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Anaerobic Transformation Kinetics and Mechanism of Steroid Estrogenic Hormones in Dairy Lagoon Water

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Supporting Information

ABSTRACT: Wastewater from concentrated animal feeding operations (CAFOs) frequently contains high concentrations of steroid estrogenic hormones. Release of these hormones into the environment may occur when CAFO wastewater is applied to agricultural lands as a nutrient and water source for crop production. To assess the potential risk of hormone contaminants derived from animal wastewater, we investigated the transformation kinetics and mechanisms of three natural estrogenic hormones (17α-estradiol, 17β-estradiol, and estrone) in aqueous solutions blended with dairy lagoon water under anaerobic conditions. Initial transformations of the three hormones in the dairy lagoon water were dominated by biodegradation and the degradation rates were temperature-dependent. The total amounts of hormones (initial concentration at 5 mg L^{-1}) remaining in the solution after 52 days at 35 °C accounted for approximately 85%, 78%, and 77% of the initial amounts of 17α-estradiol, 17β-estradiol, and estrone, respectively. This observation suggests that these hormones are relatively stable over time and may accumulate in anaerobic or anoxic environments and anaerobic CAFO lagoons. A racemization reaction between 17α-estradiol and 17β-estradiol via estrone was observed in aqueous solutions in the presence of CAFO wastewater under anaerobic conditions. The initial hormone concentrations did not affect this degradation mechanism. A reversible reaction kinetic model was applied to fit the observed transformation dynamics. The degradation and regeneration of the parent hormone and its metabolites were successfully simulated by this model. The information in this study is useful for assessing the environmental risk of steroid hormones released from CAFO wastewater and to better understand why these hormone contaminants persist in many aquatic environments.

INTRODUCTION

As the scarcity of water supplies grows, wastewater reclamation offers an essential and viable water management solution.[1] It is commonplace to reuse treated municipal wastewater for landscape irrigation. The reuse of concentrated animal feeding operation (CAFO) wastewater on agricultural fields is another feasible and economic water recycling strategy,[2] which can provide nutrients and organic matter for plant growth and offer an alternative water source to reduce the demand for high quality water. However, water derived from CAFOs usually retains many contaminants such as excess amounts of nutrients,[3,4] salinity,[5−7] pathogens,[7,8] heavy metals,[9,10] and organics (e.g., animal hormones and veterinary pharmaceuticals),[11−15] posing a potential risk both to the receiving ecosystems and drinking water resources.

Current management and regulations for CAFO wastewater reuse on agricultural lands are primarily focused on nutrients.[2] Emerging contaminants such as veterinary antibiotics and hormones in CAFO wastewater are currently unregulated and their potential adverse impacts on environmental resources and public health are poorly understood.[2,7] Unlike municipal wastewater, which is treated to remove most contaminants, CAFO wastewater receives no additional treatment before land application. Therefore, CAFOs are attracting extensive attention as an important source for the release of these emerging contaminants into the environment.[13−16]

Dairy farms are one of the most important CAFOs. It has been estimated that dairy cattle contributes 45 t of endogenous hormones annually, accounting for approximately 90% of estrogen excretion by livestock in the United States.[17] Hormones excreted from livestock species occur as free steroids, or sulfate or glucuronide conjugates. Free steroid estrogenic hormones are classified as highly potent endocrine-disrupting chemicals (EDCs), whereas most conjugated forms of these hormones are biologically inactive. In vitro studies have shown that low concentrations of steroid estrogenic hormones, even at levels as low as ng L^{-1}, can adversely affect the reproductive biology of aquatic wildlife.[18−20] High concen-

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trations of three natural estrogenic hormones, 17α-estradiol, 17β-estradiol, and estrone, are often detected in dairy manure and manure-containing wastewater.[2,12,14−16] Although all naturally occurring hormones are readily degradable in waste disposal systems,[15] significant quantities of undegraded hormones and their metabolites are introduced to soils from land application of livestock wastes[21] or from cattle feedlots by runoff.[22]

Sorption and degradation are two primary processes affecting the fate and transport of steroid hormones in the environment once they enter the soil. The sorption of hormones has been extensively studied in a variety of soils and sediments.[23−28] It has been reported that the sorption potential of steroid hormones are moderate to high depending on the fraction of organic matter content.[24,28] Batch equilibration experiments have shown that equilibrium times range from several hours to several days.[27,29] Rapid biodegradation of hormones in soils affects their sorption processes, which may account for the highly variable equilibrium times. Most hormones exhibit relatively short half-lives in a variety of environmental media including agricultural soils,[24,30,31] sewage sludge,[32,33] and sediments.[34] For example, the half-lives of 17α-estradiol, 17β-estradiol, and estrone in activated sludge and agricultural soils are often less than one day.[24,31,33] The degradation rates are also related to the hormone compounds. In a silt loam soil, estrone was found to be relatively recalcitrant to degradation compared to the two estradiol isomers.[30] In addition, the degradation rates of hormones are much higher under aerobic conditions compared to anaerobic incubation.[33−35] Previous studies have shown that both estradiol hormones can be readily oxidized to estrone under aerobic conditions and that estrone can be further degraded in the environment.[24,31,32] Thus, aerobic biodegradation is the major removal mechanism for these steroid hormones in the environment.

The release of steroid hormones into agricultural soils is usually associated with manure, manure-contaminated wastewater, or other biosolids. Addition of organic materials into soil has a significant impact on the soil biota, for example, increasing the soil organic carbon content, altering the soil microbial community structure, and improving microbial activity. Accordingly, it may also affect the transformation and transport processes of hormones in agricultural soils. Previous studies reported that animal manure amendment increased the sorption and biodegradation of 17β-estradiol and estrone in soils.[36,37] Given the rapid degradation and high affinity in manure amended soils, these estrogenic hormones were not expected to be released to surrounding water bodies. However, these hormones are frequently detected in tile drainage and ditch water from the agricultural fields receiving livestock wastes,[21] which indicates that their persistence in the environment may be underestimated. The objective of this study is to investigate the mechanisms and kinetics of anaerobic transformation for three steroid estrogenic hormones in dairy lagoon water. The study provides a better understanding of the persistence of hormones in the environment and will help to address some of the knowledge gaps about the degradation of estrogenic hormones in CAFO wastewater.

MATERIALS AND METHODS

Chemicals and Dairy Lagoon Water. The hormones 17α-estradiol, 17β-estradiol, and estrone were purchased from the Sigma-Aldrich Chemicals (St. Louis, MO) at the highest possible purity (>98%). Stock solutions of these estrogenic hormones (1.0 mg mL^{-1}) were prepared in methanol. Deionized water was supplied by a Barnstead E-pure purification system (Dubuque, IA). Other reagent chemicals were obtained from Fisher Scientific (Fair Lawn, NJ). All chemical reagents were used as received. All glassware was autoclaved prior to use.

The dairy lagoon water was collected from a dairy farm located in San Jacinto, California (CA). The farm has three large anaerobic lagoons, which are sequentially connected to store manure-contaminated water from stormwater runoff and wastewater derived from the milking parlor. The lagoon water used in this study was collected from the outlet of the tertiary lagoon at a depth of about 15 cm below the surface using a stainless steel bucket. The collected water samples were stored in 4 L solvent bottles and immediately transported to the laboratory in an ice cooler. Detailed information concerning the lagoon water can be found in our previous research.[15] The oxidation−reduction potential of dairy lagoon water is reported to be −277 ± 24 mV.[2] Laboratory analysis revealed that the total concentration of steroid hormones in this tertiary lagoon water was less than 5 ng L^{-1}.[15] The lagoon water to be used for anaerobic incubations was passed through a 2.0 μm filter to remove visible particles, flushed with nitrogen gas for 1 h, and sealed tightly. The dairy lagoon water was stored at 4 °C overnight, then thawed gradually to room temperature and used within 24 h.

Experimental Systems. To investigate the transformation processes of hormones in CAFO lagoon water, kinetic experiments were conducted in amber glass bottles with Teflon-lined screw caps under anaerobic conditions. Generally, lagoon water needs to be diluted using surface water and groundwater prior to its irrigation.[2] Similar to lagoon water application practice, the experimental solutions were prepared by thoroughly mixing the dairy lagoon water and deionized water (1:1 by volume) within an anaerobic glovebag. The aqueous solutions blended with lagoon water were purged with nitrogen gas for 30 min prior to use.

All solutions were preconditioned at a selected temperature for 1 day and were then spiked with stock hormone solutions, yielding an initial hormone concentration of 5 mg L^{-1}. Similar hormone concentration has also been chosen in previous studies.[28,38,39] All solution bottles were vigorously shaken and then incubated at 15, 25, 35, and 45 °C in the dark. At regular time intervals, aliquots of incubation solutions were withdrawn from each bottle and immediately transferred into a centrifuge tube containing an equal volume of methanol within the anaerobic glovebag. The samples were vortexed for 5 min at room temperature for extraction. The tubes were centrifuged at 4000 rpm for 10 min and then filtered through a 0.45 μm membrane (Iso-Disc, PTFE, Supelco, Bellefonte, PA) using a syringe. All filtrate samples were stored in a freezer (−21 °C) until analysis. Preliminary experiments revealed that the addition of methanol could immediately quench hormone transformation and effectively extract hormones that were sorbed to suspended manure in the lagoon water. The recoveries of the three estrogenic hormones ranged from 95 to 105% in aqueous solutions blended with 50% dairy lagoon water.

A control experiment was concurrently performed using a sterile lagoon water sample to determine abiotic degradation and thereby deduce the effect of biodegradation in nonsterile samples. Briefly, the solutions blended with lagoon water (lagoon water: deionized water, 1:1 by volume) were

autoclaved twice at 120 °C, each for 60 min within a 24 h interval. The same procedures described above were performed including solution preparation, incubation, sampling, extraction, and analysis of steroid hormones. All experiments were carried out in triplicate.

Analytical Methods. To determine the transformation kinetics of three estrogenic hormones, filtrate samples were analyzed using an Agilent 1100 series high performance liquid chromatography (HPLC) with a diode array detector (DAD) (Agilent Technologies, Palo Alto, CA). Separation for HPLC/DAD analysis was performed using an Eclipse C18 column (250 × 4.6 mm i.d., particle size 5 μm). The mobile phase consisted of acetonitrile/water (50:50, v/v), the flow rate was 1.0 mL min^{-1}, and the detector wavelength was 205 nm. Under these conditions, the retention times for 17β-estradiol, 17α-estradiol, and estrone were 6.5, 7.7, and 9.2 min, respectively. The detection limits of the method were 0.05, 0.05, and 0.08 mg L^{-1} for 17β-estradiol, 17α-estradiol, and estrone, respectively.

The biodegradation products of hormones were analyzed using an Agilent 1100 series HPLC/DAD in tandem with a mass spectrometer (MS) equipped with an electrospray ionization (ESI) source. The biodegradation products of hormones were identified using the HPLC/DAD method described above by matching retention times to their corresponding reference standards, and then quantified by external calibration. The identities of biodegradation products were further confirmed by LC/MS. LC/MS total ion current (TIC) chromatograms were recorded between m/z 100 and 500 at a rate of 2 scans per second. The negative polarity ionization mode was operated to obtain mass spectra for the identification of transformation products of estrogenic hormones. The electrospray source parameters were optimized by infusion of hormone standard solutions. The operating conditions for ESI were capillary voltage 4000 V for positive mode, drying gas (nitrogen) flow rate 10 L min^{-1} at 300 °C, and nebulizer gas pressure 45 psi.

Transformation of Hormones at Low Concentration. Additional experiments were conducted to validate the transformation of hormones at environmentally−relevant concentrations. This study employed dairy lagoon water from Champaign, Illinois (IL) and incubation solutions at an initial hormone concentration of 5 μg L^{-1}. Details for these experiments are described in the Supporting Information (SI). Specific chemical properties of this dairy wastewater are provided in the Table S1 of the SI. New analytic procedures had to be developed to determine the low hormone concentrations in this study. Details of this analytical method are summarized in Table S2 and Figure S1 of the SI.

RESULTS AND DISCUSSION

Initial Degradation of Three Estrogenic Hormones. The first transformation study focused on the initial degradation of the three steroid hormones (i.e., within 24 h for 17α-estradiol and 17β-estradiol, and 72 h for estrone) in aqueous solutions blended with dairy lagoon water. Time courses for degradation of each estrogenic hormone (initial concentration at 5 mg L^{-1}) at different temperatures are shown as semilogarithmic plots in Figure 1. No discernible hormone degradation occurred in control experiments conducted in sterile solutions mixed with the lagoon water over comparable time periods (Figure 1). The result indicates that the initial transformations of the three hormones in blended CAFO wastewater were dominated by biological degradation and that abiotic degradation such as hydrolysis was negligible.

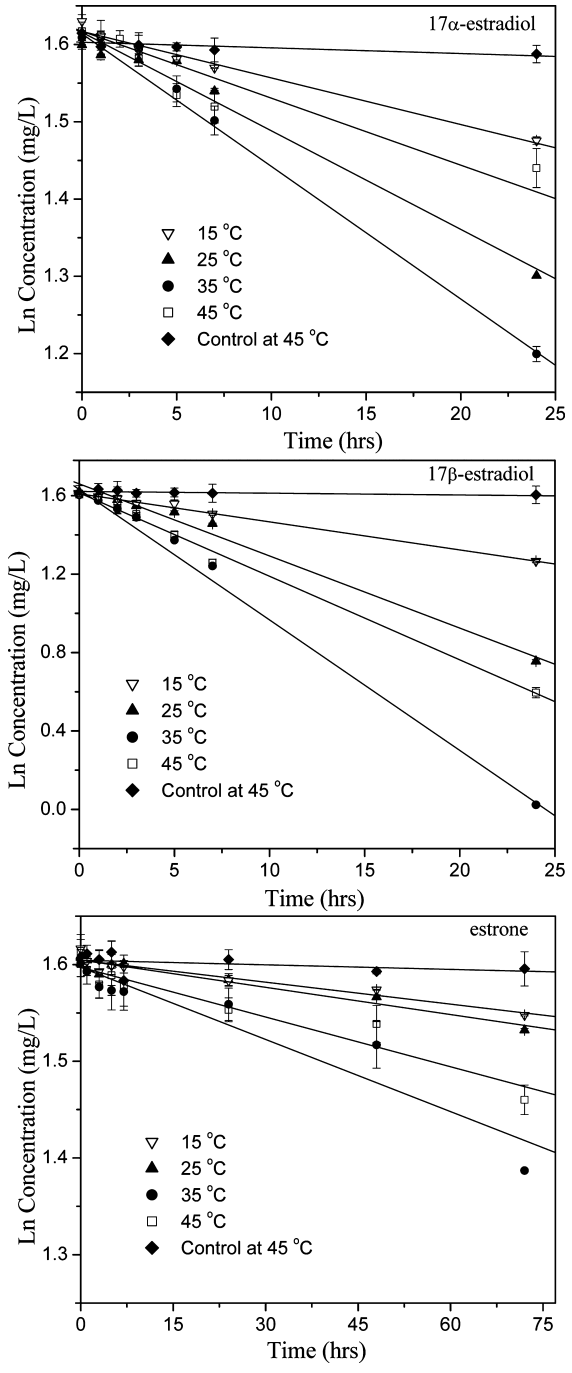

Figure 1. Time courses for initial degradation of three estrogenic hormones in aqueous solutions blended with dairy lagoon water at different temperatures under anaerobic conditions. The initial concentration of each investigated steroid hormone was 5 mg L^{-1}. Error bars represent standard deviations of triplicate samples.

For the initial degradation of three hormones at different temperatures, the biodegradation rate can be represented by a *pseudo*-first-order kinetic model:

$$\frac{d[C]}{dt} = -k_i[C] \quad (1)$$

Upon rearrangement and integration, eq 1 becomes

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Table 1. Initial *Pseudo*-First-Order Biodegradation Rate Constants (h^{-1}) and Correlation Coefficient (r) of Three Hormones in Aqueous Solutions Blended with Dairy Lagoon Wastewater at Different Temperatures[a]

	17α-estradiol		17β-estradiol		estrone	
temperature	k_i	r	k_i	r	k_i	r
15 °C	0.60 (±0.04) × 10^{-2}	0.991	1.43 (±0.05) × 10^{-2}	0.997	0.68 (±0.08) × 10^{-3}	0.963
25 °C	1.28 (±0.10) × 10^{-2}	0.988	3.68 (±0.19) × 10^{-2}	0.994	1.06 (±0.07) × 10^{-3}	0.990
35 °C	1.72 (±0.05) × 10^{-2}	0.998	6.75 (±0.22) × 10^{-2}	0.997	2.49 (±0.34) × 10^{-3}	0.950
45 °C	0.73 (±0.12) × 10^{-2}	0.938	4.30 (±0.15) × 10^{-2}	0.997	1.71 (±0.20) × 10^{-3}	0.962

[a]The initial concentration of each investigated steroid hormone was 5 mg L^{-1}.

$$\ln([C]) = -k_i t + \ln([C]_0) \quad (2)$$

where k_i is the initial biodegradation rate constant of the investigated hormone under a certain temperature, [C] is the concentration of the hormone, and $[C]_0$ is the initial concentration of the hormone in incubation solutions. Values of k_i were calculated from the slope of semilogarithmic plots of hormone concentration versus time. These plots fit a log−linear model well for the degradation of all three hormones during the initial experimental time period (Figure 1). Details pertaining to the initial biodegradation rate constants of three hormones under different incubation temperatures are summarized in Table 1.

The effect of temperature on the initial biodegradation rates of the three hormones in blended CAFO wastewater is shown in Figure 2. For all hormones, the biodegradation rate increased

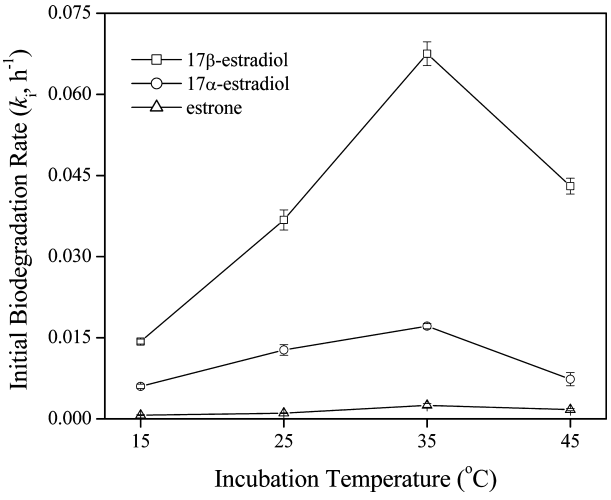

Figure 2. Effect of incubation temperature on the initial biodegradation rates of three hormones in aqueous solutions blended with dairy lagoon water under anaerobic conditions.

with increasing temperature from 15 to 35 °C, but then decreased when the incubation temperature was elevated to 45 °C. It appears that the most suitable temperature for hormone biodegradation in this study is around 35 °C, which is close to the well-known optimum temperature (~37 °C) for the growth of active microorganisms in various environmental media. Yang et al.[35] reported that the testosterone degradation at 37 °C was much faster than that at room temperature (i.e., 22 °C), because many fecal-derived enzymes have optimal activity at physiological temperature. A similar effect of temperature was observed for the biodegradation kinetics of ceftiofur in CAFO wastewater.[40]

Statistically significant differences in k_i were observed for the three hormones investigated for each incubation temperature. The initial biodegradation rate of the three hormones decreases in the following order: 17β-estradiol >17α-estradiol > estrone (Figure 2 and Table 1). This observation is consistent with a previous study, in which the degradation of these estrogenic hormones was conducted in soil.[31] For instance, the degradation rates of 17β-estradiol and 17α-estradiol were approximately 35 and 12-fold higher than estrone in aqueous solutions blended with the dairy lagoon water and incubated at 25 °C, respectively. The significant difference in the biodegradation rates of these hormones is attributed to subtle variations in their molecular structure.

Transformation Mechanism and Pathway at 35 °C under Anaerobic Conditions. Following the initial kinetic study, longer-term transformation experiments were conducted for 52 days at 35 °C for the three hormones with an initial concentration at 5 mg L^{-1}. The concentration of each estrogenic hormone is plotted as a function of time in Figure 3. The same transformations were observed for the three hormones in studies conducted at their low initial concentrations under anaerobic conditions at 35 °C (SI, Figure S2). Relevant information is reported in the SI for studies conducted at an initial hormone concentration of 5 μg L^{-1}. This section primarily focuses on discussing the transformation studies conducted at an initial hormone concentration of 5 mg L^{-1}.

To elucidate the anaerobic degradation pathways for the three hormones (initial concentration at 5 mg L^{-1}) in blended CAFO wastewater, solution aliquots were periodically withdrawn and analyzed by HPLC/DAD/MS. A representative HPLC/DAD chromatogram exemplifying the hormone transformation process is shown in Figure S3 of the SI. Peak identification was concurrently performed by LC/MS. To better identify the transformation products, three hormone standards were run to verify chromatographic separation and mass spectra with desired fragmentations.

The initial loss of 17α-estradiol was accompanied by an accumulation of a degradation product over the first two days (Figure 3a). This product was characterized as estrone according to the analysis of its mass spectrum (SI, Figure S4-a) and retention time. This observation is consistent with previous reports that estrone is the major degradation product of 17α-estradiol.[24,31−33] Interestingly, another product identified as 17β-estradiol was detected in the degradation process of 17α-estradiol after two days. 17α-Estradiol and 17β-estradiol were observed with the same mass spectra (SI, Figure S4-b) but at different retention times on the chromatogram (SI, Figure S3). This provides an explanation for why both estradiol isomers have been detected in dairy wastes,[15] although cow species only excrete 17α-estradiol.[12] A recent study investigated the degradation of 17α-estradiol in the feedlot surficial soil under simulated rainfall. It also showed that a concentration decrease of 17α-estradiol was accompanied by an equivalent increase in estrone and 17β-estradiol.[22]

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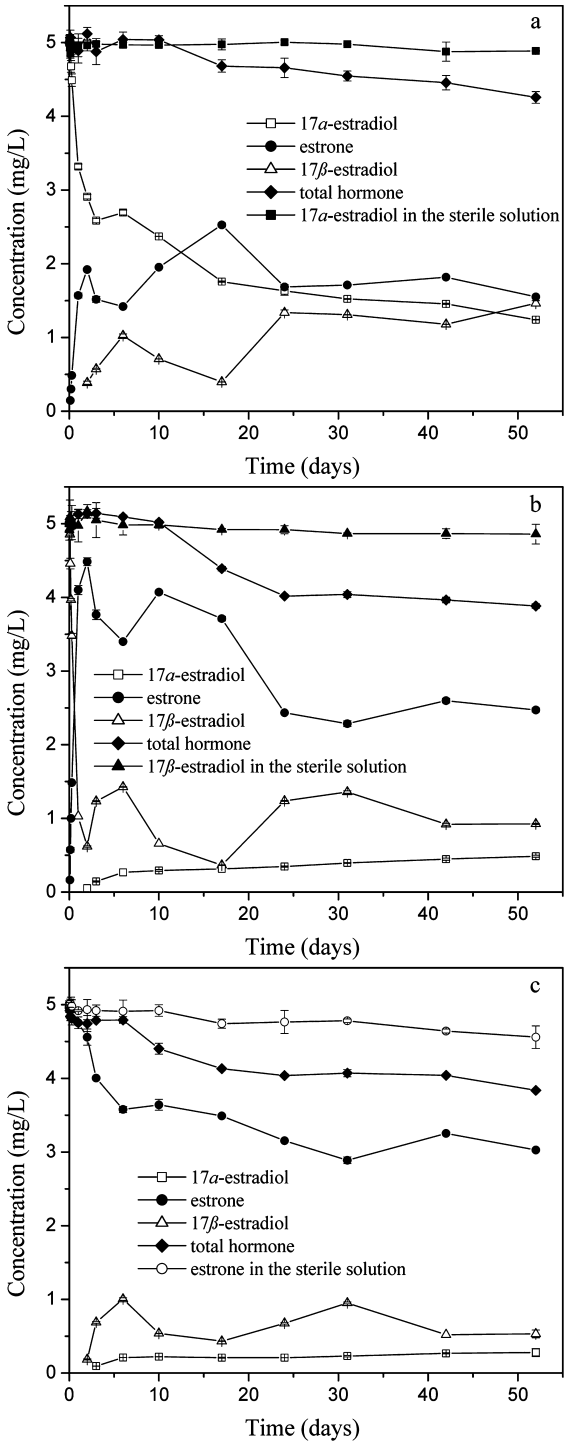

Figure 3. Anaerobic transformation of investigated hormones and formation of their degradation products in aqueous solutions mixed with dairy lagoon water at 35 °C: (a) 17α-estradiol; (b) 17β-estradiol; and (c) estrone. The initial concentration of each investigated steroid hormone was 5 mg L^{-1}. Standard deviation of triplicate samples is shown as error bars.

Similarly, estrone was immediately detected following the rapid degradation of 17β-estradiol in aqueous solutions containing dairy lagoon water (Figure 3b). The rate of estrone formation equaled the rate of 17β-estradiol consumption within the first two days indicating that it is the primary transformation product. The formation of estrone achieved a maximum when the lowest concentration of 17β-estradiol was detected in the solution after two days. The concentration of estrone then decreased with a concurrent formation of a new product—17α-estradiol, inferring that estrone was subsequently transformed to 17α-estradiol with further incubation. These two products were further confirmed using their authentic standards. Simultaneously, the concentrations of 17β-estradiol were surprisingly observed to transiently increase for 4 days, decrease over the subsequent 10 days, and then accumulate again (Figure 3b). By contrast, the time course of the major product estrone in the transformation process had a reversed change pattern compared to that of 17β-estradiol (Figure 3b). Similar degradation and formation patterns were also observed for 17α-estradiol and its degradation products (Figure 3a). These results indicate that 17α-estradiol and 17β-estradiol could be readily oxidized to estrone in aqueous solutions containing dairy wastewater, and the latter could be reduced back to both estradiol isomers under anaerobic conditions.

The transformation study of estrone in solution blended with dairy lagoon water further confirmed that both estradiol isomers were formed in its degradation process (Figure 3c). A reversible transformation pathway related to the three hormones is illustrated in Figure 4. The racemization reaction

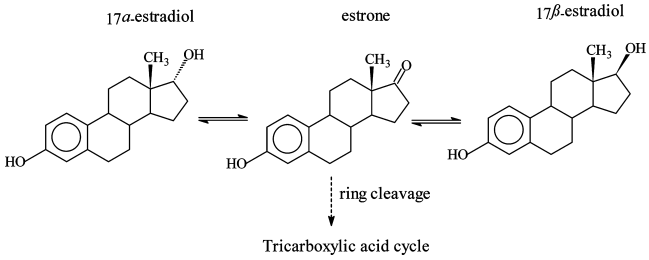

Figure 4. Reversible transformation pathway among three hormones in aqueous solutions blended with dairy lagoon water under anaerobic conditions.

between 17α-estradiol and 17β-estradiol via estrone has been suggested in previous reports.[15,34] The reversible transformation among the three estrogenic hormones under anaerobic conditions suggests that these compounds may persist in an anoxic aquatic setting, such as anaerobic CAFO lagoons and underlying sediments. For the studies conducted at an initial hormone concentration of 5 μg L^{-1}, the same reversible transformation pathway was observed under anaerobic conditions. Relevant information is provided in the SI.

The total hormone contents with time in blended CAFO wastewater under anaerobic conditions are shown in Figure 3 and Figure S2 of the SI. Throughout the test period, a small reduction in total hormone content was observed for both hormone degradation experiments conducted at 5 mg L^{-1} and 5 μg L^{-1}. For instance, the total hormones (initial concentration at 5 mg L^{-1}) remaining in the solutions after 52 days at 35 °C were 3.85, 3.90, and 4.26 mg/L, which corresponded to about 85%, 78%, and 77% of initial spiked amounts of 17α-estradiol, 17β-estradiol, and estrone, respectively. Also, the control experiments conducted in the sterile solutions revealed that the abiotic degradation of three hormones was minimal (Figure 3 and Figure S2 of the SI). These results suggest there should be other degradation pathways in addition to the reversible transformation and/or estrone could be further degraded. Previous studies revealed

Table 2. Anaerobic Transformation Rate Constants and Correlation Coefficient (r) of Hormones at 35 °C Calculated from their Consecutive Reversible First-Order Reactions Shown in eq 3

investigated hormone	k_1 (d^{-1})	k_{-1} (d^{-1})	k_2 (d^{-1})	k_{-2} (d^{-1})	r^2
17α-estradiol	0.414 ± 0.085	0.422 ± 0.111	0.064 ± 0.026	0.077 ± 0.042	0.974
17β-estradiol	1.518 ± 0.294	0.455 ± 0.135	0.010 ± 0.008	0.021 ± 0.048	0.943
estrone[a]	0.076 ± 0.077	0.013 ± 0.001	0.058 ± 0.021	0.215 ± 0.096	0.992

[a]When estrone served as the source hormone, k_1 and k_{-1} denoted transformation rate constants between estrone and 17α-estradiol; k_2 and k_{-2} represented transformation rate constants between estrone and 17β-estradiol. The initial concentration of each investigated hormone was 5 mg L^{-1}.

that estriol was one of main metabolites during estrone degradation,[14,31] but not shown in this study. Additionally, this study did not detect any other likely metabolites, presumably because estrone can be quickly degraded through a ring cleavage and mineralized to carbon dioxide by a tricarboxylic acid cycle (Figure 4).[32,41] Thus, further work is needed to elucidate all degradation pathways and mechanisms, although they are minor compared to the reversible transformation of the three hormones that occurred in this experimental system.

Transformation Kinetics of the Three Estrogenic Hormones at 35 °C under Anaerobic Conditions. As shown in Figure 4, the anaerobic transformation of the three estrogenic hormones in aqueous solutions blended with dairy lagoon water involved two consecutive reversible first-order reactions:

$$\mathrm{A} \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} \mathrm{B} \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} \mathrm{C} \tag{3}$$

The differential rate equations are

$$\frac{dC_A}{dt} = -k_1 C_A + k_{-1} C_B \tag{4}$$

$$\frac{dC_B}{dt} = k_1 C_A - (k_{-1} + k_2) C_B + k_{-2} C_C \tag{5}$$

$$\frac{dC_C}{dt} = k_2 C_B - k_{-2} C_C \tag{6}$$

where C_A is the concentration of the investigated hormone (17α-estradiol or 17β-estradiol), C_B is the concentration of estrone, C_C is the concentration of transformation product (17β-estradiol or 17α-estradiol), k_1 and k_2 are the transformation rate constants denoting the forward steps, and k_{-1} and k_{-2} are the reversible transformation rate constants denoting the backward steps.

These differential equations with constant coefficients can be solved using Laplace transform theory.[42] After taking the Laplace transform of eqs 4−6, rearranging and obtaining the inverse Laplace transform, the appropriate solutions to eqs 4−6 are given as follows, respectively

$$C_A(t) = \left[\frac{C_o k_{-1} k_{-2}}{\alpha\beta} + e^{-\alpha t} \frac{C_o k_{-1} k_{-2} - \alpha[C_{Bo} k_{-1} + C_{Ao}(m - \alpha)]}{\alpha(\alpha - \beta)} - e^{-\beta t} \frac{C_o k_{-1} k_{-2} - \beta[C_{Bo} k_{-1} + C_{Ao}(m - \beta)]}{\beta(\alpha - \beta)}\right] \tag{7}$$

$$C_B(t) = \left[\frac{C_o k_1 k_{-2}}{\alpha\beta} + e^{-\alpha t} \frac{C_o k_{-2}(k_1 - \alpha) + \alpha[C_{Ao}(k_{-2} - k_1) + C_{Bo}(\alpha - k_1)]}{\alpha(\alpha - \beta)} - e^{-\beta t} \frac{C_o k_{-2}(k_1 - \beta) + \beta[C_{Ao}(k_{-2} - k_1) + C_{Bo}(\beta - k_1)]}{\beta(\alpha - \beta)}\right] \tag{8}$$

$$C_C(t) = \left[\frac{C_o k_1 k_2}{\alpha\beta} + e^{-\alpha t} \frac{\alpha[C_{Ao}(\beta - k_{-2}) - C_{Bo}(k_2 + k_{-2} - \beta)] + C_o k_{-2}(k_2 + k_{-2} - \beta)}{\alpha(\alpha - \beta)} - e^{-\beta t} \frac{C_o k_{-2}(k_2 + k_{-2} - \alpha) - \beta[C_{Ao}(k_{-2} - \alpha) + C_{Bo}(k_2 + k_{-2} - \alpha)]}{\beta(\alpha - \beta)}\right] \tag{9}$$

where

$$\alpha + \beta = k_1 + k_{-1} + k_2 + k_{-2} \tag{10}$$

$$\alpha\beta = k_1 k_2 + k_1 k_{-2} + k_{-1} k_{-2} \tag{11}$$

$$m = k_{-1} + k_2 + k_{-2} \tag{12}$$

and C_{Ao}, C_{Bo}, and C_{Co}, respectively, are the initial concentrations of $[C_A]$, $[C_B]$, and $[C_C]$. The mass balance relationship for three differential equations in the process of hormone transformation is $C_T = C_A + C_B + C_C$, where C_T is the total hormone concentration in solution.

The rate constants of the transformation reactions for the three hormones exhibited in eq 3 were obtained by optimizing the fit of the analytic solution to the corresponding concentration−time data using nonlinear regression. The reversible first-order kinetic model produced a good fit to the data ($r > 0.97$). Table 2 lists the transformation rate constants at 35 °C for each spiked hormone (initial concentration at 5 mg L^{-1}) in blended dairy lagoon water under anaerobic conditions. The transformation rate constants for 17α-estradiol or 17β-estradiol from the reversible kinetic model (k_1) were close to their initial degradation rate constants (Table 1). Similarly, the initial biodegradation rate constant of estrone nearly equals the sum of its transformation rate constant to both estradiol isomers. This result indicates that the transformation kinetics of the three hormones can be well-described by eqs 7−9. Additionally, the rate constant for the transformation from estrone to 17β-estradiol was approximately 4.5-fold higher than that of the conversion from estrone to 17α-estradiol. This result is in agreement with the formation of both estradiol isomers during the degradation of estrone, in which the formation concentration of 17β-estradiol was higher than 17α-estradiol (Figure 3c).

Similarly, simulations based on the reversible first-order reactions provided a good description of the data for studies

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conducted at an initial hormone concentration of 5 $\mu g\ L^{-1}$ ($r > 0.97$) (SI, Table S2). The effect of initial hormone concentrations on the transformation rates is provided in the SI.

Environmental Significance. In this study, a reversible transformation process among 17α-estradiol, 17β-estradiol, and estrone is clearly illustrated in the blended dairy lagoon water. The racemization of the two estradiol isomers readily occurred under anaerobic conditions due to the reduction of the ketone group of estrone to the hydroxyl group of 17α-estradiol or 17β-estradiol (Figure 4). 17α-Estradiol and 17β-estradiol have been suggested as principal indicators to distinguish different livestock sources of estradiol in environmental samples, since cattle primarily excretes 17α-estradiol while 17β-estradiol mainly occurs in the excreta of swine or poultry.[12] However, the stereotransformation of the two estradiol isomers through estrone may result in inaccurate source apportionment analyses if they are used to identify the livestock species contributing to waterway contamination.[14] More importantly, this transformation mechanism may alter the endocrine-disrupting activity of dairy lagoon water because 17β-estradiol and estrone have much higher estrogenic potency compared to 17α-estradiol. For example, a toxicological study on aquatic species revealed that 17β-estradiol is generally several orders of magnitude more potent than 17α-estradiol.[43] Therefore, the alteration of the biological activity resulting from the reversible transformation of the three estrogenic hormones in CAFO lagoons should be considered when addressing their potential adverse impact on the environment.

Results from this study demonstrate that the total amount of hormones slowly decreased in the aqueous solutions containing animal wastewater under anaerobic conditions (Figure 3), although the three estrogenic hormones could be readily transformed during their initial degradation processes (Table 1). It can be surmised that these estrogenic hormones will persist and possibly accumulate in anaerobic or anoxic environments. For instance, aquatic sediments and groundwater have the potential to be a reservoir for these endocrine chemicals, especially when they are anoxic.[34] This finding is noteworthy because it may help explain why estrogenic hormones are frequently detected in many natural aquatic systems. By contrast, aeration is very effective at eliminating hormone contaminants because the biodegradation of these endocrine chemicals under aerobic conditions is very rapid.[44,45] Unlike aerobic ponds, CAFO lagoons typically function as anaerobic reactors. Moreover, CAFO wastewater does not require additional treatment as long as it does not discharge directly into surface waters. This suggests that steroid hormones frequently detected in aquatic systems may be primarily attributable to anaerobic animal wastewater. Further research is needed to better understand the contamination potential related to releases of steroid hormones when reusing CAFO lagoon water on agricultural lands.

■ ASSOCIATED CONTENT

Supporting Information

Detailed description of transformation of hormones at a low concentration (5 $\mu g\ L^{-1}$) and a high concentration (5 $mg\ L^{-1}$), main compositions and hormone concentrations of the dairy wastewater, MRM setting and chromatograms of hormones and their degradation products, anaerobic transformation rate constants of hormones at the initial concentration of 5 $\mu g\ L^{-1}$, plots of degradation of hormones and formation of their metabolites, an HPLC/DAD chromatogram showing three estrogenic hormones, and electrospray LC/MS mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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