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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