*50*). Based on




(a) Biodegradation by sewage bacteria (47)

(b) Degradation by $T_iO_2$ photocatalyst (52)

**FIGURE 1. Pathways of estrogen (E2, E1) degradation.**

terminal restriction fragment length polymorphism (T-RFLP) assay, Yu et al. (*51*) identified a frequent occurrence of *E. coli*, *Pseudomonas fluorescens* (gram negative), and *Bacillus thuringiensis* (gram positive) strains, which were linked to high estrogen-degrading capacity in municipal sludge.

The degradation pathway of E2 and E1 with microbial enzyme and industrial catalyst is illustrated in Figure 1 (parts (a) and (b), respectively). E2 was oxidized from the cyclopentane ring D at C17 into E1 during enzymatic degradation and then further degraded into metabolite X1 and finally to carbon dioxide through a tricarboxylic acid (TCA) cycle (Figure 1(a)). Alternatively, in $TiO_2$-assisted photocatalytic degradation, E2 was first chemically degraded at the phenol ring A, and then into intermediate product DEO, and finally to carbon dioxide through the TCA cycle (Figure 1(b)).

*Adsorption.* Natural estrogen compounds are mainly removed from the aqueous phase by adsorption onto associated solid phases, such as sludge in wastewater treatment or soil in the case of land application. Free estrogens, which are nonvolatile and moderately hydrophobic, partition readily from the liquid phase onto the solid phase to a large extent. The sorption behavior of steroidal estrogens has been modeled using the empirical Freundlich isotherm (*53*).

Table S3 (see Supporting Information) lists adsorption capacity coefficient ($K_f$) values of different kinds of soil for E1 and E2, where $K_f$ varies from 4 (sand) to as high as 667 (LaDelle silt loam). It is apparent that $K_f$ is strongly correlated to the organic content of the sorbent, as pure sand, silt, and clay contain very low organic matter; whereas LaDelle silt loam, which contains the highest organic matter, was a strong adsorbent of free estrogens. Furthermore, $K_f$ values are also governed by the specific surface area of the adsorbent. $K_f$ values are somewhat correlated to clay and silt contents of the soil, since fine-textured soils usually have a higher organic matter content than those with coarse textures. More systematic research is needed to further understand estrogen−soil−water interactions under different soil conditions, temperatures, salinities, and moisture contents.

**6.2. Removal of Free Estrogen Compounds from Solid Phase.** Estrogenic compounds with octanol−water partition coefficient ($K_{ow}$) of 2.6−4.0 are readily adsorbed onto solids, including soil, river sediment, etc. The removal of free estrogens from the solid phase occurs mainly through biodegradation by soil microbes. Compared to the biodegradation by sewage microbes, which is fast and complete, biodegradation by soil microbes is rather slow and incomplete. The half-lives of E2 in a loam soil with 3.2% organic matter and a silt loam soil with 2.9% organic matter were 61 and 72 h, respectively, at room temperature. The mineralization efficiency measured in terms of carbon dioxide released did not exceed 15% in both loam and silt soils (*54*).

Bacteria in animal manure are capable of degrading estrogens. Soils amended with swine manure facilitate the biodegradation of estrogens, mainly due to the presence of fecal bacteria. Raman et al. (*55*, *56*) tested estrogen degradation in cattle and swine manures. The authors found that the E2 concentration dropped sharply during the first 24 h of incubation under aerobic conditions; while E1 was accumulated and reached a peak concentration in 48 h. The total estrogenic activity measured by yeast screen decayed following first-order kinetics, and the rate constants increased with temperature from 0.03 $day^{-1}$ at 3 °C to 0.12 $day^{-1}$ at 5 °C. The manure microorganism *Cornybacterium* spp. was believed to be responsible for the biodegradation of both E1 and E2. The complete biodegradation of estrogen compounds is largely dependent on the destruction of the phenolic ring (Table 1). The fungus group *Paecilomyces lilacinus* was found to be capable of cleaving the phenolic ring of biphenyl into five di- and trihydroxylated metabolites and thus is believed to have the capacity to degrade estrogen compounds as well (*57*).

The aerobic biodegradation of phenolic organic compounds in the solid phase occurs at a much faster rate than the anaerobic route. Normally, the gas phase of soil surface horizons has an oxygen content close to that of the atmosphere [∼ 20% (v/v)]. In deeper horizons, the oxygen content is variable and depends particularly on the degree of water saturation. In water-saturated subsoil, microbial activity can rapidly deplete oxygen, leading to anaerobic conditions (*59*). Biodegradation is expected to proceed much faster in aerobic surface horizons. Accumulation of estrogen compounds in the deeper layer of soils might be possible because of low estrogen biodegradation due to oxygen limiting conditions. However, no published research particularly elucidates the estrogen degradation profile at

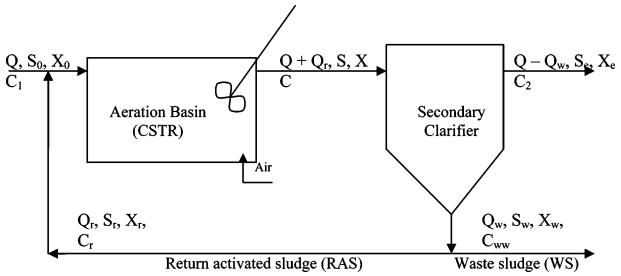



**FIGURE 2. Mass balance around a typical activated sludge process.**

different soil depths. Biodegradation of estrogens are further inhibited by the presence of antibiotics such as sulfamethazine, tylosin, and chlorotetracycline. Antibiotics inhibit estrogen biodegradation by deactivating the microbial degradation capacity and competing as the primary carbon source with the estrogens. Since coexistence of estrogens and antibiotics is ubiquitous in biosolids and manure-amended soil, more research is needed to understand the competitive biodegradation pathway between estrogens and antibiotics.

## 7. Fate of Estrogenic Compounds in an Engineered System

**7.1. Fate of Estrogens during Biological Wastewater Treatment.** *Preliminary Treatment.* Preliminary treatment involves the screening of raw sewage through bar screens to remove large objects, including rags, plastic bottles and bags, branches, etc. A large amount of organic material remains in dissolved form and in suspension and is poorly removed during preliminary treatment. Thus, essentially no free estrogen removal takes place during preliminary treatment (*1*). Glucuronide conjugates are immediately deconjugated into free forms upon entry into the wastewater treatment system, and sulfate conjugates have a longer life (*1, 37*).

*Primary Treatment.* Primary treatment mainly removes organic solids by gravity. Natural estrogens are removed from the water phase by partitioning onto fat, oil, and grease (FOG) and/or onto primary sludge. Solid-bound estrogens are then removed through solid−liquid separation, such as flotation or sedimentation.

A mass balance of estrogen compounds for a primary sedimentation tank is given below (*41*)

$$C_0 \times Q = (C_1 \times Q) + (C_{ps} \times M_{ps}) \quad (3)$$

where $C_0$ is the estrogen concentration in raw sewage, in ng/L; $C_1$ is the estrogen concentration in primary effluent, in ng/L; $Q$ is the flow rate, in $m^3$/day; $C_{ps}$ is the estrogen uptake by primary sludge, in ng/kg; and $M_{ps}$ is the total mass of primary sludge, in kg.

At water-sludge partition equilibrium

$$C_{ps} = K_{d,ps} \times C_1 \quad (4)$$

where $K_{d,ps}$ is the estrogen partition coefficient between primary sludge and water, in L/kg.

For primary treatment, $C_{inf} = C_0$, $C_{eff} = C_1$; by substituting eqs 3 and 4 into removal fraction $= (C_{inf} - C_{eff})/C_{inf}$, we have

$$\text{removal fraction} = (K_{d,ps} \times M_{ps})/(Q + K_{d,ps} \times M_{ps}) \quad (5)$$

Based on eq 5, estrogen removal during primary treatment is governed by three factors, namely (1) mass of primary sludge, $M_{ps}$; (2) partition coefficient of estrogen between the water phase and primary sludge, $K_{d,ps}$; and (3) hydraulic retention time (HRT), i.e., the shorter the HRT (high $Q$), the lower the removal efficiency.

*Secondary Treatment.* Natural estrogens are removed from the aqueous phase by adsorption onto floc/biofilm and are further degraded by microbes within the flocs/biofilm. The activated sludge process has been reported to have better estrogen removal efficiency than trickling filters (*59*). To assess the fate of estrogens in an activated sludge process, a mass balance assuming it as a completely mixed system with sludge recycling is illustrated in Figure 2.

To simplify the mass balance, the following assumptions may be made: (1) the influent and effluent biomass concentrations are negligible ($X_0 = X_e = 0$); (2) aeration basin is an ideal continuously stirred tank reactor (CSTR), and all reactions occur within the basin (i.e., $S = S_w = S_r = S_e$, $C = C_{ww} = C_e = C_2$); and (3) the two-phase transfer model is employed to describe the estrogen removal in a completely mixed activated sludge system, in both liquid and sludge phase (*60*).

In the liquid phase

$$d[C_{ww}]/dt = -k_{tr}(K_{d,ss}C_{ww} - C_{ss})X \quad (6)$$

In the sludge phase

$$d[C_{ss}X]/dt = k_{tr}(K_{d,ss}C_{ww} - C_{ss})X - k_1C_{ss}X \quad (7)$$

where $C_{ww}$ is the estrogen concentration in WS, in ng/L; $C_{ss}$ is the estrogen uptake on the secondary sludge, in ng/kg; $k_{tr}$ is the estrogen water-sludge mass transfer rate constant, in $day^{-1}$; $k_1$ is the biodegradation rate constant, in $day^{-1}$; and $K_{d,ss}$ is the estrogen partition coefficient between secondary sludge-water, in L/kg.

By incorporating the above assumptions in biomass and substrate mass balances of a typical activated sludge process, we have

$$C_2 = C_{ww} = \frac{K_s(1 + k_d \bullet \text{SRT})}{\text{SRT} \bullet \lambda \bullet (\mu_{max} - k_d) - 1} \quad (8)$$

where $C_2$ is the estrogen concentration in secondary effluent, in ng/L; $K_s$ is the half saturation constant, in mg/L; $\mu_{max}$ is the maximum specific growth rate, in $day^{-1}$; $k_d$ is the biomass decay coefficient, in $day^{-1}$; and $\lambda$ is the estrogen cometabolism coefficient, ng estrogen/mg BOD.

The estrogen level in secondary effluent, $C_2$, is governed by several factors: (1) An increase in solids retention time (SRT) would lead to the decrease of $C_2$ in the effluent, and thus an increase in estrogen removal. The removal efficiency of E2 and E1 increased from 75 to 96% and 64 to 94%, respectively, when the SRT was increased from 6 to 11 days in activated sludge systems (*1*). (2) An increase in the estrogen partition coefficient between liquid and secondary sludge $K_{d,ss}$ would decrease $C_2$ and thus increase the removal of estrogens. $K_{d,ss}$ correlates with the secondary sludge solid content; therefore, a higher mixed liquor suspended solids (MLSS) would lead to a better removal of estrogens from the liquid phase. E2 and E1 levels in the secondary effluent decreased from 7.9 to 2.2 ng/L and 26.3 to 10.3 ng/L, respectively, in a bench-scale experiment when MLSS was increased from 1000 to 10 000 mg/L (*61*). (3) An increase in biodegradation rate constant, $k_{biol}$, and an increase in mixed liquor volatile suspended solids (MLVSS) would decrease the $C_2$ half-life in sludge and thereby enhance the estrogen removal from the aqueous phase. The biodegradation in the sludge phase was found to follow a pseudo-first-order reaction as given below (*42*)

$$\frac{dC}{dt} = k_{biol} \bullet \text{MLVSS} \bullet t \quad (9)$$

where $k_{biol}$ is the first-order biological degradation rate constant, in day$^{-1}$; MLVSS is the mixed liquor volatile suspended solids, in kg/L; and $t$ is the time of degradation in sludge, in day.

The integration of eq 9 will result in

$$C_2 = C_1 \cdot \exp(-k_{biol} \bullet \text{MLVSS} \bullet t) \quad (10)$$

where $C_1$ is the estrogen concentration in primary effluent, in ng/L.

There is significant removal of natural estrogens in the case of nitrification, which is mainly attributed to two reasons based on eq 10; first, nitrifying bacteria have been shown to possess superior estrogen removing capability (*62*) and thus possess a high $k_{biol}$, leading to a smaller $C_2$; second, a nitrification process usually requires a longer SRT than a conventional activated sludge system; therefore, an extended SRT would also lead to a smaller $C_2$. The orders of biodegradation rate for free and conjugated estrogens are as follows: E2 − GLU > E2 > E2 − SUL >> E1 − GUL >> E1 > E1 − SUL (*42*). For nitrified returned sludge with E2 and E1 concentrations of 20 ng/L each, it took about 10 min for E2 to be degraded to 1 ng/L; however, it needed as long as 5 h for E1 to reach 1 ng/L. Some earlier studies also reported that E1 removal efficiency is inferior in comparison to E2 (*19*). Streams receiving WWTP effluents were found to have an elevated E1 level downstream, compared to the background E1 level upstream, which in turn indicated that the WWTPs were not able to remove E1 as efficiently as other natural estrogens (*63*). The estrogen removal efficiencies of activated sludge systems for nine countries from 1995 to 2004 are summarized in Table S4 (Supporting Information).

**7.2. Fate of Estrogens during Advanced Treatment.** *Activated Carbon (AC).* E2 was quickly adsorbed onto granular activated carbon (GAC) as the adsorption reached equilibrium within 3 h in a series of bench-scale adsorbent evaluation tests (*64*). The removal efficiency of GAC was found to decrease with the decrease in initial concentration of E2. When the initial concentration of E2 was decreased from 100 to 1 ng/L, the removal efficiency decreased from 81 to 49% (*65*). The presence of other soluble organics measured as total organic carbon (TOC) would compete with natural estrogen adsorption onto GAC. In a comparative study, Fukuhara et al. (*66*) found that the adsorption capacity for E2 onto GAC was reduced from 21.3 to 67.7 mg/g in pure water with 1 $\mu$g/L of E2 to only 0.1−1 $\mu$g/g in river and secondary effluent containing the same estrogen level. Thus, the use of GAC for estrogen removal from wastewater is not a good option. By contrast, powdered activated carbon (PAC) was found to remove over 90% of E2 at initial concentrations ranging from 27 to 135 ng/L, and the efficiency was further improved by increasing the retention time (*67, 68*). However, the PAC-based system requires a continuous supply of media, which makes the application suitable only for temporary or seasonal use (*53*).

**Membrane Bioreactor (MBR).** MBR is able to maintain an extremely long SRT and diverse microbial community, which facilitates the degradation of estrogen compounds (*69*). Microfiltration (MF) and ultrafiltration (UF) membranes have a pore size 100 to 10 000 times larger than the estrogen molecules, which have a molecular mass ranging from 270 to 288 Da. Therefore, the removal of estrogens in the membrane system is achieved by sorption on suspended and colloidal particles and biological degradation (*70*). Liu et al. (*71*) employed cross-flow UF membranes to determine the partition coefficient of EDCs, including estrogen compounds in molecular and colloidal forms. The authors reported a removal efficiency of over 82% for estrogens (E2, E1, and EE2).

The removal mechanism of estrogens by UF membranes is primarily through filtration in which the estrogen-bound colloids are formed by the binding of free estrogen molecules to the existing colloids in aqueous phase

$$\text{EDC}_{free} + \text{colloids} \rightarrow \text{EDC}_{colloids} \quad (11)$$

with an equilibrium constant for eq 11, $K_p = [\text{EDC}_{colloids}]/[\text{EDC}_{free}][\text{colloids}]$.

The binding equilibrium constant $K_p$ is independent of the octanol water partition coefficient $K_{ow}$ but is correlated to a normalized organic carbon partition coefficient, $K_{coc}$, as follows

$$K_p = K_{coc} \times f_{coc} \quad (12)$$

where $f_{coc}$ is the fraction of organic carbon in the colloids.

The effectiveness of a nanopore MBR for estrogen removal was demonstrated in a comparative experiment by Wintgens et al. (*69*). The authors observed that an MBR system was able to remove 28% more estrogens than a GAC system. Since the MBR is able to maintain longer SRT and a much higher MLSS concentration than a conventional activated sludge process, it can achieve better estrogen removal. An MF-based MBR with 10 000 mg/L MLSS and 83.3 days SRT achieved effluent estrogen concentrations of 2.2 ng/L as E2 and 10.3 ng/L as E1, whereas the conventional activated sludge process with MLSS of 1000 mg/L and SRT of 4.8 days could only achieve effluent estrogen levels of 7.9 ng/L as E2 and 26.3 ng/L as E1 (*61, 64*).

**Chemical Oxidation and Other Advanced Oxidation Processes.** The use of chemical oxidants has been found highly efficient for estrogen removal from the aqueous phase in several bench-scale studies. The time for oxidation of E2 into E1 was reduced from 48 h to 10 min and 2 h, respectively, when ozone and chlorine were employed (*72*). Ozone dosages of 10−50 mg/L provided excellent removal of estrogen compounds (*70*). Westerhoff et al. (*68*) reported that the implementation of ozone was more efficient in oxidizing compounds with phenolic rings including estrogens than those without phenolic rings. An ozone dosage of 5 mg/L successfully reduced the initial concentration of 3.0 ng/L E2 and 13 ng/L E1 to below detection limits of 1 ng/L. Furthermore, ozone oxidation kinetics have been studied recently, and an ozone dosage of 2 $\mu$g min/L achieved more than 95% E2 and E1 reduction at neutral pH and room temperature (*73*). Ferrate (Fe(VI)) has also been employed as a strong oxidant in batch test, and Fe(VI) at a dosage of 13−17 mg/L proved to be a better oxidation method than electrochemical oxidation at 2−200 mV/s sweep rate (*74*).

Photodegradation of E2 occurred with direct exposure to UV lamps above 30 W, and that of E1 required direct exposure above 12 W. The degradation of estrogens at the initial concentration of 3−20 mg/L followed first-order kinetics. The rate was higher with an increase in pH from 7.0 to 8.0, but it was lowest at a pH around 5.0 (*75*). E1 required a higher energy input for degradation than E2, and the degradation of E1 using a 250 W UV lamp system coupled with Fe(III) and $H_2O_2$ at an initial concentration of 18.5 $\mu$M E1 reached over 98% removal within 160 min (*76*). Photodegradation of E2 could be achieved at a much lower energy requirement in the presence of $TiO_2$ (*50*). E2 at an initial concentration of $10^{-6}$ M was completely mineralized to $CO_2$ under UV radiation at 18 mJ/cm$^2$ during a 3-h column test in the presence of 1.0 g/L $TiO_2$.

**7.3. Solids Handling.** Natural estrogens have $K_{ow}$ values ranging from 2.8 to 3.6 which render them readily adsorbable onto organic solids. The sorbed fraction of natural estrogens

directly affects the estrogen concentration in biosolids and is governed by (*77*)

$$C_{\text{sorbed}} = \frac{K_d \bullet \text{MLSS}}{1 + K_d \bullet \text{MLSS}} \quad (13)$$

where $C_{\text{sorbed}}$ is the sorbed fraction of natural estrogens.

The $K_d$ values ranged from 376 to 528 L/kg for E1 and from 284 to 668 L/kg for E2, as determined by a series of experiments. In a typical activated sludge process with a suspended solid concentration of 4 g/L, about 52−70% of E1 and 53−79% of E2 were removed from the aqueous phase by adsorption onto the sludge phase (*78*). The estrogen adsorbed by the sludge would subsequently go through solids handling processes that include thickening, stabilization, and dewatering before landfill, land application, or incineration.

There is a lack of research that specifically predicts the fate of estrogen compounds during solids handling processes. Sludge stabilization, particularly aerobic and anaerobic digestion, has the potential to remove some estrogen hormones due to a long SRT. One study reported that the biodegradation of natural estrogen during anaerobic digestion was relatively low compared to aerobic conditions (*79*). In another study in Japan, untreated raw nightsoil (human excreta) and septic tank sludge had E2 levels of 303 and 274 μg/kg, respectively, whereas in the treated solids, the E2 level was 101 μg/kg, and the extracted liquor had an E2 concentration of 38.9 ng/L (*80*). Studies need to be conducted to examine the efficiency of aerobic and anaerobic digestion processes to degrade estrogen compounds in the solids phase. Such studies will eventually predict the residual estrogens in the treated biosolids and their ultimate risk to human beings and animals.

## 8. Estrogens in Livestock Manure and their Fate

Like human beings, livestock excrete steroidal estrogens in the urine and feces irrespective of their sex and age. It is estimated that estrogen contribution by livestock manure would account for at least 90% of the total estrogen in the environment (*58*). Larsson et al. (*81*) reported that estrogens derived from animals are excreted in urine as conjugates of sulfate or glucuronide and in feces in the unconjugated form. These conjugates are biologically inactive; however, they act as a precursor hormone reservoir that can be converted to the free active form by microbes present in waste such as *E. coli* (*1, 19*). Different livestock species excrete different estrogens. For example, cattle waste contains a large amount of 17α, E2, and E1 (*25*, *28*), whereas poultry or swine waste rarely contains 17α (*82*−*84*). Estrogenic compounds are excreted by different routes. Cattle excrete estrogens mainly in feces, while swine and poultry excrete estrogens mainly in urine (*84*).

Estrogenic hormones are frequently administered to livestock as growth promoters. This may increase their urine output of estrogens (*85*). Callantine et al. (*86*) found that giving livestock E2 could result in a 5−6-fold increase in urine estrogen production. There are, however, limited data available with regard to the daily excretion rates of estrogens from various animal types. Most of the available data represent only sexually mature, female animals; therefore, the usefulness of these data to understand estrogen fate in the environment is limited.

Land application is widely viewed as an economic way of disposing animal manure and recycling nutrients. Agricultural lands, however, could be a potential source of environmental steroidal estrogenic compounds when animal manure is applied over long periods. Recent studies have indicated that runoff from applied fields, where manure has been applied, can enter adjacent streams or infiltrate through the soil into groundwater, resulting in detectable levels of estrogens that could affect wildlife (*31, 87*). Nichols et al. (*88*) found an average E2 concentration of 3500 ng/L in surface runoff following poultry litter application to grassland. Furthermore, an E2 concentration of 37.6 ng/L was detected in aquifers underlying areas where animal manure was applied (*32*). Peterson et al. (*89*) measured E2 concentrations ranging from 6 to 66 ng/L in five springs located in northwest Arkansas where poultry litter and cattle manure were applied.

Limited data exist regarding the fate and transport of estrogens derived from animal manure. Such data are essential to assess the impact of estrogens on natural ecosystems and human health. Colucci et al. (*90*) reported that E2, E1, and 17α were rapidly biotransformed in agricultural soils. Colucci and Topp (*91*) and Colucci et al. (*90*) found that E2 could be abiotically degraded, whereas E1 and 17α were only biotically degraded. Schlenker et al. (*92*) observed 80% estrogen removal in cattle manure following 12 weeks of incubation at 20−23 °C. However, it has recently been reported that <10% of estradiol and estrone added to poultry litter was mineralized after incubation for 25 weeks at a variety of moisture contents and temperatures (*93*). The observation could mean that the estrogens or their metabolites were protected from biodegradation by adsorption to the solid phases in the litter or that the low pH of the litter (pH 5.2) precluded significant microbial activity. However, complete mineralization of E2 and E1 occurs at an extremely minute proportion of all biodegradation processes, as only up to 7.8% radio-labeled E2 and E1 was mineralized to carbon dioxide during a 150-day exposure in broiler litter under room temperature (*93*).

Free estrogens are moderately hydrophobic with log $K_{ow}$ values ranging from 2.6 to 4.0, which suggests that sorption may govern their transport (*15*). The rate of biotransformation of estrogens in the environment was reported to take place until log $K_{ow}$ reaches 3.0−3.5; after that, sorption becomes predominant in determining their ultimate fate (*94*). Thus, E1 and E2 with greater log $K_{ow}$ values are likely to show a preference for solid partitioning (*95*). Experiments conducted using pure E2 compound have also documented that E2 sorption onto soil correlates with the organic carbon content of soil (*54*). However, there are no studies that have investigated how manure-associated estrogens interact with soil components when they are added to agricultural soils.

## 9. Future Research

There has been considerable progress on steroidal estrogen research, particularly monitoring of estrogen in aquatic systems. Although a wide variety of methods is available for detection of steroidal estrogens, there is still a need for a new analytical method which is simple, affordable, and sensitive enough for detection at levels which might interrupt the endocrine systems of aquatic organisms. Research is required to obtain a complete material balance for estrogen compounds in WWTP in order to identify and optimize the process/operation to maximize their removal. A systematic biodegradability study needs to be conducted under aerobic, anaerobic, and anoxic conditions using mixed microbial cultures to understand estrogen removal mechanisms and pathways in an engineered system. There is a continuing need to quantify the estrogen removal by biosorption on sludge and the subsequent biodegradation within the sludge. Currently, there is no comprehensive model that focuses on fate, transport, and removal of estrogen compounds in engineered systems and the environment. Development of models will help to predict estrogen removal. There is a need to understand the degradability of estrogen compounds in the manure-amended soils and their sorption and transport within the soil matrix.

Tertiary treatment of wastewater based on physical and chemical methods has a potential to significantly reduce the estrogen concentration in the treated effluent. However, more research is needed to find the cost-effectiveness of each method and its applicability in field applications. Livestock is a major contributor of estrogen hormones and manure from CAFOs and is not regulated—at least in the United States. The fate, transport, and degradation of manure-associated estrogens in the environment have not been well investigated.

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## Supporting Information Available

Additional relevant information, particularly sources of estrogen compounds, their release by human beings and livestock, their determination in solid and liquid samples, and their removal efficiency in engineered systems (Tables S1−S4 and Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

## Literature Cited

(1) Ternes, T. A.; Stumpf, M.; Mueller, J.; Haberer, K.; Wilken, R. D.; Servos, M. Behavior and occurrence of estrogens in municipal sewage treatment plants. I. Investigations in Germany, Canada and Brazil. *Sci. Total Environ.* **1999**, *225*, 81−90.

(2) Matsui, S.; Takigami, H.; Taniguchi, N.; Adachi, J.; Kawami, H.; Shimizu, Y. Estrogen and estrogen mimics contamination in water and the role of sewage treatment. *Water Sci. Technol.* **2000**, *42*, 173−179.

(3) Anderson, H.; Siegrist, H.; Halling-Sørensen, B.; Ternes, T. A. Fate of estrogens in a municipal sewage treatment plant. *Environ. Sci. Technol.* **2003**, *37*, 4021−4026.

(4) Duguet, J. P.; Bruchet, A.; Mallevialle, J. Pharmaceuticals and endocrine disruptors in the water cycle. *IWA Yearbook 2004* **2004**, pp 41−46.

(5) Tyler, C. R.; Spary, C.; Gibson, R.; Santos, E. M.; Shears, J.; Hill, E. M. Accounting for differences in estrogenic responses in rainbow trout and rouch exposed to effluents from wastewater treatment works. *Environ. Sci. Technol.* **2005**, *39*, 2599−2607.

(6) Purdom, C. E.; Hardiman, P. A.; Bye, V. J.; Eno, N. C.; Tyler, C. R.; Sumpter, J. P. Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.* **1994**, *8*, 275−285.

(7) Water 21 (April, 2004). Disruptive Influences. *Magazine of the International Water Association,* pp 20−22.

(8) Hanselman, T. A.; Graetz, D. A.; Wilkie, A. C. Manure-Borne estrogens as potential environmental contaminants: A review. *Environ. Sci. Technol.* **2003**, *37*, 5471−5478.

(9) Jobling, S.; Nolan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **1998**, *32*, 2498−2506.

(10) Panter, G. H.; Thompson, R. S.; Sumpter, J. P. Adverse reproductive effects in male fathead minnows (Pimephales promelas) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. *Aquat. Toxicol.* **1998**, *42*, 243−253.

(11) Tabata, A.; Kashiwada, S.; Ohnishi, Y.; Ishikawa, H.; Miyamoto, N.; Itoh, M.; Magara, Y. Estrogenic influences of estradiol-17b, p-nonylphenol and bis-phenol-A on Japanese Medaka (Oryzias latipes) at detected environmental concentrations. *Water Sci. Technol.* **2001**, *43*, 109−116.

(12) Irwin, L. K.; Gray, S.; Oberdorster, E. Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol. *Aquat. Toxicol.* **2001**, *55*, 49−60.

(13) http://www.latimes.com.

(14) Lai, K. M.; Johnson, K. L.; Scrimshaw, M. D.; Lester, J. N. Binding of waterborne steroid estrogens to solid phases in river and estuarine system. *Environ. Sci. Technol.* **2000**, *34*, 3890−3894.

(15) Lai, K. M.; Scrimshaw, M. D.; Lester, J. N. Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. *Sci. Total Environ.* **2002**, *289*, 159−168.

(16) Huang, C.; Sedlak, D. L. Analysis of estrogenic hormones in municipal wastewater effluent and surface water using ELISA and GC/MS/MS. *Environ. Technol. Chem.* **2001**, *20*, 133−139.

(17) Desbrow, C.; Routledge, E. J.; Brighty, G. C.; Sumpter, J. P.; Waldock, M. Identification of estrogenic chemicals in STW effluents. *Environ. Sci. Technol.* **1998**, *32*, 1549−1558.

(18) Baronti, C.; Curini, R.; D' Ascenzo, G.; Di Corcia, A.; Gentili, A.; Samperi, R. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environ. Sci. Technol.* **2000**, *34*, 5059−5066.

(19) Isobe, T.; Shiraishi, H.; Yasuda, M.; Shinoda, A.; Suzuki, H.; Morita, M. Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry. *J. Chromatogr., A.* **2003**, *984*, 195−202.

(20) Snyder, S. A.; Keith, T. L.; Verbrugge, D. A.; Synder, E. M.; Gross, T. S.; Kannan, K.; Giesty, J. P. Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. *Environ. Sci. Technol.* **1999**, *33*, 2814−2820.

(21) Ferguson, P. L.; Iden, C. R.; McElroy, A. E.; Brownawell, B. J. Determination of steroid estrogens in wastewater by immunoaffinity extraction coupled with HPLC-ESI-MS. *Anal. Chem.* **2001**, *73*, 3890−3895.

(22) Kuch, H. M.; Ballschmiter, K. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. *Environ. Sci. Technol.* **2001**, *35*, 3201−3206.

(23) Burnison, B. K.; Hartmann, A.; Lister, A.; Servos, M.; Ternes, T.; Kraak, G. V. D. A toxicity identification evaluation approach to studying estrogenic substances in hog manure and agricultural runoff. *Environ. Toxicol. Chem.* **2003**, *22*, 2243−2250.

(24) Ternes, T. A.; Andersen, H.; Gilberg, D.; Bonerz, M. Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. *Anal. Chem.* **2002**, *74*, 3498−3504.

(25) Gomes, R. L.; Avcioglu, E.; Srimshaw, M. D.; Lester, J. N. Steroid estrogen determination in sediment and sewage sludge: a critique of sample preparation and chromatographic/mass spectrometry considerations, incorporating a case study in method development. *Trends Anal. Chem.* **2004**, *23*, 737−744.

(26) Shore, L. S. and Shemesh, M. Naturally produced steroid hormones and their release into the environment. Pure Appl. Chem. **2003**, *75*, 1859−1871.

(27) Möstl, E.; Choi, H. S.; Wurm, W.; Ismail, M. N.; Bamberg, E. Pregnancy diagnosis in cows and heifers by determination of estradiol-17α in feces. *Br. Vet. J.* **1984**, *140*, 287−291.

(28) Lorenzen, A.; Hendel, J. G.; Conn, K. L.; Bittman, S.; Kwabiah, A. B.; Lazarovita, G.; Massé, D.; McAllister, T. A.; Topp, E. Survey of hormone activities in municipal biosolids and animal manures. *Environ. Toxicol.* **2004**, *19*, 216−255.

(29) Lange, I. G.; Daxenberger, A.; Schiffer, B.; Witters, H.; Ibarreta, D.; Meyer, H. H. D. Review − sex hormones originating from different livestock production systems: Fate and potential disrupting activity in the environment. *Anal. Chem. Acta* **2002**, *473*, 27−37.

(30) Hoffman, B.; Depinho, T. G.; Schuler, G. Determination of free and conjugated estrogens in peripheral blood plasma, feces and urine of cattle throughout pregnancy. *Exp. Clin. Endocrinol. Diabetes* **1997**, *105*, 296−303.

(31) Koplin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999−2000, a national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.

(32) USEPA. (2002). 40 CFR Parts 9, 122 & 412, *Concentrated Animal Feeding Operation − Final Rule.* (http://cfpub1.epa.gov/npdes/afo/cafofinalrule.cfm?program_id=7).

(33) Dyer, A. R.; Raman, D. R.; Mullen, M. D.; Burns, R. T.; Moody, L. B.; Layton, A. C.; Sayler, G. S. Determination of 17$\beta$-estradiol concentrations in runoff from plots receiving dairy manure. *ASAE Meeting paper*; No. 01-2107; ASAE: St. Joseph, MI, 2001.

(34) Finlay-Moore, O.; Hartel, P. G.; Cabrera, M. L. 17$\beta$-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. *J. Environ. Qual.* **2000**, *29*, 1604−1611.

(35) Shore, L. S.; Correll, D. L.; Chakraborty, P. K. Relationship of fertilization with chicken manure and concentration of estrogens in small streams. *Animal waste and the land-water interface;* Boca Raton, FL, Lewis: 1995; pp 49−56.

(36) Belfroid, A. C.; Van der Horst, A.; Vethaak, A. D.; Schafer, A. J.; Rijs, G. B. J.; Wegener, J.; Cofino, W. P. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and wastewater in the Netherlands. *Sci. Total Environ.* **1999**, *225*, 101−108.

(37) D' Ascenzo, G.; Di Corcia, A.; Gentili, A.; Mancini, R.; Mastropasqua, R.; Nazzari, M.; Samperi, R. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. *Sci. Total Environ.* **2003**, *302*, 199−209.

(38) Liebig, M.; Egeler, P.; Oehlmann, J.; Knacker, T. Bioaccumulation of 14C-17α-ethinylestradiol by the aquatic oligochaete *Lumbriculus variegates* in spiked artificial sediment. *Chemosphere* **2004**, *59*, 271−280.

(39) Fenske, M.; van Aerle, R.; Brack, S.; Tyler, C. R.; Segner, H. Development and validation of a homologous zebrafish (Danio rerio Hamilton-Buchanan) vitellogenin enzyme-linked immunosorbent assay (ELISA) and its application for studies on estrogenic chemicals. *Comp. Biochem. Physiol.* **2001**, *129*, 271−232.

(40) Sheahan, D. A.; Bucke, D.; Matthiessen, P.; Sumpter, J. P.; Kirby, M. F.; Neall, P.; Walldock, M. The effects of low levels of 17α-ethinylestradiol upon plasma vitellogenin levels in male and female rainbow trout, *Oncorhynchus mykiss* held at two acclimation temperatures. In *Sublethal and chronic effects of pollutants on freshwater fish;* Blackwell Scientific: Cambridge, U.K., 1994; pp 99−112.

(41) Schoenberg, T. H.; Helmig, E. G.; Fettig, J. D.; Cordone, L. In *Fate of estrogens in a nitrifying sequencing batch reactor receiving a high organic strength, nitrogen-rich industrial wastewater,* CD-ROM Proceedings of 78th Annual Conference & Exposition (WEFTEC), Washington, DC, Oct 29−Nov 2, 2005; 2005; pp 5125−5139.

(42) DEPA document. *Degradation of estrogens in sewage treatment processes*; Danish Environmental Protection Agency: Report No. 899; 2004.

(43) Lai, K. M.; Scrimshaw, M. D.; Lester, J. N. Biotransformation and bioconcentration of steroid estrogens by Chlorella vulgaris. *Appl. Environ. Microbiol.* **2001**, *68*, 859−864.

(44) Matsuoka, S.; Kikuchi, M.; Kimura, S.; Kurokawa, Y.; Kawai, S. Determination of estrogenic substances in the water of Muko river using *in Vitro* assays, and the degradation of natural estrogens by aquatic bacteria. *J. Health Sci.* **2005**, *51*, 178−184.

(45) Jürgens, M. D.; Holthaus, K. I. E.; Johnson, A. C.; Smith, J. J. L.; Hetheridge, M.; William, R. J. The potential for estradiol and ethinylestradiol degradation in English rivers. *Environ. Toxicol. Chem.* **2001**, *21*, 480−488.

(46) Nghiem, L.; Schäfer, A.; Elimelech, M. Removal of natural hormones by nanofiltration membranes: measurement, modeling and mechanisms. 2004. (http://www.yale.edu/env/elimelech/PosterRemovalHormonesNF.pdf).

(47) Lee, H. B.; Liu, D. Degradation of 17$\beta$-estradiol and its metabolites by sewage bacteria. *Water, Air, Soil Pollut.* **2001**, *134*, 353−368.

(48) Fujii, K.; Kikuchi, S.; Satomi, M.; Ushio-Sata, N.; Morita, N. Degradation of 17$\beta$-estradiol by a Gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan. *Appl. Environ. Microbiol.* **2002**, *68*, 2057−2060.

(49) Yoshimoto, T.; Nagai, F.; Fujimoto, J.; Watanabe, K.; Mizukoshi, H.; Makino, T.; Kimura, K.; Saino, H.; Sawada, H.; Omura, H. Degradation of estrogens by *Rhodococcus zopfii* and *Rhodococcus equi* isolated from activated sludge in wastewater treatment plants. *Appl. Environ. Microbiol.* **2004**, *70*, 5283−5289.

(50) Layton, A. C.; Gregory, B. W.; Seward, J. R.; Schultz, T. W.; Sayler, G. S. Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee USA. *Environ. Sci. Technol.* **2000**, *34*, 3925−3931.

(51) Yu, Z.; Huang, W. Competitive sorption between 17α-ethinyl estradiol and naphthalene/phenanthrene by sediments. *Environ. Sci. Technol.* **2005**, *39*, 4878−4885.

(52) Ohko, Y.; Iuchi, K.; Niwa, C.; Tatsuma, T.; Nakashima, T.; Iguchi, T.; Kubota, Y.; Fujishima, A. 17$\beta$-estradiol degradation by $T_iO_2$ photocatalysis as a means of reducing estrogenic activity. *Environ. Sci. Technol.* **2002**, *36*, 4175−4181.

(53) Casey, F. X. M.; Larsen, G. L.; Hakk, H.; Simunek, J. Fate and transport of 17$\beta$-estradiol in soil-water system. *Environ. Sci. Technol.* **2003**, *37*, 2400−2409.

(54) Jacobson, A.; Lorenzen, A.; Chapmen, R.; Topp, E. Persistence of testosterone and EE2 in soils receiving swine manure or municipal biosolids. *J. Environ. Qual.* **2005**, *34*, 861−871.

(55) Raman, D. R.; Layton, A. C.; Moody, L. B.; Easter, J. P.; Sayler, G. S.; Burns, R. T.; Mullen, M. D. Degradation of estrogens in dairy waste solids: effect of acidification and temperature. *Trans. ASAE* **2001**, *44*, 1881−1888.

(56) Raman, D. R.; Williams, E. L.; Layton, A. C.; Burns, R. T.; Easter, J. P.; Daugherty, A. S.; Mullen, M. D.; Sayler, G. S. Estrogen content of dairy and swine wastes. *Environ. Sci. Technol.* **2004**, *38*, 3567−3573.

(57) Gesell, M.; Hammer, E.; Specht, M.; Francke, W.; Schauer, F. Biotransformation of biphenyl by *Paecilomyces lilacinus* and characterization of ring cleavage products. *Appl. Environ. Microbiol.* **2001**, *67*, 1551−1557.

(58) Maier, R. M., Pepper, I. L.; Gerba, C. P. Terrestrial Environment. In *Environmental Microbiology*; Academic Press: 2000; pp 61−80.

(59) Ziegler, T. E.; Wittwer, D. J. Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing and analysis. *Am. J. Primatol.* **2005**, *67*, 159−174.

(60) Huang, Y.; Pineau, I.; Chang, H.; Azzi, A.; Bellemare, V.; Laberge, S.; Lin, S. Critical residues for the specificity of cofactors and substrates in human estrogenic 17$\beta$ − hydroxysteroid dehydrogenase. *Mol. Endocrinol.* **2005**, *15*, 2010−2020.

(61) Kikuta, T.; Urase T. *Removal of endocrine disruptors in membrane separation activated sludge process*; International Membrane Science and Technology Conference (IMSTEC'03), Sydney, Australia, 2003.

(62) Vader, J. S.; Finkel, C. G.; Sperling, F. M. G. M.; Jong, J.; Boer, W.; Graaf, J. S.; Most, M.; Stokman, P. G. W. Degradation of ethinyl estradiol by nitrifying activated sludge. *Chemosphere* **2000**, *41*, 1239−1243.

(63) Holbrook, R. D.; Love, N. G.; Novak, J. T. Sorption of E2 and EE2 by colloidal organic carbon derived from biological wastewater treatment systems. *Environ. Sci. Technol.* **2004**, *38*, 3322−3329.

(64) Snyder, S. A.; Westerhoff, P.; Yoon, Y.; Sedlak, D. Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. *Environ. Eng. Sci.* **2003**, *20*, 449−459.

(65) Boyd, G. R.; Reemtsma, H.; Grimm, D. A.; Mitra, S. Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA, and Ontario, Canada. *Sci. Total Environ.* **2003**, *311*, 135−149.

(66) Fukuhara, T.; Iwasaki, S.; Kawashima, M.; Shinohara, O.; Abe, I. Adsorbability of estrone and 17$\beta$-estradiol in water onto activated carbon. *Water Res.* **2005**, in press.

(67) Yoon, Y.; Westerhoff, P.; Snyder, S. A. Adsorption of 3H-labeled 17$\beta$-estradiol on powdered activated carbon. *Water, Air, Soil Pollut.* **2005**, *166*, 343−351.

(68) Westerhoff, P.; Yoon, Y.; Snyder, S.; Wert, E. Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environ. Sci. Technol.* **2005**, *39*, 6649−6663.

(69) Wintgens, T.; Gallenkemper, M.; Melin, T. Endocrine disrupting removal from wastewater using membrane bioreactor and nanofiltration technology. *Desalination* **2002**, *146*, 387−391.

(70) Larson, T.; Lienert, J.; Joss, A.; Siegrist, H. How to avoid pharmaceuticals in the aquatic environment. *J. Biotechnol.* **2004**, *113*, 295−299.

(71) Liu, R.; Wilding, A.; Hibberd, A.; Zhou, J. L. Partition of endocrine-disrupting chemicals between colloids and dissolved phase as determined by cross-flow ultrafiltration. *Environ. Sci. Technol.* **2005**, *39*, 2753−2761.

(72) Alum, A.; Yoon, Y.; Westerhoff, P.; Abbaszadegan, M. Oxidation of BPA, E2 and EE2 and byproduct estrogenicity. *Environ. Toxicol.* **2004**, *19*, 257−264.

(73) Deborde, M.; Rabouan, S.; Duguet, J.; Legube, B. Kinetics of aqueous ozone-induced oxidation of some endocrine disrupters. *Environ. Sci. Technol.* **2005**, *39*, 6086−6092.

(74) Jiang, J. Q.; Yin, Q.; Zhou, J. L.; Pearce, P. Occurrence and treatment trials of endocrine disrupting chemicals (EDCs) in wastewaters. *Chemosphere* **2005**, *61*, 544−550.

(75) Liu, B.; Liu, X. Direct photolysis of estrogens in aqueous solutions. *Sci. Total Environ.* **2004**, *320*, 269−274.

(76) Feng, X.; Ding, S.; Tu, J.; Wu, F.; Deng, N. Degradation of estrone in aqueous solution by photo-Fenton system. *Sci. Total Environ.* **2005**, *345*, 229−237.

(77) Ternes, T. A.; Janex-Habibi, M.; Knacker, T.; Kreuzinger, N.; Siegrist, H. Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse. *POSEIDON*, detailed reported related to the overall duration; 2005. http://www.eu-poseidon.com

(78) Anderson, H. R.; Hansen, M.; Kjølholt, J.; Stuer-Lauridsen, F.; Ternes, T.; Halling-Sørensen, B. Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. *Chemosphere* **2005**, *61*, 139−146.

(79) Johnson, A. C.; Sumpter, J. P. Critical review: Removal of endocrine-disrupting chemicals in activated sludge treatment works. *Environ. Sci. Technol.* **2001**, *35*, 4697−4703.

(80) Takiugami, H.; Taniguchi, N.; Matsuda, T.; Yamada, M.; Shimizu, Y.; Matsui, S. The fate and behavior of human estrogens in a night soil treatment process. *Water Sci. Technol.* **2000**, *42*, 45−49.
(81) Larsson, D. G. J.; Adolfsson-Erici, M.; Parkkonen, J.; Pettersson, M.; Berg, A. H.; Olsson, P. E.; Forlin, L. Ethinyloestradiol - an undesired fish contraceptive? *Aquat. Toxicol.* **1999**, *45*, 91−97.
(82) Ainsworth, L.; Common, R. H.; Carter, A. L. Chromatographic study of some conversion products of estrone-16-C14 in urine and feces of laying hen. *Can. J. Biochem. Physiol.* **1962**, *40*, 123−135.
(83) Common, R. H.; Mathur, R. S.; Mulay, S.; Henneberry, G. O. Distribution patterns of in vivo conversion products of injected estradiol-17$\beta$-4-14C and estrone-4-14C in urines of nonlaying and laying hen. *Can. J. Biochem.* **1969**, *47*, 539−545.
(84) Ivie, G. W.; Christopher, R. J.; Munger, C. E.; Coppock, C. E. Fate and residues of [$_4$C$^{14}$] estradiol-17$\beta$ after intramuscular injection into holstein steer calves. *J. Anim. Sci.* **1986**, *62*, 681−690.
(85) Herschler, R. C.; Olmsted, A. W.; Edwards, A. J.; Hale, R. L.; Montgomery, T.; Preston, R. L.; Bartle, S. J.; Sheldon, J. J. Production responses to various doses and rations of estradiol benzoate and trenbolone acetate implants in steers and heifers. *J. Anim. Sci.* **1995**, *73*, 2873−2882.
(86) Callantine, M. R.; Stob, M.; Andrews, F. N. Fecal elimination of estrogens by cattle treated with diethylstilbestrol and hexestrol. *Am. J. Vet. Res.* **1961**, *22*, 462−465.
(87) Bushěe, E. L.; Edwards, D. R.; Moore, P. A. Quality of runoff from plots treated with municipal sludge and horse bedding. *Trans. ASAE* **1998**, *41*, 1035−1041.
(88) Nichols, D. J.; Daniel, T. C.; Edwards, D. R.; Moore, P. A.; Pote, D. H. Use of grass filter strips to reduce 17 beta-estradiol in runoff from fescue-applied poultry litter. *J. Soil Water Conserv.* **1998**, *53*, 74−77.
(89) Peterson, E. W.; Davis, R. K.; Orndorff, H. A. 17$\beta$-estradiol as an indicator of animal waste contamination in mantled karst aquifers. *J. Environ. Qual.* **2000**, *29*, 826−834.
(90) Colucci, M.; Bork, H.; Topp, E. Persistence of estrogenic hormones in agricultural soils: I. 17 beta-estradiol and estrone. *J. Environ. Qual.* **2001**, *30*, 2070−2076.
(91) Colucci, M.; Topp, E. Persistence of estrogenic hormones in agricultural soils: II. 17α-ethynylestradiol. *J. Environ. Qual.* **2001**, *30*, 2077−2080.
(92) Schlenker, G.; Muller, W.; Glatzel, P. S. Analysis for the stability of sexual steroids in feces of cows over 12 weeks. *Berl. Muench. Tieraerztl. Wochenschr.* **1998**, *111*, 248−252.
(93) Hemmings, S. N. J.; Hartel, P. G. Mineralization of hormones in breeder and broiler litters at different water potentials and temperatures. *J. Environ. Qual.* **2006**, *35*, 701−706.
(94) Danielsson, L. G.; Zhang, Y. H. Methods for determining *n*-octanol-water partition constants. *Trends Anal. Chem.* **1996**, *15*, 188−196.
(95) Johnson, K. The partitioning of natural and synthetic oestrogens between aqueous and solid phases, M.Sc. Dissertation, Imperial College London, U.K., 1999.

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