Sorption and Biodegradation of 17β-estradiol by Acclimated Activated Sludge under Anaerobic Conditions

Qingling Zeng, Yongmei Li*, Guowei Gu
State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai, China

Abstract—The sorption and biodegradation of 17β-estradiol (E2) was investigated by spiking E2 into acclimated activated sludge under anaerobic conditions in laboratory-scale. The effect of temperature on the sorption and biodegradation was also studied. Results showed that, E2 was removed 99% after 2 h for the tested concentrations (5-15 μg·L⁻¹). E2 sorption followed both the Freundlich and linear sorption models. The distribution coefficient ($K_d$) was 543.7 L·kg⁻¹ at 20°C. E2 degradation was well described by first-order reaction kinetics and the rate constant was 2.765 h⁻¹ at 20°C. Temperature has significant effect on both the sorption and biodegradation of E2 by activated sludge. Distribution coefficient decreased but reaction rate constant increased with the increasing temperature. Partitioning played a significant role in the transfer of E2 between the aqueous phase and the sludge phase. The biodegradation behavior was inside the sludge phase.

Keywords- 17β-estradiol; anaerobic; sorption; biodegradation; temperature

I. INTRODUCTION

The fates and effects of endocrine disrupting chemicals (EDCs) entering the environment have gained increasing concern in recent years [1, 2]. 17β-estradiol (E2), released by humans and livestock, is a potential endocrine disruptor even at ng·L⁻¹ levels [3]. It has been found in many aquatic systems, and raises serious problems in aquatic organisms and animals [4, 5].

It has been reported that E2 is one of the major contributors to the estrogenic activity observed in many sewage treatment plants [6]. Due to their harmful effects on the hormonal system and the difficulty to remove it by the common wastewater treatment methods, many researchers have endeavored to develop more efficient removal techniques [7]. A better understanding of factors that impact the fates of E2 during the wastewater treatment process is critical to the development and implementation of suitable wastewater control strategies.

Usually, sorption and biodegradation are the two major processes removing E2 from the wastewater [8]. Therefore, most researches investigating the removal of estradiol focused on this two processes [8, 9]. High sorption potential of E2 has been observed [10]. As E2 is liable to sorb onto activated sludge, its biodegradation behavior might be significantly influenced by sorption. The removal could be attributed by the biodegradation of E2 by microorganisms after E2 was transferred from the aqueous phase to the sludge phase [11]. Therefore, measuring E2 concentrations in aqueous phase and sludge phase separately might illuminate the mechanism of E2 transference between the two phases as well as the biodegradation behavior in sludge.

In this paper, batch experiments were performed to investigate the sorption and biodegradation of E2 by activated sludge under anaerobic conditions. By measuring the concentration in aqueous phase and sludge phase separately, the sorption isotherms and degradation kinetics under anaerobic conditions were investigated at different temperatures.

II. MATERIALS AND METHODS

A. Chemicals

17β-estradiol (CAS 50-28-2, >98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were supplied by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

B. Activated sludge

The seed sludge was obtained from the returning activated sludge tanks of Shanghai Changqiao Municipal Wastewater Treatment Plant. The activated sludge was acclimated using Sequencing Batch Reactor (SBR) mode. The detailed acclimation and the synthetic wastewater referred to Zhao et al. [12] except that no oxygen was supplied and the reactor was filled with nitrogen gas in the headspace and was capped during the reaction. The reactor was placed in a temperature-controlled room at 20±1°C. After two months of acclimation, the performance of activated sludge was stabilized. The mixed liquid suspended solid (MLSS) was 2968 mg·L⁻¹. The removal efficiencies of both CODCr and total nitrogen (TN) were greater than 70%.

C. Degradation experiments

Degradation experiments were conducted in a thermostatic rotary shaker (125 rpm) at the temperature of 20±0.5°C. A series of 500-mL conical flasks were used. The acclimated activated sludge was taken form the SBR and
washed three times. After decanting the supernatant, the sludge was distributed to each flask. The synthetic wastewater was added to the flasks to the volume of 300 mL. MLSS in the flasks was 3000 mg·L⁻¹. E2 was spiked to the flasks to make the initial concentrations of E2 were 5, 10 and 15 μg·L⁻¹, respectively. The flasks were sealed with butyl rubber stoppers after spiking, and headspaces of the flasks were purged with nitrogen for 20 min. Samples were collected from each flask after 5, 15, 30, 45, 60, 75, 90 and 120 min. The samples were immediately separated into the aqueous and solid phases by centrifugation (6000 rpm) for 10 min. The aqueous phases were collected in 500-mL glass bottles, and acidified to pH 3.0±0.1 using 1 mol·L⁻¹ HCL. The aqueous samples were then stored at 4°C in the dark until solid-phase extraction (SPE). The solid phases were frozen after centrifugation. The aqueous phases and solid phases were analyzed separately. To determine the effect of temperature on the sorption and biodegradation of E2, experiments were conducted at the temperature of 10, 20 and 30°C, respectively, at the initial concentration of E2 was 10 μg·L⁻¹.

D. Sample preparation and analysis

The aqueous phases were prepared with solid-phase extraction (SPE) according to the method described by Zhao et al. [12]. After E2 enrichment on the cartridges, it was eluted sequentially using 4 mL of methanol and 10 mL of dichloromethane. After the eluate dryness, the residue was dissolved in 1 mL of methanol. The solid phases were freeze-dried. E2 in the solid phase was extraction using ultrasonic extraction method described by Zeng et al. [11]. The concentration of E2 was determined using high performance liquid chromatography (HPLC) equipped with a fluorescence detector (Prostar, Varian) according to the method described by Zeng et al. [11].

Recovery experiments were performed by spiking E2 into the synthetic wastewater with E2 concentration in the range of 50 ng·L⁻¹ to 15 μg·L⁻¹. The sample preparation procedure was the same as the aqueous phases described above. The recovery percentages were in the range of 95-104%.

III. RESULTS AND DISCUSSION

A. Sorption isotherms

Fig. 1 shows the concentration profiles of E2 in aqueous phases (Cw) and in solid phases (Cs) during its anaerobic degradation. The content of E2 in the mix liquor (C) was equal to the sum of E2 contents in aqueous phase and solid phase. In the beginning of the reaction, as E2 concentration in the aqueous phase decreased rapidly, E2 content in the solid phase increased significantly. This was due to sorption of E2 on the solid phase. Interestingly, the E2 concentration in the solid phase peaked in 5 min, and it began to decrease, suggesting a spontaneous degradation in the system.

Ren et al. [14] has shown that the adsorption equilibrium of E2 was approached within 10 min at 20°C with inactivated sludge. Clara et al. [15] indicated that within a contact time of 24 h, no difference between the sorption to activated and inactivated sludge could be detected. The preliminary sorption equilibrium experiment of E2 onto sterilized sludge indicated that the sorption capacity of E2 on the inactivated sludge at 5 min approached 90% of its equilibrium sorption capacity. Therefore, it was assumed that sorption of E2 on the activated sludge was in the state of dynamic pseudo-equilibrium after 5 min. The experimental data after 5 min were subjected to
regression using the Freundlich model and linear distribution model. The results showed that the concentrations in the aqueous phases and the corresponding E2 quantities on the sludge were well correlated by both the Freundlich and linear sorption models. The Freundlich parameters and distribution coefficients were shown in Table I. The Freundlich exponent was close to 1. Therefore, the sorption can be well described by the linear partitioning model. The relative standard deviation (RSD) for the replicate experiments was less than 2%. The results indicated that the hypothesis for the dynamic pseudo-equilibrium of E2 on the activated sludge after 5 min is reasonable.

B. Degradation kinetics

At the initial concentrations of 5-15 \( \mu g \cdot L^{-1} \), E2 was able to be removed more than 99% within 2 h. E2 remaining both in the effluent and in the solid phase was less than 0.5%. This demonstrated that E2 was liable to be sorbed by activated sludge and further biodegraded under anaerobic conditions. The biodegradability of E2 under anaerobic conditions was also reported by Sarmah et al. [16] and Czajka et al. [17]. Sarmah et al. [16] investigated the degradation of E2 in river water-sediment under anaerobic conditions, and showed rapid degradation of E2 within the first 2 to 4 d under anaerobic conditions. Czajka et al. [17] indicated that E2 could be readily transformed to E1 under anaerobic conditions.

The biodegradation of estrogens by activated sludge could be described by first-order reaction [8, 18]. The first-order rate constants of E2 were shown in Table II. The average first-order rate constant was 2.765 h\(^{-1}\). In general, the relative standard deviation (RSD) for the replicate experiments was less than 2.0%.

The biodegradation was inside the sludge phase and the reaction in the aqueous phase was neglected because decrease in E2 concentration was not observed in the absence of sludge. Therefore, the removal mechanism of E2 by activated sludge is as follow: E2 transferred from the aqueous phase to the sludge phase, and was further biodegraded by the microorganisms in sludge phase. The transfer of the compound between the aqueous phase and the sludge phase followed the partitioning model.

C. Effect of temperature on E2 sorption and biodegradation

The concentration profiles of E2 at the temperature of 10, 20 and 30°C were similar to those in Fig. 1. After 5 min contact between E2 and sludge, the peak concentration of E2 on the solid phases were 1731, 1401, and 1268 \( \mu g \cdot kg^{-1} \) at 10, 20, and 30°C, respectively. These results suggested that lower temperature benefited sorption and the sorption of E2 on the sludge was an exothermic reaction.

The sorption isotherms of E2 at 10-30°C were shown in Fig. 2. The Freundlich coefficients (\( K_f \)) were 565.1, 445.5 and 318.3 L·kg\(^{-1}\); the Freundlich exponents (1/n) were 1.011, 1.035 and 1.064 at 10, 20, and 30°C, respectively. Since the Freundlich exponents were close to 1, partitioning played a dominant role in the sorption of E2 on the activated sludge. The distribution coefficients (\( K_d \)) were 611.0, 543.7 and 431.4 L·kg\(^{-1}\) at 10, 20, and 30°C, respectively. The distribution coefficients decreased with the increasing temperature. When the temperature increased from 10 to 30°C, \( K_d \) was decreased by 42%. The results also confirmed lower temperature benefited the sorption of E2 on sludge.

The residual contents of E2 in activated sludge at different temperatures were showed in Fig. 3. After regressing the experimental data with first-order reaction kinetics, the correlation coefficients (\( R^2 \)) were 0.8965, 0.9570 and 0.9376 at 10, 20, and 30°C, respectively. Therefore, it can be concluded that the first-order reaction was fairly accurate description for the degradation of E2 under anaerobic conditions in the investigated temperature range (10-30°C).

In general, increasing temperature enhanced the first-order reaction constants [19]. The first-order reaction constants were 2.175, 2.765 and 3.216 h\(^{-1}\) at 10, 20, and 30°C, respectively. Lowering the temperature by 10°C (from 20 to 10°C) decreased the rate constant by 26%. In contrary, elevating the temperature by the same magnitude (from 20°C to 30°C) only enhanced the rate constant by 16%. A suitable temperature can be critical for bacteria to degrade E2 and achieve an optimized economic efficiency. The results indicated that E2 biodegradation under anaerobic conditions was fast in the range of 20 to 30°C, and there was little E2 accumulated in the solid phase after 2 h.

<table>
<thead>
<tr>
<th>Initial concentration (( \mu g \cdot L^{-1} ))</th>
<th>First-order kinetics</th>
<th>rate constant (h(^{-1}))</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>( y = 2.786 t )</td>
<td>2.786</td>
<td>0.9055</td>
</tr>
<tr>
<td>10</td>
<td>( y = 2.748 t )</td>
<td>2.748</td>
<td>0.9570</td>
</tr>
<tr>
<td>15</td>
<td>( y = 2.761 t )</td>
<td>2.761</td>
<td>0.9176</td>
</tr>
</tbody>
</table>

Figure 2. Sorption isotherms of E2 at different temperatures
For the tested concentrations (5-15 μg·L⁻¹), E2 was removed 99% by activated sludge in 2 h under anaerobic conditions. There was less than 0.5% of E2 residue observed on either aqueous or solid phase. E2 sorption on activated sludge was well correlated by both the Freundlich and linear sorption models. Partitioning played a significant role in the sorption of E2 on the activated sludge. E2 biodegradation can be described by first-order reaction kinetics under anaerobic conditions.

Temperature has significant effect on the sorption and biodegradation of E2 in activated sludge process. Lower temperature was benefit to the sorption of E2 on sludge but decrease the biodegradation rate. Elevating the temperature was benefit to the sorption of E2 in aerobic activated sludge process. Lower conditions.

Partitioning played a significant role in the sorption of E2 on the activated sludge. E2 biodegradation can be described by first-order reaction kinetics under anaerobic conditions.

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**REFERENCES**


Figure 3. The residual content of E2 in activated sludge under anaerobic conditions at different temperatures