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The microbial transformation of 17β estradiol in an anaerobic aqueous environment is mediated by changes in the biological properties of natural dissolved organic matter

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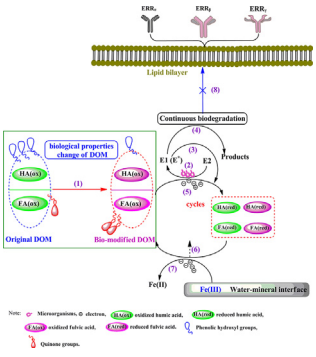
HIGHLIGHTS

- Fe(III) and dissolved organic matter synergistically promote microbial degradation of steroid estrogens in aqueous media.
- Dissolved organic matter serves as an electron shuttle mediator in the biodegradation.
- Biodegradation efficiency was enhanced by biological properties change of dissolved organic matter.
- This explains why steroid estrogens do not accumulate in natural aqueous environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Dissolved organic matter (DOM) is shown to act as an electron shuttle mediator which enhances the microbial degradation of steroid estrogens in natural water. Batch studies were conducted with 17β estradiol (E2), quinone-reducing bacteria, DOM, and Fe(III) as a terminal electron acceptor. The results show that anthraquinone 2 disulfonate (AQS) approximately doubles the microbial degradation of E2 by DOM alone. The effect decreases sharply at AQS concentrations above 1.0 mmol·L^{-1}. Over three oxidation-reduction cycles, the electron-shuttling ability of AQS and the E2 biodegradation rate decreased step by step. Changes in the biological properties of the dissolved organic matter increased its aromaticity, its quinone content, and its fulvic-like fluorescence while significantly improving the electron transfer between DOM and the microbes, which made the degradation more effective. This explains why steroid estrogens do not accumulate in natural aqueous environments. Moreover, the estrogenic activity of steroid estrogens is inhibited at low concentrations through the activity of DOM.

1. Introduction

Steroid estrogens (SEs), especially 17β estradiol (E2), are normally found in water only at nanogram–per-litre levels, but field and laboratory studies have demonstrated that even at those levels they can still alter normal hormone functions and the physiological status of wildlife (Liu et al., 2011, 2012; Huang et al., 2015). Thus, the fate and behavior of SEs in natural water environments have generated extensive concern. Previous studies have shown that high concentrations of residual SEs (4.2–86.5 ng·L^{-1}) are discharged into aquatic environments every day through the effluent of eight municipal wastewater treatment plants (Huang et al., 2013), with the total amount of 35.8 g·day^{-1}. However, SE accumulation in water and sediment is not very obvious despite

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regular monitoring (Huang et al., 2013), which means some natural degradation process is at work.

Anaerobic biodegradation of residual SEs has been identified as one of the predominant natural degradation process in anaerobic water environments (Bagnall et al., 2012), but the process is very slow, and the complete degradation of SEs may take weeks or even months (Ying et al., 2003). There are, however, some active substances in anaerobic aqueous environments that can enhance biodegradation and bioconversion of pollutants by microorganisms. DOM and mineral species (such as Fe(III)) can be co-metabolised in anaerobic water and sediment, an important process by which microorganisms transform organic and inorganic contaminants (Wu et al., 2014; Gu et al., 2016; Xu et al., 2014; Huang et al., 2016). They usually serve as competing electron acceptors during quinone respiration by microorganisms (Kotik et al., 2013). For instance, anthraquinone 2 disulfonate (AQS) apparently promotes Cr(VI) reduction (Huang et al., 2016). Fe(III) and DOM together exhibit better redox function than either alone, and their coupled abiotic action can significantly enhance Cr(VI) reduction by quinone-reducing bacteria. Humic substances and quinone in DOM have also been shown to increase the abiotic reductive dechlorination of polychlorinated pollutants in water and sediment (Xu et al., 2014). DOM is able to accelerate the microbial reduction of Fe(III) minerals by acting as an electron shuttle between the cells and Fe(III) minerals, which results in increased generation of biogenic Fe(II) and anaerobic transformation of organic matter in the iron(III) reduction.

Previous research has shown that the electron transfer is influenced by the quinone structure in DOM (Wu et al., 2014). The mechanism has been shown to involve oxidation of carbonyl structures cycling with the reduction of hydroxyl quinone (Lovley et al., 1996). DOM can also serve as a terminal electron acceptor to mediate the oxidation of phenolic compounds (Cervantes et al., 2000), or act as a redox intermediate to effect the reduction of heavy metals (Brookshaw et al., 2014) and organic contaminants by microbes (Jiang and Kappler, 2008). To date, almost all research into these matters has been limited to DOM serving as a terminal electron acceptor enhancing oxidative degradation (Cervantes et al., 2000; Gu et al., 2016), or DOM serving as an electron shuttle to promote the reduction of organic pollutants in water and sediment (Xu et al., 2014; Meng et al., 2014; Klupfel et al., 2014). Little published research has addressed the potential role of natural DOM in mediating microorganisms' degradation of SEs when Fe(III) is present as a competitive matrix in an anaerobic aqueous environment.

DOM in natural water is derived from plant and animal residues which have decayed (Page et al., 2002; Page, 2003). Its biological decomposition processes of course may influence its reactivity and other characteristics. So, it would be important to know to what extent DOM's biological property changes affect its efficiency in promoting the biodegradation of SEs and whether its activity changes upon microbial modification. That motivated this series of experiments to elucidate the roles of DOM with Fe(III) in the microbial biodegradation of 17β estradiol, which is very important to the ecology of natural surface water. The study's objectives were 1) to explore the mechanisms underlying the action of DOM and Fe(III) in E2's microbial degradation, 2) to investigate the composition and structure changes in DOM after biomodification and its effect on E2 biodegradation with Fe(III), 3) to explain why steroid estrogens do not accumulate in natural waters, and 4) to assess estrogenic activity when modified DOM mediates E2's anaerobic biodegradation.

2. Material and methods

2.1. Reagents and chemicals

The anthraquinone 2 sodium sulfonate (AQS), Fe_2O_3 and HPLC-grade E2 used were supplied by Sigma-Aldrich. All of the other reagents were analytical grade if not otherwise mentioned. The sediment was collected from ErHai Lake in China's Yunnan Province. It was sieved using a 4 mm sieve and rinsed with deionized water. The prepared sediment was then treated with a 1:10 (w/v) mixture of NaOH and $Na_4P_2O_7$ (0.1 mol L^{-1}) to concentrate it. Water collected from ErHai Lake was used in the E2 biodegradation experiments. The composition and characteristics of the ErHai Lake water are summarized in Table S1 of the supplementary information. Humic acid (LHA) and fulvic acid (LFA) were separated using the alkali-acid method of the International Humic Substances Society. The LHA and LFA stock solutions were stored in polyethylene containers at 4 °C in the dark and used within 4 weeks.

2.2. The effect of AQS on E2 biodegradation

Prior to use, all of the materials, including the serum bottles, the sealing rubber and the solutions were sterilized in an autoclave at 121 °C for 25 min. The quinone-reducing bacteria were also isolated from ErHai Lake sediment and then enriched. Standard anaerobic culturing techniques were used throughout the study (Lovley and Phillips, 1988; Lovley et al., 1996). The enriching mineral salts medium (MSM) was modified from that recommended by Lovley and Phillips (1988). It contained (in $mg \cdot L^{-1}$): NaCl, 1000; NH_4Cl, 800; KH_2PO_4, 500; K_2HPO_4, 600; $MgCl_2$, 200; $CaCl_2 \cdot 2H_2O$, 50; and then 5 mL each of a vitamin solution and a trace mineral solution were added. The medium was supplemented with 1 $mmol \cdot L^{-1}$ AQS as an electron acceptor and 5 $mmol \cdot L^{-1}$ sodium formate as a substrate. To isolate quinone-reducing bacteria from the enriched MSM, the medium was diluted serially and incubated on agar plates containing 1 $mmol \cdot L^{-1}$ AQS and 5 $mmol \cdot L^{-1}$ sodium formate. Selected well-developed colonies were streaked three times with new agar and then preserved for further study.

DOM model (AQS) mediates E2. Batch anaerobic biodegradation experiments using AQS to model DOM were performed in rubber-sealed 250 mL serum bottles. The experiments exploring AQS's role were performed with 250 mL of MSM under the following conditions: (1) Sterile + E2; (2) Sterile + Fe(III) + E2; (3) Sterile + AQS + E2; (4) Sterile + Fe(III)/AQS + E2; (5) Strain + E2; (6) Strain + Fe(III) + E2; (7) Strain + AQS + E2; and (8) Strain + Fe(III)/AQS + E2. In these tests, 0.5 $mmol \cdot L^{-1}$ AQS, 10 mg Fe_2O_3 (simulating the iron in natural waters), 1 $mg \cdot L^{-1}$ E2 and 0.1 $g \cdot L^{-1}$ of the quinone-reducing bacteria were added to the MSM solution. The experiments tested AQS concentrations of 0 to 2 $mmol \cdot L^{-1}$ with 10 mg Fe_2O_3, 1 $mg \cdot L^{-1}$ E2, and 0.1 $g \cdot L^{-1}$ of the quinone-reducing bacteria. Each experiment was performed in triplicate, and all of the experiments were conducted statically in anaerobic and dark conditions at pH 8.0 and 30 °C for 120 h. Bacterial growth was monitored by measuring the protein concentration with Bradford's method using bovine serum albumin as a standard (Bradford, 1976). The Fe(II) concentration was recorded at selected time points using the 1,10 phenanthroline chemical method (Lovley et al., 1996).

Cyclic experiments were also performed with 250 mL of the mineral salts medium that contained 0.5 $mmol \cdot L^{-1}$ AQS, and again 10 mg Fe_2O_3, 1 $mg \cdot L^{-1}$ E2, and 0.1 $g \cdot L^{-1}$ of the quinone-reducing bacteria. After 120 and 240 h the E2 concentration was restored to 1 $mg \cdot L^{-1}$ by adding the E2 methanol solution. Previous research in our laboratory (Gu et al., 2016) has shown that quinone-reducing bacteria work best under anaerobic conditions and in the dark at pH 8.0 and 30 °C, so each experiment was performed in triplicate under those conditions for 120 h. The absorbance of AQS was measured at 336 nm using a UV–vis spectrophotometer, and the concentration of AH_2QS (the reduction of AQS) was calculated from the change in the AQS concentration (Huang et al., 2016).

2.3. DOM properties and E2 biodegradation

Microbial action can change DOM's properties. This was tested using the humic and fulvic acids from the lake. One litre of LHA (or LFA) solution was placed in an acid-washed 3 L glass container for microbial modification. Sufficient nutrients (NH_4NO_3 and K_2HPO_4) were added to yield a C:N:P mass ratio of 30:10:3 in order to ensure that nutrition