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Fate of estrogen conjugate 17α-estradiol-3-sulfate in dairy wastewater: Comparison of aerobic and anaerobic degradation and metabolite formation

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HIGHLIGHTS

- Investigate the environmental fate of 17α-estradiol-3-sulfate in dairy wastewater.
- Compare aerobic and anaerobic degradation rates of estrogen 17α-estradiol-3-sulfate.
- Explore aerobic and anaerobic degradation mechanisms of the estrogen in wastewater.
- Assess the potential risk of using dairy wastewater for agricultural irrigation.

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ABSTRACT

Irrigation with concentrated animal feeding operation (CAFO) wastewater on croplands has been identified as a major source discharging steroid hormones into the environment. To assess the potential risks on this irrigation practice, the degradation kinetics and mechanisms of 17α-estradiol-3-sulfate were systematically investigated in aqueous solutions blended with dairy wastewater. Dissipation of the conjugated estrogen was dominated by biodegradation under both aerobic and anaerobic conditions. The half-lives for the biodegradation of 17α-estradiol-3-sulfate under aerobic and anaerobic conditions from 15 to 45 °C varied from 1.70 to 415 d and 22.5 to 724 d, respectively. Under the same incubation conditions, anaerobic degradation rates of 17α-estradiol-3-sulfate were significantly less than aerobic degradation rates, suggesting that this hormone contaminant may accumulate in anaerobic or anoxic environments. Three degradation products were characterized under both aerobic and anaerobic conditions at 25 °C, with estrone-3-sulfate and 17α-estradiol identified as primary metabolites and estrone identified as a secondary metabolite. However, the major degradation mechanisms under aerobic and anaerobic conditions were distinctly different. For aerobic degradation, oxidation at position C17 of the 17α-estradiol-3-sulfate ring was a major degradation mechanism. In contrast, deconjugation of the 17α-estradiol-3-sulfate thio-ester bond at position C3 was a major process initiating degradation under anaerobic conditions.

1. Introduction

The occurrence of endocrine-disrupting chemicals (EDCs) in natural waters has been recognized as an emerging environmental issue. Steroid hormones including estrogens, androgens, and gestagens are classified as the most potent EDCs, which are either excreted endogenously from humans and animals or are derived from widespread use in clinical practices. Among known endocrine-disruptors, steroid estrogenic hormones (e.g., estradiol and estrone) possess the highest estrogenic potency compared to other hormones and exogenous EDCs [1,2]. In aquatic environments, even extremely low levels of these steroid estrogens (e.g., ng/L), may interfere with the endocrine systems of a variety of freshwater species, thereby adversely impacting their reproduction and development [3–5].

Synthetic and naturally occurring estrogens have been widely detected in the effluents and sludge of sewage treatment plants (STPs) at concentrations typically ranging from a few parts per trillion to several parts per million [6–9]. It has been well documented that the discharge of STP effluents increases the

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occurrence of steroid hormones and elevates their concentrations in receiving watersheds [7,10–12]. Recently, concentrated animal feeding operations (CAFOs) such as dairy and swine facilities have been identified as another major source of natural hormones and veterinary pharmaceuticals in the environment [13–17]. For example, it has been estimated that approximately 45 t of natural estrogens would be introduced into the environment annually if all dairy wastes were applied on agricultural fields [18].

It is known that estrogen molecules are usually conjugated in human and animal bodies. Steroid estrogens are excreted as either free estrogens or as sulfate or glucuronide conjugates, with the conjugated forms being predominant in the urine of humans and animals [1,19]. Although estrogen conjugates have very low estrogenic potencies, they can readily undergo chemical or enzymatic dissociation and convert into highly active free estrogens [1,19–21]. The occurrence of free estrogens as dominant forms in STP effluent suggests that deconjugation occurs between excretion and sewage effluent discharge [22]. In contrast, another study has shown that estrogen conjugates account for a significant percentage of the total estrogens in various CAFO lagoons [14]. For example, it was estimated that the corresponding contributions of estrogen conjugates in the dairy lagoon samples were 57% of the total estrogen equivalents [14]. These estrogen conjugates may enter the environment once lagoon water is applied to crops. Thus, it is important to determine the environmental fate of estrogen conjugates since they are potential sources of active free estrogens. Additionally, most conjugated estrogens are more polar than the free compounds, suggesting that they may be more mobile in the environment [1].

While considerable research has focused on the fate and behavior of free estrogens in a variety of environmental media, estrogen conjugates have largely been ignored. It has been speculated that fecal microorganisms such as *Escherichia coli* (*E. coli*) are capable of hydrolyzing estrogen conjugates to free estrogen by sulfatase and glucuronidase [1,19,23]. Such microorganisms are present in STPs and therefore are likely to be responsible for estrogen conjugate degradation [19,20,24]. Moreover, degradation rates are also related to the conjugated estrogen species. It has been reported that the estrogen sulfate forms are more recalcitrant to deconjugation than the glucuronide form in STPs [22,24], leading to the more frequent occurrence of the sulfate estrogen conjugates relative to glucuronide estrogen species in STP effluents and receiving watersheds. The aerobic degradation of estrogen conjugates in soils has also been reported [25,26]. Deconjugation of estrone-3-sulfate in agricultural soils leads to the formation of estrone, which is mediated by naturally occurring arylsulfatases present in the soil environment [25]. In contrast, the aerobic degradation of 17β-estradiol-3-sulfate in three soils involves oxidation and deconjugation mechanisms, resulting in the formation of estrone-3-sulfate as a major primary metabolite and 17β-estradiol as a secondary metabolite [26]. The degradation rate constants of 17β-estradiol-3-sulfate across soils were suggested to significantly correlate with arylsulfatase activity. To the best of our knowledge, there is virtually no information on the degradation of estrogen conjugates in CAFO wastewater, especially for the steroid estrogen 17α-estradiol-3-sulfate.

The objective of this study was to investigate the degradation kinetics and mechanisms of 17α-estradiol-3-sulfate in dairy wastewater, and to evaluate the effects of incubation conditions including temperature and oxygen presence on its degradation rates. 17α-Estradiol-3-sulfate appears to be the major steroid estrogen in cattle urine during pregnancy and is likely to act as a precursor to other estrogens (e.g., 17α-estradiol) found in dairy wastes [27]. This estrogen conjugate has been frequently detected in dairy lagoons [14] and dairy shed effluents [28].

2. Materials and methods

2.1. Chemicals

The free estrogens 17α-estradiol and estrone were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA) at the highest available purity (>98%). The conjugated estrogens 17α-estradiol-3-sulfate and estrone-3-sulfate were obtained from Steraloid (Newport, RI, USA). A stock solution of 17α-estradiol-3-sulfate (1.0 mg mL^{-1}) was prepared in methanol. Deionized water (>17.6 MΩ-cm) was supplied by a Labconco Water Pro Plus system (Kansas City, MO, USA). Other reagent chemicals were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All chemical reagents were used as received. All glassware used in this study was baked overnight at 450 °C.

2.2. Dairy wastewater

The fresh dairy wastewater was collected from a University of Illinois dairy farm located in Urbana, IL, USA. This dairy farm has more than 250 milking cows. Large volumes of water are used daily to flush the milking parlor and barns, which generates manure-containing wastewater. This wastewater is treated in a settling pit to remove coarse solids and then stored in a lagoon. The dairy lagoon water is usually blended with surface water and applied to surrounding fields for crop production, when needed. The dairy wastewater used in this study was taken from the outlet adjacent to the lagoon at about 10 cm below the surface using a self-made collector. The sample was stored in a 4-L solvent bottle and immediately transported to the laboratory in an ice-filled cooler. The collected dairy wastewater was passed through a 2.0-μm filter to remove visible particles. The water was stored at 4 °C overnight, then thawed gradually to room temperature and extracted within 24 h of sample collection. The physical–chemical parameters and main composition of the collected dairy wastewater are summarized in Table S1 of Supplementary Information (SI). Four estrogenic hormones were detected in the collected water samples and their concentrations are also shown in Table S1 of the SI.

2.3. Degradation experiments

To investigate the degradation processes of 17α-estradiol-3-sulfate in dairy wastewater, kinetic experiments were conducted in amber glass bottles (250 mL) with Teflon-lined screw caps. First, 1% (by volume) of the dairy wastewater was added to deionized water and mixed thoroughly, yielding incubation solutions with concentrations of all endogenous estrogens less than their detection limits of the method described below. The amber glass bottles were filled with incubation solutions for the anaerobic and aerobic experiments.

For anaerobic degradation experiments, the aqueous solutions blended with dairy wastewater were purged with nitrogen gas for 2 h and then preconditioned at a selected incubation temperature for 1 d. To ensure anaerobic conditions, Na_2S (1.0 mM) was added to the incubation solutions, and the anaerobic degradation experiments were conducted in an anaerobic glove bag. Kinetic experiments were initiated by spiking the stock solution of 17α-estradiol-3-sulfate into the incubation bottles, yielding an initial hormone concentration of 5 mg L^{-1}. All solution bottles were vigorously shaken and then incubated in the dark at various temperatures (15 °C, 25 °C, 35 °C, 45 °C). At regular time intervals, aliquots of incubation solution were withdrawn from each bottle and immediately transferred to a centrifuge tube containing an equal volume of methanol within the anaerobic glove bag. The samples were vortexed for 5 min at room temperature to facilitate extraction, centrifuged at 4000 rpm for 10 min, and then

passed through a 0.45-μm syringe filter (Iso-Disc, PTFE, Supelco, Bellefonte, PA). Filtrates were stored in a freezer (−21 °C) until analysis. All experiments were carried out in triplicate. Preliminary experiments revealed that the addition of methanol immediately quenched hormone degradation and effectively extracted hormones that were sorbed to suspended particles in the wastewater. The recovery of 17α-estradiol-3-sulfate ranged from 98 to 102% in aqueous solutions blended with dairy wastewater using the above-described experimental procedure.

For aerobic degradation experiments, the aqueous solutions blended with dairy wastewater were purged with air for 2 h. Aerobic experiments were carried out entirely under atmospheric conditions. Except for the addition of Na_2S, procedures for aerobic degradation were the same as those for anaerobic degradation, including solution preconditioning, estrogen spiking, incubation, and extraction.

A control experiment was concurrently performed in sterile solutions blended with the dairy wastewater to determine abiotic degradation. Briefly, the solutions blended with 1% dairy wastewater were autoclaved twice for 60 min at 121 °C, with a 24-h interval between autoclaving. Subsequently, experimental procedures followed those used for the anaerobic and aerobic experiments.

2.4. Analysis methods

To determine the degradation of 17α-estradiol-3-sulfate and formation of metabolites, the extracts were analyzed using a Waters 2695 Separations Module high performance liquid chromatograph (HPLC) equipped with a Waters 996 PDA detector. Separation was performed using a Phenomenex Luna C18 column (250 mm × 4.6 mm i.d.; particle size, 5 μm). A gradient elution method was performed with a binary mobile phase consisting of acetonitrile (mobile phase A) and 5.0 mmol/L ammonium sulfate in water (mobile phase B). The following gradient program (with respect to mobile phase A) was used 0–2.0 min, 28% B; 2–3 min, 28–60% B; 3–15 min, 60% B; 15–20 min, re-equilibrate with 28% B. The flow rate was 0.8 mL/min and the detector wavelength was 205 nm. Under these conditions, the retention times for 17α-estradiol-3-sulfate, estrone-3-sulfate, 17α-estradiol, and estrone were 7.6, 8.1, 11.4, and 12.9 min, respectively. The method detection limits were 0.05, 0.08, 0.05, and 0.04 mg L^{-1} for 17α-estradiol-3-sulfate, estrone-3-sulfate, 17α-estradiol, and estrone, respectively.

The degradation products of 17α-estradiol-3-sulfate were confirmed by a Waters liquid chromatograph/tandem mass spectrometer (LC/MS/MS) equipped with an electrospray ionization (ESI) source (Quattro Macro QA1140, Waters). For the HPLC analysis, a Symmetry C18 column (3.5 μm particle size, 2.1 mm × 150 mm, Waters) was used. A gradient method with a binary mobile phase consisting of acetonitrile with 5 mM ammonium hydroxide (mobile phase C) and water with 5 mM ammonium hydroxide (mobile phase D) was used for the separation. Initial mobile phase composition was 10% C and 90% D, which was maintained for 2 min. The mobile phase was ramped up to 95% C and down to 5% D by a linear gradient in 13 min and maintained for 8 min. Afterward, the column was re-equilibrated at 10% C and 90% D for 5.5 min. The flow rate was 0.2 mL/min. The MS/MS was operated in negative ESI mode simultaneously with optimized instrument conditions: source temperature at 120 °C; desolvation gas flow rate at 650 L/min, and capillary voltage at 3.5 kV. Multiple reactions monitoring (MRM) was applied for detection. Optimized collision energies, cone voltage, and retention times for each compound are listed in Table S2 of the SI.

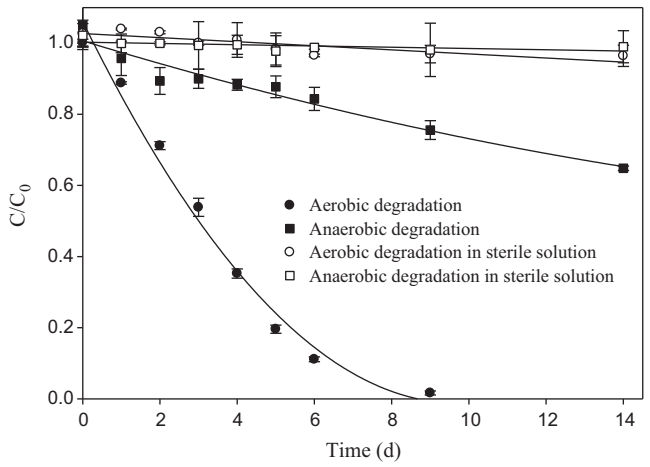

Fig. 1. Degradation of 17α-estradiol-3-sulfate in aqueous solutions blended with 1% dairy wastewater at 35 °C under aerobic and anaerobic conditions. Standard deviation of triplicate samples is shown as error bars.

3. Results and discussion

3.1. Aerobic and anaerobic degradation of 17α-estradiol-3-sulfate in dairy wastewater

Representative time courses for 17α-estradiol-3-sulfate degradation in aqueous solutions blended with 1% dairy wastewater at 35 °C are shown in Fig. 1. No discernible degradation of 17α-estradiol-3-sulfate occurred in control experiments conducted in the sterile solutions under either aerobic or anaerobic conditions over comparable time periods (Fig. 1). The results indicate that biodegradation is the primary degradation mechanism of 17α-estradiol-3-sulfate in blended CAFO wastewater and that abiotic degradation, such as hydrolysis, was negligible.

The effect of aerobic conditions on 17α-estradiol-3-sulfate degradation is also apparent from Fig. 1. The dissipation of the estrogen conjugate in 1% dairy wastewater is much more rapid under aerobic conditions than anaerobic conditions. At 35 °C, over 95% of the 17α-estradiol-3-sulfate was degraded under aerobic conditions within 9 d, compared to only 25% degradation under anaerobic conditions (Fig. 1). This indicates that the manure-borne facultative bacteria present in the dairy wastewater are more efficient at transforming the estrogen conjugate under aerobic conditions than anaerobic conditions. Similarly, many studies have shown that the biodegradation of most free estrogens under aerobic conditions is very rapid, with half-lives of less than one day in a variety of environmental media [29–31]. Therefore, aeration as an effective wastewater treatment practice can be used to eliminate hormone contaminants. By contrast, the less efficient degradation of the hormones under anaerobic conditions could result in their persistence under some environmental scenarios [16], especially in anoxic systems such as aquatic sediments [32]. Accordingly, the accumulation of hormone contaminants in sediments may potentially impact water quality and cause adverse effects for aquatic species, especially those that are sediment dwelling or feeding.

3.2. Effect of temperatures on degradation of 17α-estradiol-3-sulfate

The biodegradation kinetics of 17α-estradiol-3-sulfate in aqueous solutions blended with dairy wastewater under aerobic and anaerobic conditions were assessed under different incubation temperatures. The degradation rates of the estrogen conjugate at

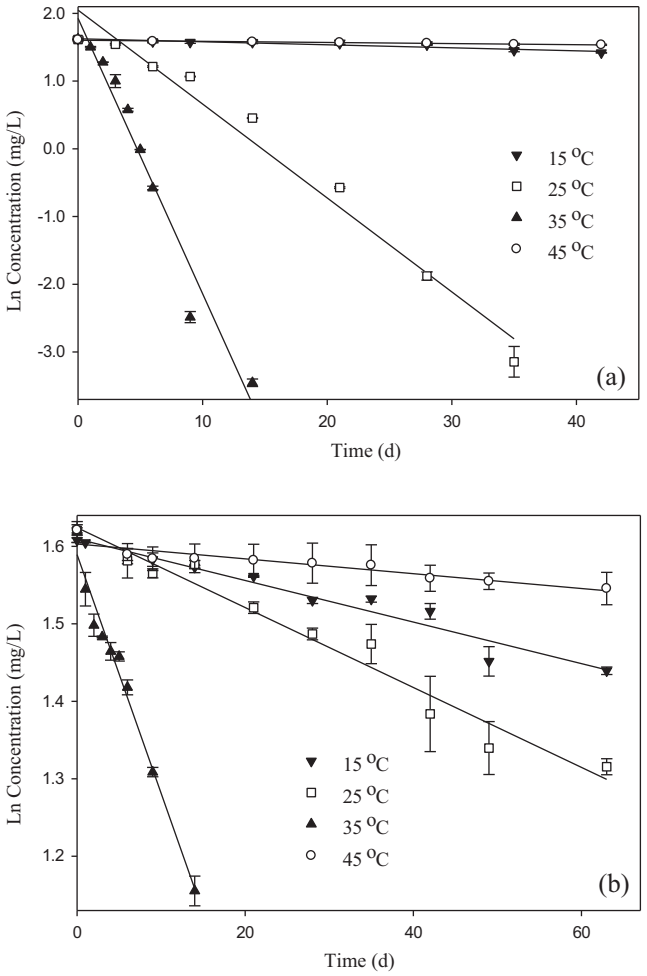

Fig. 2. Degradation of 17α-estradiol-3-sulfate in aqueous solutions blended with 1% dairy wastewater at different incubation temperatures: (a) aerobic conditions and (b) anaerobic conditions. Standard deviation of triplicate samples is shown as error bars.

different temperatures can be represented by a *pseudo*-first-order kinetic model:

$$\ln([C]) = -kt + \ln([C]_0) \quad (1)$$

where k is the temperature dependent biodegradation rate constant of 17α-estradiol-3-sulfate, [C] is the hormone concentration, and $[C]_0$ is the initial hormone concentration. Values of k were calculated as the slope of semi-logarithmic plots of hormone concentration versus time. The results follow a log-linear model well for both aerobic and anaerobic degradations of 17α-estradiol-3-sulfate during the observed time period (Fig. 2). High regression coefficients suggest that the biodegradation of 17α-estradiol-3-sulfate under aerobic and anaerobic conditions followed a *pseudo*-first-order reaction kinetic model (Table 1). By contrast, a previous study showed that the degradation of the hormone testosterone by swine manure-borne bacteria followed a zero-order reaction kinetic model under anaerobic conditions and a *pseudo*-first-order kinetic model under aerobic conditions [30], which suggests that testosterone degradation proceeds differently under aerobic than anaerobic conditions.

To compare the degradation rates for 17α-estradiol-3-sulfate at different incubation conditions, the rate constants (k) and half-lives ($t_{1/2}$) are summarized in Table 1. The half-lives of 17α-estradiol-3-sulfate under aerobic and anaerobic conditions varied from 1.70 to 415 d and 22.5 to 724 d, respectively, in the dairy wastewater within the range of incubation temperatures studied in this study. Multiple comparisons were conducted using ANOVA ($\alpha = 0.025$) to analyze the difference between degradation rate constants under different conditions. For each temperature, the degradation rate of the hormone under aerobic conditions was significantly higher (p value < 0.025) than that under anaerobic incubation. A previous study reported that the half-lives of 17β-estradiol-3-sulfate in soils ranged from 3.25 to 0.424 h under incubation temperatures of 15 and 25 °C [26], which were a few orders of magnitude smaller than the $t_{1/2}$ value (2.5 d) obtained from a sewage wastewater [20]. The values for $t_{1/2}$ in the present study were 5.0 and 150 d under aerobic conditions at 15 and 25 °C, respectively. These differences in degradation rates may be attributable to the different molecular structures of two hormone compounds and the different experimental matrices and conditions.

The effect of temperature on the degradation of 17α-estradiol-3-sulfate for solutions blended with 1% dairy wastewater is shown in Fig. S1 of the SI. For both aerobic and anaerobic experiments, degradation rates increased with increasing temperature from 15 to 35 °C. Over the temperature range of 15 to 35 °C, temperature effects on the degradation rates of the estrogen conjugate can be quantified by the Arrhenius equation:

$$\ln k = \frac{-E_a}{RT} + \ln A \quad (2)$$

where A is the pre-exponential factor, E_a (J/mol) is the activation energy, R (J/K/mol) is the universal gas constant, and T is the absolute temperature (K). The activation energies (E_a) of the hormone were calculated from logarithms of degradation rate constants versus the reciprocal of the absolute temperatures. The E_a values of 17α-estradiol-3-sulfate under aerobic or anaerobic conditions are approximately 168 kJ mol^{-1} ($r = 0.96$) and 89.5 kJ mol^{-1} ($r = 0.96$), respectively. From a thermodynamic perspective, an increase in temperature may accelerate the rate of a reaction with a high E_a value more significantly than a reaction with a smaller E_a value. In this study, the aerobic degradation rate of 17α-estradiol-3-sulfate increased by about 92 times when the temperature increased from 15 to 35 °C. In contrast, its degradation rate under anaerobic conditions increased by only 11 times.

As the incubation temperature was further elevated to 45 °C, both aerobic and anaerobic degradation rates of 17α-estradiol-3-sulfate decreased greatly (SI, Fig. S1). For example, the half-lives of the hormones at 35 °C were observed to be 1–3 orders of magnitude

Table 1
Aerobic and anaerobic degradation rate constants and corresponding half-lives of 17α-estradiol-3-sulfate in aqueous solutions blended with 1% dairy wastewater at different incubation temperatures.

Temperature (°C)	Aerobic condition		Anaerobic condition	
	k (d^{-1})	$t_{1/2}$ (d)	k (d^{-1})	$t_{1/2}$ (d)
15	$4.44 \pm 0.58 \times 10^{-3}$ (0.95)	156	$2.68 \pm 0.19 \times 10^{-3}$ (0.98)	258
25	$1.39 \pm 0.01 \times 10^{-1}$ (0.99)	5.00	$5.16 \pm 0.35 \times 10^{-3}$ (0.98)	134
35	$4.07 \pm 0.30 \times 10^{-1}$ (0.99)	1.70	$3.08 \pm 0.16 \times 10^{-2}$ (0.99)	22.5
45	$1.67 \pm 0.13 \times 10^{-3}$ (0.99)	415	$9.58 \pm 0.94 \times 10^{-4}$ (0.92)	724

Regression coefficients are showed in parentheses.

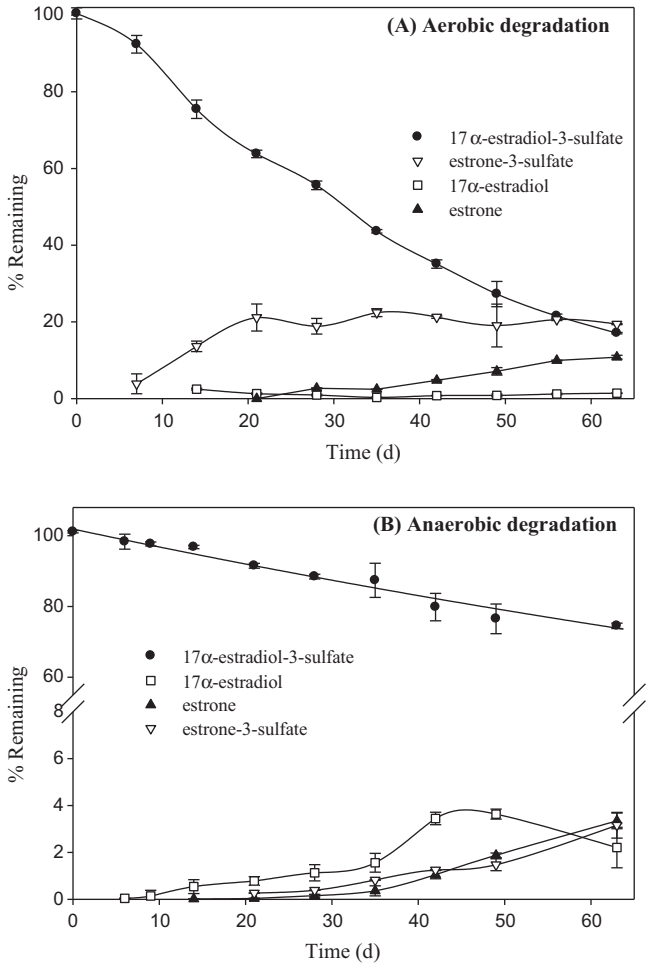

Fig. 3. Aerobic (A) and anaerobic (B) degradation of 17α-estradiol-3-sulfate and formation of its metabolites in aqueous solutions mixed with dairy wastewater at 25 °C. Standard deviation of triplicate samples is shown as error bars.

less than those at 45 °C. These results indicate that the most suitable temperature for 17α-estradiol-3-sulfate biodegradation in this study is near 35 °C. Similar temperature effects on degradation rates of three unconjugated estrogens were also observed in dairy lagoon water [16], suggesting many manure-borne microorganisms have optimal activity at physiological temperatures [30,33].

3.3. Identification of metabolites formed during 17α-estradiol-3-sulfate degradation at 25 °C

To identify major degradation products of 17α-estradiol-3-sulfate in blended dairy wastewater under aerobic and anaerobic conditions, solution aliquots were periodically withdrawn and extracted. The hormone 17α-estradiol-3-sulfate and its metabolites were identified in the extracts by matching retention times to commercially available reference standards and quantified by external calibration by HPLC/PDA. The degradation products were further confirmed using accurate mass data from MS analysis of the extracts. To better identify these degradation products, their standards were run concurrently to verify chromatographic separation and mass spectra with desired fragmentations by LC/MS/MS.

At 25 °C, the formation of degradation products under aerobic and anaerobic conditions is shown in Fig. 3 along with the loss of 17α-estradiol-3-sulfate in aqueous solutions blended with 1% dairy wastewater. Three major metabolites were identified in the aerobic and anaerobic degradation experiments. The mass spectra of these metabolites and 17α-estradiol-3-sulfate are exhibited in Fig. S2 of the SI. The mass spectrum of 17α-estradiol-3-sulfate exhibits a major ion of $[M-23]^-$ at 350.8 (SI, Fig. S2-A) that is consistent with its authentic standard.

Under aerobic conditions at 25 °C (Fig. 3A), the initial degradation of 17α-estradiol-3-sulfate was accompanied by formation of a degradation product after one week. This degradation product was identified as estrone-3-sulfate according to the analysis of its mass spectrum (SI, Fig. S2-B), which was further verified using an authentic standard. The other two degradation products were detected after 2 and 3 weeks of incubation under aerobic conditions. They were identified as 17α-estradiol (SI, Fig. S2-C) and estrone (SI, Fig. S2-D) based on their mass spectra in MS and retention times in LC, which is consistent with their authentic standards and a previous study [16]. Similarly, three metabolites, 17α-estradiol, estrone, and estrone-3-sulfate, were detected as degradation products of 17α-estradiol-3-sulfate under anaerobic conditions (Fig. 3B).

3.4. Aerobic and anaerobic degradation mechanisms of 17α-estradiol-3-sulfate at 25 °C

On the basis of identified degradation products, the primary aerobic and anaerobic degradation pathways of 17α-estradiol-3-sulfate in solutions blended with dairy wastewater at 25 °C are proposed in Fig. 4. There are two major degradation mechanisms for the dissipation of 17α-estradiol-3-sulfate in dairy wastewater. One is a deconjugation mechanism in which the thio-ester bond at position C3 of 17α-estradiol-3-sulfate is cleaved and 17α-estradiol is formed (Fig. 4). Previous studies have demonstrated that certain bacteria present in sewage wastewater can synthesize enzymes that cleave conjugated moieties and release free hormones into the ambient environment [10,19,22]. The other degradation mechanism is an oxidation reaction in which 17α-estradiol-3-sulfate is oxidized to estrone-3-sulfate under aerobic conditions (Fig. 4).

The degradations of 17α-estradiol-3-sulfate proceed differently under aerobic and anaerobic conditions. Under aerobic conditions, the initial and main product is estrone-3-sulfate. To our knowledge, this is the first study to report the oxidation of 17α-estradiol-3-sulfate to estrone-3-sulfate in dairy wastewater. Free hormones are generally considered to be the primary degradation products for estrogen conjugates [22]. In this study, only a small amount of 17α-estradiol was detected at 14 d after incubation (Fig. 3A). The percentage of estrone-3-sulfate remaining at the end of the degradation period was much higher than that of 17α-estradiol, with maximum values of 23% for the former and only 2.5% for the latter. These results suggest that the oxidation at position C17 of the 17α-estradiol-3-sulfate ring was the major degradation mechanism rather than deconjugation of the thio-ester bond at position C3 under aerobic conditions. This is similar to the aerobic degradation of 17β-estradiol-3-sulfate in New Zealand pasture soils [26], in which estrone-3-sulfate was identified as the major primary metabolite and 17β-estradiol was found in lesser amounts. In addition, estrone can be formed by the deconjugation of estrone-3-sulfate and the oxidation of 17α-estradiol (Figs. 3 and 4). A recent report showed that 17α-estradiol readily converted to estrone in aerobic soils with half-lives ranging between 4 and 12 h [34].

By contrast, the initial and major product of 17α-estradiol-3-sulfate degradation in dairy wastewater under anaerobic conditions is 17α-estradiol (Fig. 3B), indicating that deconjugation was the primary anaerobic degradation mechanism. The oxidative conversion of 17α-estradiol-3-sulfate to estrone-3-sulfate was significantly suppressed under anaerobic conditions, which caused its anaerobic degradation rate to be slower than that of its aerobic degradation (Fig. 1). In addition, estrone, identified as a secondary degradation product, was a common metabolite of both 17α-estradiol and estrone-3-sulfate in this study (Fig. 3B). Similarly, it

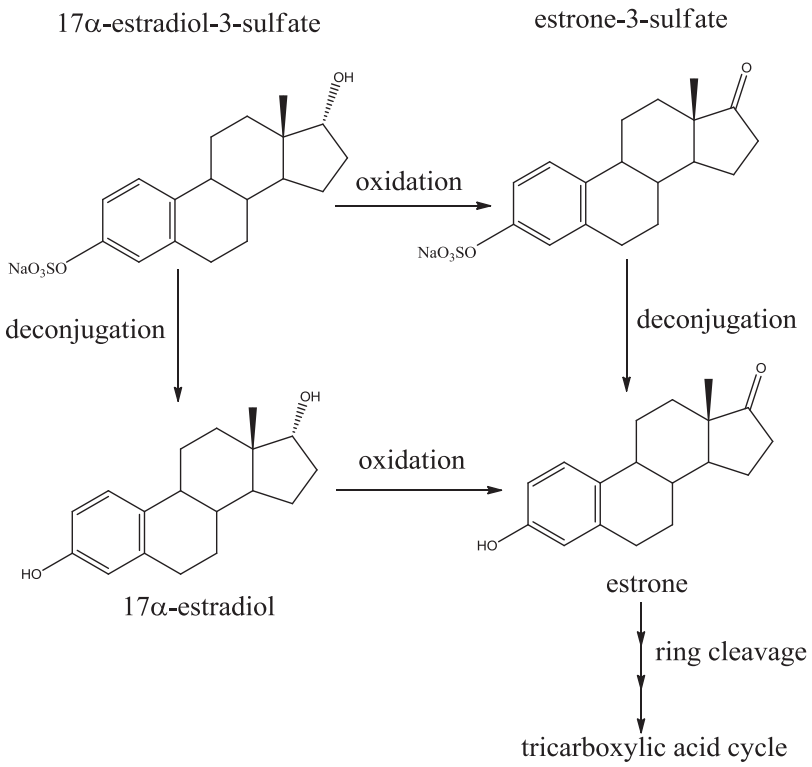

Fig. 4. Degradation pathway of 17α-estradiol-3-sulfate in aqueous solutions blended with dairy wastewater at 25 °C.

has been reported that 17α-estradiol can be oxidized to estrone in CAFO wastewater [16], and estrone-3-sulfate can be converted to its free counterpart – estrone in soils and sewage wastewater treatment plants [22,25].

3.5. Environmental significance

CAFO wastewater is becoming an important source to supplement increasingly scarce fresh water for crop irrigation. The use of CAFO wastewater on agricultural fields can also prevent direct discharge of excessive nutrients into receiving watersheds and instead utilize the nutrients for plant growth. However, CAFO wastewater often retains many contaminants such as pathogens, heavy metals, veterinary pharmaceuticals, and animal hormones. These contaminants are readily released into soils during irrigation with CAFO wastewater, and may subsequently enter the aquatic environment by leaching and/or runoff. The knowledge gained from this study provides useful information help to assess the potential risk of using CAFO wastewater for agricultural irrigation.

Generally, dairy wastewater contains relatively high concentrations of free estrogens and their conjugates. Free steroid estrogens are considered highly potent EDCs. By contrast, most conjugated forms are less biologically active, but these compounds can act as EDC precursors since they may be deconjugated to their corresponding active free estrogens. In this study, two active estrogens 17α-estradiol and estrone were formed during the degradation process of 17α-estradiol-3-sulfate in dairy wastewater, suggesting that endocrine-disrupting activity could increase in the water used for agricultural irrigation. Additionally, the anaerobic degradation rate of 17α-estradiol-3-sulfate is much less than its aerobic degradation rate, suggesting this estrogen conjugate may persist in anaerobic or anoxic environments. Dairy wastewaters are usually stored in CAFO lagoons which typically function as anaerobic reactors. Consequently, CAFO lagoons could be a potential reservoir for conjugated estrogens. When CAFO lagoon water is used for agricultural irrigation, it may result in the loading of hormone contaminants into the environment. Thus, future work needs to develop feasible and effective approaches to eliminate these conjugated estrogen contaminants in CAFO lagoons, e.g., using aerobic biological treatment or up flow anaerobic sludge blanket and step-fed sequencing batch reactor (UASB-SFSBR) treatment [35], and thereby minimize their release into the environment.

4. Conclusions

17α-Estradiol-3-sulfate is a naturally occurring steroid estrogen derived from cattle urine. This study quantifies the aerobic and anaerobic degradation of 17α-estradiol-3-sulfate in aqueous solutions amended with dairy wastewater. This estrogen conjugate was biodegraded significantly faster under aerobic rather than anaerobic conditions, and abiotic degradation was insignificant. The degradation process of 17α-estradiol-3-sulfate under both aerobic and anaerobic conditions followed *pseudo*-first-order kinetics. The degradation rates were temperature-dependent with rates increasing between 15 and 35 °C, and decreasing at 45 °C. Degradation products were characterized and quantified by LC/MS/MS and HPLC/PDA using their authentic standards. Oxidation and deconjugation were the two major degradation mechanisms for 17α-estradiol-3-sulfate degradation in dairy wastewater-containing solutions. Compared to the parent estrogen, the degradation products exhibited higher estrogenic potencies, suggesting the biological potency of dairy wastewater may increase as degradation occurs. Therefore, the occurrence and potential risk to environmental health from estrogen conjugates derived from CAFO wastewater should not be ignored. Further studies need to address the fate and transport of estrogen conjugates under field conditions, for example, after the use of dairy wastewater for agricultural irrigation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.04.038.

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