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Dissolved organic matter as a terminal electron acceptor in the microbial oxidation of steroid estrogen

Lipeng Gu, Bin Huang*, Zhixiang Xu, Xiaodong Ma, Xuejun Pan

Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming, Yunnan 650500, PR China

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ABSTRACT

Steroid estrogen in natural waters may be biodegraded by quinone-reducing bacteria, dissolved organic matter (DOM) may serve as a terminal electron acceptor in this process. The influence of temperature, pH, dissolved oxygen and light illumination on the reduction efficiency of anthraquinone-2-disulfonate (AQS) was investigated using 17β-estradiol (E2) as the target species. The optimum reduction conditions were found to be in the dark under anaerobic conditions at pH 8.0 and 30 °C. Quinone-reducing bacteria can use the quinone structure of DOM components as a terminal electron acceptor coupling with microbial growth to promote biodegradation. Compared with other DOM models, AQS best stimulated E2 biodegradation and the mediating effect was improved as the AQS concentration increased from 0 to 0.5 mM. However, further increase had an inhibiting effect. Natural DOM containing lake humic acid (LHA) and lake fulvic acid (LFA) had a very important accelerating effect on the degradation of E2, the action mechanism of which was consistent with that defined using DOM models. The natural DOM contained more aromatic compounds, demonstrating their greater electron-accepting capacity and generally more effective support for microorganism growth and E2 oxidation than Aldrich humic acid (HA). These results provide a more comprehensive understanding of microbial degradation of steroid estrogens in anaerobic environments and confirm DOM as an important terminal electron acceptor in pollutant transformation.

1. Introduction

Natural and synthetic steroid estrogens (SEs) such as estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethynylestradiol (EE2) have been recognized as potential endocrine disruptors (Huang et al., 2013). Their incomplete removal in sewage treatment plants (STPs) and indeed direct discharge have led to their being widely detected in aquatic environments (Huang et al., 2014). Although those compounds are normally found in water at only ng per litre levels, field and laboratory studies have demonstrated that 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.

Among the various fates of environmental SEs, biodegradation has been identified as one of the predominant removal mechanisms from both natural water and sediment. However, it has been shown that SE biodegradation by heterotrophic bacteria is very slow. SEs have been reported to have a biodegradation half-life of 20–40 days under aerobic conditions (Clouzot et al., 2008), and longer could be expected under anaerobic conditions (Ying et al., 2003). According to previous studies in our laboratory, even when SEs are continuously discharged into surface water from STPs, their accumulation is not very obvious (Huang et al., 2013). Therefore, it is speculated that some active substances in natural aquatic environments enhance the biodegradation of SEs.

Many studies have shown that the fast degradation of pollutants in natural waters can usually be attributed to the accelerating effects of dissolved organic matter (DOM). Quinone groups in DOM can promote microbes to degrade heavy metals (Reijonen et al., 2016) and organic pollutants (Guha et al., 2001). For instance, such groups have recently been reported to play an active role in the de-colorization of azo dyes (Meng et al., 2014) and in the reduction of Cr(VI) (Guo et al., 2012; Huang et al., 2016) under anaerobic conditions. But those studies have been restricted to considering organic acids as electron donors. There are no published reports regarding DOM as a terminal electron acceptor acting

* Corresponding author.
E-mail address: huangbin@kmust.edu.cn (B. Huang).

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to accelerate SEs' biodegradation by quinone-reducing bacteria in aquatic environments. That was the phenomenon explored in this study.

Quinone-reducing bacteria have attracted much interest for their respiration diversity, their wide distribution in diverse environments and their potential application in pollution bioremediation through biodegradation. Recently, extensive studies have assessed the catalytic effect of redox mediators on the reduction of heavy metals and organic contaminants by quinone-reducing bacteria isolated from terrestrial or freshwater sources (Cao et al., 2013; Pearce et al., 2006). Recent studies in our own laboratory have demonstrated for the first time their ability to degrade E2 under alkaline conditions. Both DOM and DOM models were considered as terminal electron acceptors in that work. The testing of DOM derived from natural water was a new attempt to mediate E2 biodegradation, and no such results have been published so far.

The aim of this study was to test the mediating mechanism of four models of DOM and two actual samples of DOM on the microbial degradation of E2. The influences of temperature, pH, dissolved oxygen and light illumination on the reduction efficiency of the DOM models were assessed using sodium formate as an electron donor. Also investigated were the ability of quinone-reducing bacteria to accelerate DOM reduction and E2 biodegradation, the coupling of E2 as the electron donor and DOM as a terminal electron acceptor to support microbial growth, as well as the effects of DOM concentration on the anaerobic microbial oxidation of E2.

2. Materials and methods

2.1. Reagents and chemicals

Anthraquinone-2-sodium sulfonate (AQS), anthraquinone-2-carboxylate (AQC), 5-hydroxy-1,4-naphthoquinone (juglone, JQ), 2-hydroxy-1,4-naphthoquinone (lawsone, LQ), E2 and Aldrich humic acid (HA) were supplied by Sigma-Aldrich. The E2 used was high performance liquid chromatograph grade. Its key physicochemical properties are shown in Table 1 (Lai et al., 2000). The natural DOM was extracted from sediment collected from ErHai Lake in Yunnan Province of China. It was sieved using a 4.0 mm sieve and rinsed with deionized water. Lake humic acid (LHA) and lake fulvic acid (LFA) were extracted using the alkali-acid method of the International Humic Substances Society. The HA, LHA and LFA stock solutions were stored in polyethylene containers at 4 °C in the dark and used within 3 weeks. All of the other reagents are analytical grade if not otherwise mentioned.

2.2. Bacteria and culturing conditions

The quinone-reducing bacteria were also enriched and isolated from the ErHai Lake sediment. The enriched mineral salts medium (MSM) was supplemented with AQS (1 mM) as an electron acceptor and sodium formate (5 mM) as a substrate. To isolate quinone-reducing bacteria from the enriched MSM, the enrich MSM was diluted serially and incubated on agar plates containing AQS (1 mM) and sodium formate (5 mM). Selected well-developed colonies were streaked three times with new agar and then preserved for further study. The quinone-reducing bacteria were inoculated into a medium, which contained 10 g L^{-1} of tryptones, 5 g L^{-1} of yeast extract and 10 g L^{-1} of NaCl at 30 °C on a rotary shaker and agitated at 160 rpm for 24 h. Subsequently, the strains were separated from the medium by centrifugation at 10,000 rpm for 10 min. The harvested biomass was washed three times with buffer solution before being employed to the experiments. Standard anaerobic culturing techniques were used throughout the study. The base medium was modified from that recommended by Lovley and Phillips, 1988. It contained (in mg 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 yeast extract, 10.

2.3. Reducing characteristics of the AQS

Serum bottles containing 100 mL MSM, 0.5 mM AQS, 5 mM sodium formate and 0.1 g L^{-1} of quinone-reducing bacteria were agitated on a rotary shaker at 160 rpm for 120 h to determine the conditions delivering the best AQS reduction efficiency. The effects of temperature, pH, dissolved oxygen and illumination conditions were assessed. The temperatures tested were 0, 5, 10, 15, 25, 30, 35 and 45 °C conducted at pH 7.0, and the pH values were 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 conducted at 30 °C. The dissolved oxygen levels were 0, 0.5, 1.0, 2.0 and 3.0 mg L^{-1} and agitation under natural and ultraviolet light and in the dark were tested. Sterile controls were tested under the same conditions. They were prepared by autoclaving at 121 °C for 25 min. Each treatment was set up in triplicate.

2.4. E2 degradation with model and natural DOM

In order to explore the mediating mechanisms of the four DOM models (AQS, AQC, LQ and JQ) and the natural DOM (LHA, LFA and HA) in the microbial degradation of E2, batch experiments were performed assessing: (1) AQS reduction coupling with the oxidation of E2 to support microbial growth; (2) Any effects of the DOM models and their concentrations on the microbial degradation of E2; (3) DOM-mediated microbial degradation of E2; (4) The reduction of DOM by quinone-reducing bacteria with E2 as the electron donor; and (5) The effects of DOM concentration on E2 biodegradation. The experiments were conducted in rubber-sealed 100 mL serum bottles. 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 DOM model experiments were performed in the dark with 100 mL of MSM that contained different concentrations of AQS, AQC, LQ and JQ (from 0 to 2 mmol L^{-1}), different concentrations of E2 (0.5, 1, 1.5 and 2 mg L^{-1}), and 0.1 g L^{-1} of quinone-reducing bacteria under anaerobic conditions at pH 8.0 and 30 °C for 120 h. The natural DOM experiments were performed with different concentrations of LHA, LFA and HA (from 0 to 8 mgC L^{-1}), 1 mg L^{-1} of E2, and 0.1 g L^{-1} of quinone-reducing bacteria under the same conditions.

2.5. Analytical methods and data analysis

AQS absorbance was measured at 336 nm using a UV–Vis spectrophotometer, and the reduction efficiency of the AQS was then calculated using the change in AQS absorbance as follows.

$$\text{The reduction efficiency of AQS}(\%) = (A_0 - A_t)/A_0 \times 100\% \quad (1)$$

Table 1
Selected physiochemical properties of the 17β-estradiol.

Estrogen	Molecular formula	Molecular weight (g·mol^{-1})	Water solubility at 20 °C (mg L^{-1})	logK_{ow}	pk_a
17β-estradiol (E2)	$C_{18}H_{24}O_2$	272.4	3.9–13.3	3.1–4.0	10.5–10.7