

Removal and fate of estrogens in an anaerobic-anoxic-oxic activated sludge system

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ABSTRACT

Laboratory-scale experiments were conducted to investigate the removal and fate of estrogens 17 β -estradiol (E2) and 17 α -ethinylestradiol (EE2) in an anaerobic-anoxic-oxic (AAO) activated sludge system. Estrogen concentrations in the aqueous and solid phases in each reactor of AAO system were analyzed separately. E2 was not detected in the final effluent. The anaerobic, anoxic and oxic reactors accounted for 71%, 7% and 22% of the overall E2 removal, respectively. The overall EE2 removal efficiency was about 80%, and the anaerobic, anoxic and oxic reactors were responsible for 44%, 8% and 48% of the overall EE2 removal, respectively. In anaerobic unit, sorption was the dominant mechanism for the removal of E2 and EE2. While E2 was degraded in all the three units of the AAO system, EE2 was only degraded in the anoxic and aerobic units. Biodegradation is important for the fate of E2 compared to sorption. Of the total influent E2 in the AAO system, 99.99% was biodegraded and 0.01% remained in the waste sludge. Nevertheless, both sorption and biodegradation play important roles in the removal of EE2. Of the total influent EE2, 79.1% was degraded by activated sludge, 19.9% was discharged in the effluent and 1% remained in the waste sludge.

Key words | 17 α -ethinylestradiol, 17 β -estradiol, AAO activated sludge system, biodegradation, fate, sorption

INTRODUCTION

The natural estrogen 17 β -estradiol (E2) and the synthetic estrogen 17 α -ethinylestradiol (EE2), recognized as endocrine disrupting chemicals (EDCs), are released by humans and livestock and usually discharged into surface waters via wastewater treatment plants (WWTP) (Khanal *et al.* 2006; Zuo *et al.* 2006). Thus, the removal and fate of estrogens in the wastewater treatment processes have gained increasing concern (Esperanza *et al.* 2007; Pholchan *et al.* 2008; Ying *et al.* 2008a; Hashimoto & Murakami 2009).

Sorption and biodegradation are important mechanisms for the removal of estrogens in biological wastewater treatment systems (Joss *et al.* 2004; Khanal *et al.* 2006; Ying *et al.* 2008a). Estrogens are removed from the aqueous phase by sorption onto floc and are further degraded by microbes with flocs (Khanal *et al.* 2006; Zeng *et al.* 2009b). The activated sludge system has been reported to have better estrogen removal efficiency than trickling filters (Ziegler & Wittwer 2005). Ying *et al.* (2008a) investigated the fate and removal of estrogens in four sewage treatment plants, indicating that EE2 was

more persistent during treatment compared with E2. Hashimoto & Murakami (2009) have studied the removal of natural and synthetic estrogens by activated sludge in batch experiments and observed rapid removal and degradation of E2, whereas the removal of EE2 showed the lag phase although EE2 was finally removed and degraded completely after 24 h. The aerobic degradation of E2 and EE2 was investigated in batch experiments with activated sludge from a conventional and a membrane sewage treatment plant by Weber *et al.* (2005). They indicated that E2 was completely transformed within 3 days, but EE2 was persistent in both sludges.

Although more than 90% removal efficiency of estrogens in activated sludge treatment systems had been achieved, they are still major contributors of effluent estrogenic activity in a large number of WWTPs (Joss *et al.* 2004; Furuichi *et al.* 2006; Nakada *et al.* 2006). Due to their harmful effects on the hormonal system (Sharp & Skakkebaek 1993; Tyler *et al.* 2005), many researchers have endeavored to develop more efficient removal techniques (Balest *et al.* 2008; Dytczak

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et al. 2008). Balest *et al.* 2008 used a sequencing batch biofilter granular reactor (SBBGR) to treat endocrine disrupter compounds including E2 and EE2, and found that SBBGR performed better than a conventional activated sludge process in removing E2, but it is not an ideal method for EE2. Dytczak *et al.* (2008) indicated that higher removal rates of estrogens were associated with higher nitrification rates, but EE2 was persistent under anoxic conditions. However, a recent study by Zeng *et al.* (2009b) found that EE2 could be degraded under anoxic conditions. Due to the difficulty to completely remove estrogens by the common wastewater treatment processes, a better understanding of the fate of estrogens during the wastewater treatment process is critical to the development and implementation of suitable control strategies for WWTP.

In the present study, a laboratory-scale anaerobic-anoxic-oxic (AAO) activated sludge system was established to investigate the removal and fate of E2 and EE2. Removal mechanisms including sorption and biodegradation in the activated sludge process were studied. Mass balance was calculated to assess the fate of the two estrogens during the biological treatment process.

MATERIALS AND METHODS

Chemicals

17 β -estradiol (CAS 50-28-2, >98%) and 17 α -ethinylestradiol (CAS 57-63-2, >98%) were purchased from Sigma-Aldrich (St. Louis, USA). Other reagents of analytical grade were supplied by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

AAO reactor system

The laboratory-scale anaerobic-anoxic-oxic (AAO) activated sludge system consisted of a 2-L anaerobic reactor, a 5-L anoxic reactor, a 9-L aerobic reactor and a settling reactor

(Figure 1), which were all made of polyethylene. Mixing in the anaerobic and anoxic reactors was achieved using motor-driven mixers. The aerobic reactor was equipped with a set of diffusion aerators to maintain the dissolved oxygen in the range of 2–4 mg/L. The sludge was returned from the bottom of the settling reactor to the anaerobic reactor. The mixed liquor was recycled from the aerobic reactor to the anoxic reactor. The system was placed in a temperature-controlled room at 20°C, and was conducted under the following conditions: hydraulic retention time (HRT) = 8 h, sludge retention time (SRT) = 20 d, sludge circulation ratio = 100%, the mixed liquor circulation = 300%.

Wastewater and seed sludge

The wastewater was taken from Shanghai Quyang Municipal WWTP. The conventional parameters of the wastewater during the experiment were as follows: COD = 248–293 mg/L, NO₃⁻-N = 0.38–0.53 mg/L, NH₄⁺-N = 23–24 mg/L, TN = 25–45 mg/L, TP = 2.8–4.4 mg/L. The average concentrations of E2 and EE2 were 50 and 8 ng/L, respectively.

The seed sludge was obtained from the returning activated sludge tank of Shanghai Changqiao Municipal WWTP. It was acclimated with the above wastewater. In order to investigate the fate of E2 and EE2 during the treatment with AAO system, E2 and EE2 were spiked with the concentrations of 15 μ g/L and 5 μ g/L, respectively. After 1 month's acclimation, the performance of the system was stable and the mixed liquor suspended solid (MLSS) in the system was about 3000 mg/L.

Sample preparation and analytical methods

The aqueous phase and sludge phase samples were prepared according to the method described by Zeng *et al.* (2009a). Briefly, 250 mL aqueous sample was filtered through a 0.45 μ m glass microfiber filter (GF/C filter) to eliminate suspended solid matter. The filtrate was extracted with C18 solid-phase extraction (SPE) cartridges using a Visiprep

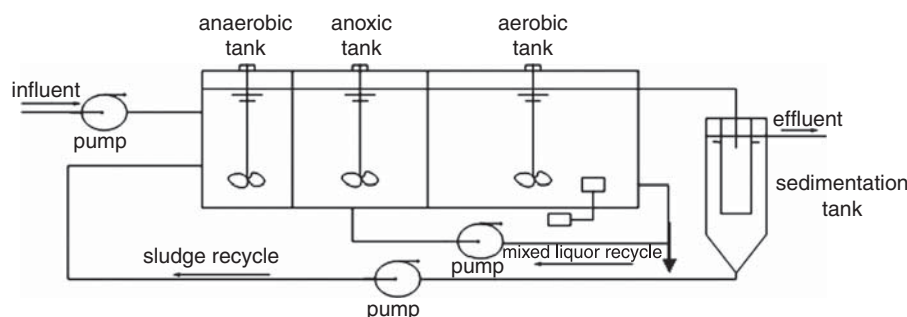


Figure 1 | Schematic diagram of the anaerobic-anoxic-oxic (AAO) activated sludge system.

system (Supelco, Sigma-Aldrich, USA). Then, the cartridges were eluted sequentially using 4 mL of methanol and 10 mL of dichloromethane. After the eluate dryness with nitrogen, the residue was dissolved in 1 mL of methanol. The sludge phases were freeze-dried. The dried pellets were ground into powder before extraction. E2 and EE2 in the sludge phase were extracted ultrasonically and subsequently separated by centrifugation. The supernatants were mixed and followed by solvent evaporation. After evaporation, the residue was dissolved in 300 mL of distilled water and go through the same procedure as the aqueous samples.

The concentration of E2 and EE2 was determined using high performance liquid chromatography (HPLC) equipped with a fluorescence detector (Prostar, Varian) according to the method described by Zeng *et al.* (2009a). The water-acetonitrile solutions (65/35 for E2; 61/39 for EE2; v/v) were employed as the mobile phase with a constant flow rate of 1 ml/min at room temperature. The excitation/emission wavelengths were 280/307 nm. The volume injection was 20 μ L. The detection limit of instrument was lower than 1 μ g/L, and a linear calibration was obtained in the range of 5–2500 μ g/L.

Mass balance calculation

The mass balance of estrogens in AAO system was shown in Figure 2. The methodology presented by Joss *et al.* 2005 was used for reference. To simplify the mass balance, the following assumptions are made: (1) the influent and effluent biomass concentrations are negligible; (2) the reactors are ideal continuously stirred tank reactors (CSTR), and all reactions occur within the reactors (i.e. $C_3 = C_e = C_{ww} = C_r$). The possible fates of estrogens in the AAO system include discharging with final effluent and waste sludge and being degraded by activated sludge. The fate of estrogens is calculated based on the following mass balance:

$$M_i = M_e + M_{ww} + M_{biol} \quad (1)$$

Where M_i , M_e , and M_{ww} are estrogen masses in the influent,

final effluent, and waste sludge of the AAO system, respectively (μ g/d). They were calculated based on the relevant measured estrogen concentrations. M_{biol} is estrogen mass degraded by the activated sludge in the system (μ g/d), which was calculated from the difference in the masses of the inluent, effluent and waste sludge.

RESULTS AND DISCUSSION

Removal of conventional parameters

As AAO activated sludge system is usually designed to remove nutrients in the wastewater, conventional parameters were first measured, as shown in Table 1. The results indicated that the average removal efficiencies of NH_4^+ -N, TN, TP and COD_{cr} were 99.2%, 81.0%, 90.6%, 86.3%, respectively. The effluent concentrations of the above parameters are up to the national first-class standard for the discharge of municipal wastewater (GB 18918 2002).

Estrogen removal

In each reactor, a sample of mixed liquor was collected, and the concentrations of estrogens in the aqueous and solid phases were determined separately. The concentrations of E2 and EE2 in the influent, final effluent, and the aqueous and solid phases of each reactor in the AAO system are listed in Table 2. E2 was not detected in the final effluent. The overall removal efficiency of EE2 was $80.1 \pm 0.7\%$. The removal percentage by anaerobic, anoxic and aerobic reactors of the total removed E2 and EE2 were shown in Figure 3. Anaerobic, anoxic and aerobic reactors accounted for $71 \pm 0.4\%$, $7 \pm 0.3\%$ and $22 \pm 0.6\%$ of the total removed E2; the three reactors respectively contributed $44 \pm 0.3\%$, $8 \pm 0.4\%$ and $48 \pm 1.5\%$ to the totally EE2 removal.

E2 was significantly eliminated in the anaerobic reactor, and then it was further removed in the anoxic and oxic reactors. E2 concentration in the solid phase of the aerobic

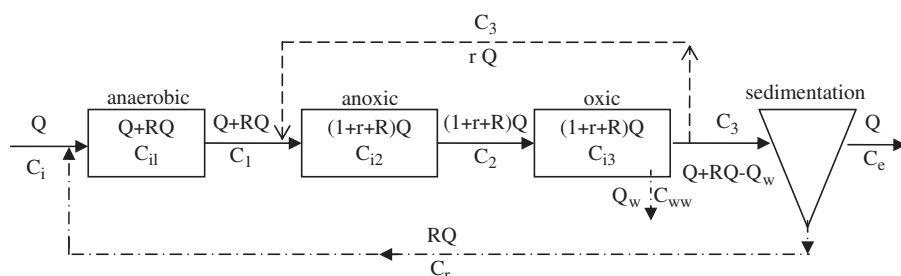


Figure 2 | Flow chart of AAO system for mass balance calculation.

Table 1 | Removal of conventional parameters in the AAO system

	COD _{cr}	TN	TP	NH ₄ ⁺ -N	NO ₃ ⁻ -N
Influent (mg/L) ^a	248–293 (2 7 1)	25.1–45.2 (32.4)	2.80–4.40 (3.63)	23.9–24.3 (24.1)	0.38–0.53 (0.44)
Effluent (mg/L) ^a	36.4–38.0 (37.2)	4.78–8.10 (6.15)	0.17–0.49 (0.34)	0.17–0.22 (0.19)	4.3–7.3 (5.4)
Removal efficiency (%) ^b	85.0–87.0 (86.3)	79.8–82.2 (81.0)	89.7–93.9 (90.6)	99.1–99.3 (99.2)	/

^aConcentration range and average in the parentheses.

^bRemoval efficiency range and average in the parentheses.

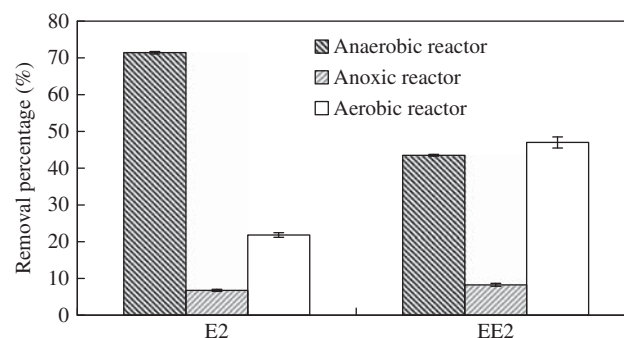
reactor was 0.057 ± 0.004 µg/L but not detected in the aqueous phase. The residual E2 on the sludge phase in the aerobic reactor suggested that E2 may remain in the sludge and discharged with the waste sludge. In addition, the solid phase contained higher levels of estrogens compared with those in the aqueous phases in each reactor. This indicated that E2 and EE2 were easily sorbed onto the sludge. It had been reported by some researchers (Furuichi *et al.* 2006; Suzuki & Maruyama 2006; Zeng *et al.* 2009a) that estrogens were easy to be sorbed onto the activated sludge and be further biodegraded. Calculated using the E2 concentrations in the inlet and the outlet mixed liquor of each reactor, the degradation efficiencies in the anaerobic, anoxic and aerobic reactors were $10 \pm 0.5\%$, $37 \pm 1\%$, $97 \pm 0.1\%$, respectively, indicating E2 was mainly degraded in the aerobic reactor, but could be biodegraded under anaerobic conditions. Czajka & Londry (2006) indicated that E2 could be readily transformed to E1 under anaerobic conditions and the oxidation of E2 was not inhibited by the presence of E1. The complete degradation of estrogens under

anaerobic conditions was minimal, suggesting that they would accumulate in anaerobic environments. Hashimoto & Murakami (2009) indicated that the anoxic condition was not favorable to the effective removal of estrogens as compared with the aerobic condition. In the present study, the much higher degradation efficiency of E2 in the aerobic reactor indicated the faster degradation rate of E2 under aerobic conditions compared to that under anaerobic conditions.

EE2 was not as easily biodegraded as E2. The total concentration of EE2 in the mixed liquor in the anaerobic reactor was even greater than the influent concentration, indicating the significant accumulation of EE2 in the solid phase in the system. It appeared that EE2 was not degraded in the anaerobic reactor, but was degraded in the following anoxic reactor and the aerobic reactor. Calculated using the EE2 concentrations in the inlet mixed liquor and the outlet mixed liquor of each reactor, its degradation efficiencies in the anoxic and aerobic reactors were $10 \pm 0.9\%$ and $27 \pm 2\%$, respectively. It has been indicated that under aerobic conditions, biodegradation played a significant role in removing EE2 from wastewater (Dytczak *et al.* 2008; Hashimoto & Murakami 2009). However, the practical use of EE2 degradation under anaerobic conditions was debatable. (Ying *et al.* 2008b) pointed out that EE2 was persistent under denitrifying conditions. (Czajka *et al.* 2006) insisted that no anaerobic

Table 2 | The concentrations of E2 and EE2 at different sampling points in AAO system

Estrogens concentration		E2 (µg/L)	EE2 (µg/L)
Influent		15.80.9	5.0340.58
Anaerobic reactor	Aqueous phase	2.260.16	2.140.22
	Solid phase	4.870.26	4.660.44
	Mixed liquor	7.130.41	6.800.66
Anoxic reactor	Aqueous phase	0.690.06	1.400.12
	Solid phase	1.120.08	2.630.23
	Mixed liquor	1.810.14	4.030.35
Aerobic reactor	Aqueous phase	n.d.	1.020.07
	Solid phase	0.0570.004	1.900.14
	Mixed liquor	0.0570.004	2.930.22
Final effluent		n.d.	1.000.08

**Figure 3** | The removal percentage from each biological reactor of the overall estrogen removal.

degradation of EE2 at 5 mg/L was observed in multiple trials over long incubation periods (more than three years), even reduction of electron acceptors such as nitrate, sulfate, and iron were provided. However, the results of Sarmah & Northcott (2008) showed rapid degradation (greater than 90%) of EE2 within the first 2 to 4 d under these anaerobic conditions although the degradation rate was extremely low for the remaining period. Our previous study also found that EE2 removal under anoxic conditions was mostly due to biodegradation (Zeng *et al.* 2009b). The results of the present study further verified the biodegradation potential of EE2 under anoxic conditions.

Distribution coefficient of estrogens on the activated sludge

In each reactor, the solid phase contained higher level of estrogens, indicating that sorption was the key factor controlling the removal of estrogens in activated sludge systems. It has been indicated that partitioning played a dominant role in the sorption of E2 and EE2 on the activated sludge (Strenn *et al.* 2003; Andersen *et al.* 2005; Zeng *et al.* 2009a,b). Distribution coefficients (K_d) between water and activated sludge particles of E2 and EE2 in anaerobic, anoxic and oxic reactors in AAO system were listed in Table 3. It shows that the distribution coefficients of estrogens on the activated sludge in the aerobic and anoxic reactors were lower than those in the anaerobic reactor, suggesting that estrogens are more easily sorbed onto the activated sludge under anaerobic conditions. This is consistent with the previous study using batch reactors (Zeng *et al.* 2009a). Carballa *et al.* (2004) proposed to use the distribution coefficient (K_d) to calculate the concentrations in the sludge from those measured in the aqueous phase. K_d in Table 3 may be used to evaluate the fate of E2 and EE2 in the biological treatment process, thus avoiding the expensive and time-consuming analysis of these chemicals in the sludge phase.

Fate of E2 and EE2 in the AAO system

Mass balance calculation for the fate of E2 and EE2 in the AAO system is shown in Table 4. While $99.99 \pm 0.0002\%$ of the total influent E2 was biodegraded, only $0.010 \pm 0.0002\%$ of the total influent E2 was discharged with the waste sludge. This indicates that biodegradation is important for the fate of E2 in WWTP compared to sorption. In contrast, only $79.13 \pm 0.77\%$ of the total influent EE2 was biodegraded in the AAO system. The percentage of EE2 discharged with the

Table 3 | The distribution coefficients of estrogens on the activated sludge in each reactor

Estrogen	Reactor in AAO system	Average value (L/kg)
E2	Anaerobic reactor	719 ± 18
	Anoxic reactor	538 ± 13
EE2	Anaerobic reactor	727 ± 9
	Anoxic reactor	624 ± 7
	Aerobic reactor	620 ± 3

effluent and waste sludge were $19.9 \pm 0.7\%$ and $0.97 \pm 0.04\%$, respectively. The relatively higher percentage of EE2 on the waste sludge suggests its recalcitrant property. Therefore, both sorption and biodegradation play important roles in the removal of EE2 in WWTP. These results are similar to those reported in other studies (Carballa *et al.* 2004; Khanal *et al.* 2006; Esperanza *et al.* 2007; Ying *et al.* 2008a; Liu *et al.* 2009). EE2 remaining in the sludge phase might go back to the system with the return sludge, and thus make the EE2 concentration in the mixed liquor in the anaerobic reactor greater than that in the influent, as shown in Table 2.

CONCLUSIONS

E2 was completely removed and the removal efficiency of EE2 was about 80% during the treatment by AAO activated sludge system. The removal of estrogens in the activated sludge system was attributed to sorption and biodegradation. According to the distribution coefficients, the sorption ability of activated sludge was greater in the anaerobic reactor than in the anoxic and aerobic reactors. While E2 was degraded in all the three units of the AAO system, EE2 was only degraded in the anoxic and aerobic units. Mass balance calculation indicated that biodegradation is important for the fate of E2 in WWTP compared to sorption, and 99.99% of the total influent E2 was biodegraded in the AAO system; little E2 was accumulated in the sludge. Nevertheless, both sorption and

Table 4 | The fate of E2 and EE2 in AAO system

Fate (%)	E2	EE2
In the final effluent	0	19.90 ± 0.74
In the waste sludge	0.010 ± 0.0002	0.97 ± 0.04
Biodegraded by activated sludge	99.99 ± 0.0002	79.13 ± 0.77

biodegradation play important roles in the removal of EE2 in WWTP. In the present AAO system, 79.1% EE2 was degraded by activated sludge, 19.9% was discharged in the effluent, and 1% remained in the waste sludge.

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