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# Anaerobic biodegradation characteristics of estrone, estradiol, and 17α-ethinylestradiol in activated sludge batch tests

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ABSTRACT

The anaerobic degradation characteristics of estrone ($E_1$), estradiol ($E_2$), and 17α-ethinylestradiol ($EE_2$) under single and combined conditions were investigated in batch tests and the influence of initial estrogen and sludge concentrations on the biodegradation process was studied. The results indicated that the degradation process of three estrogens was biphasic. The first phase was fast and the second phase was slow. The two phases could be described by the first-order degradation kinetics. In the single degradation experiments, at the fast stage, $E_2$ degradation rate was the highest for the three estrogens, which was 2 and 5.17 times higher than $E_1$ and $EE_2$, respectively. However, at the slow stage, $EE_2$ almost could not be biodegraded, and the degradation rate constant of $E_1$ and $E_2$ was 0.18 and 0.34 times lower than that at fast degradation phase, respectively. Meanwhile, $E_1$ was accumulated in the process of $E_2$ degradation, which indicated that $E_1$ was a main intermediate product of the $E_2$ anaerobic biodegradation. In the combined degradation experiments, the degradation rate constants of estrogens had the order of $E_1 > E_2 > EE_2$, and the contribution of fast degradation stage was crucial to the removal of estrogens under anaerobic conditions. The degradation rate constant of estrogens decreased linearly with the initial estrogen concentrations and increased linearly with the sludge concentrations, which indicated that the higher sludge concentrations benefited the removal of estrogens under anaerobic conditions in the real wastewater treatment practice.

*Keywords:* Estrogen; Anaerobic sludge; Biodegradation; Kinetics

## 1. Introduction

In recent years, the estrogen problems in environment have aroused comprehensive concerns, mainly including natural and synthetic estrogens [1,2]. Natural estrogens, such as estrone ($E_1$), estradiol ($E_2$), and estriol ($E_3$), which are mainly released by humans and livestock, are discharged into sewage system at microgram levels [3]. 17α-ethinylestradiol ($EE_2$), which is a recalcitrant synthetic estrogen and the active composition of contraceptive pill, presents in the urine at the form of conjugated glucuronide or sulfate complexes [4]. These estrogens widely exist in urban

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water system. Previous studies indicated that the maximum $E_2$ concentration in the influent and effluent of the sewage treatment plants (STPs) could reach at the level of 150 and 64 ng/L [5,6], while the level of free $EE_2$ were 7 and 42 ng/L [7,8], respectively. Although the concentrations of these estrogens are very low, they can disrupt the endocrine system of the aquatic animals and cause extensive influence on the ecosystem [9]. For example, previous studies showed that very low $E_2$ concentration (even at 0.1 ng/L) could stimulate and induce the production of vitellogenin in male fish [10,11]. Moreover, Larsson et al. also found $EE_2$ at the level of 0.1–10 ng/L could cause potential danger to fish and other aquatic organisms [12].

Removal of estrogen from aqueous phase in the sewage treatment process can be achieved by three pathways, including biodegradation, solid adsorption, and volatilization [3]. Due to the very low henry constant of estrogens it is very difficult for them to volatilize from the aqueous phase, so the volatilization of estrogen can be ignored. Meanwhile, many researchers detailedly investigated the aerobic biodegradation and adsorption behaviors of estrogens in activated sludge system [13–16]. However, little study was conducted to investigate the anaerobic biodegradation characteristics of estrogen in activated sludge. In our previous studies, we found that anaerobic tank had an important role in removing estrogen in anaerobic-anoxic-oxic process. Therefore, it is very necessary to study the biodegradation characteristics of estrogen under anaerobic condition.

In this study, three widely existed estrogens ($E_1$, $E_2$, and $EE_2$) were selected to investigate the anaerobic biodegradation behaviors and kinetics under the single and combined conditions. In addition, the influence of sludge and estrogen concentrations on the biodegradation behaviors was also evaluated. It had important directive significance to determine the removal mechanisms of estrogens in practical wastewater treatment processes.

## 2. Materials and methods

### 2.1. Reagents and chemicals

$E_1$, $E_2$, and $EE_2$ were all of chromatographic pure and purchased from Sigma, China. The structural formulas of the three estrogens were shown in Fig. 1. The concentration of estrogen stock solution with methanol as co-solvent (water/methanol = 9:1) was 1,000 mg/L. Methanol (High performance liquid chromatography, grade) was obtained from Dikma Ltd., Co. All other chemicals, such as $NH_4Cl$, $MgCl_2{\cdot}6H_2O$, $KH_2PO_4$, $NaHCO_3$, $FeCl_2$, $Na_2S$, and HCl, were of analytical grade. Distilled water was used in all experiments.

### 2.2. Experimental set-up

#### 2.2.1. Source of activated sludge

The anaerobic sludge was taken from the anaerobic tank of Xinyigou STP, Harbin, China, which was operated at an anaerobic/anoxic/aerobic mode and mainly designed for treating civil domestic wastewater from Harbin urban area. The sludge was sampled and stored in a 10 L water container and was transported to lab within 2 h. After sludge settlement, the supernatant was removed and the distilled water was refilled to the container. This process was repeated 3–4 times to remove the organic matters adsorbing on the sludge. Then, the sludge concentration was adjusted to 10,000 mg/L and stored at 4℃ before using.

#### 2.2.2. Experimental designs

The estrogens degradation experiments were conducted in 100 mL serum bottles which operated in batch mode. Strict anaerobic microbial techniques were adopted throughout the experiment manipulations as previously described [17]. Mineral salt medium consisted of $NH_4Cl$ (1.5 g/L), $CaCl_2$ (0.1 g/L),

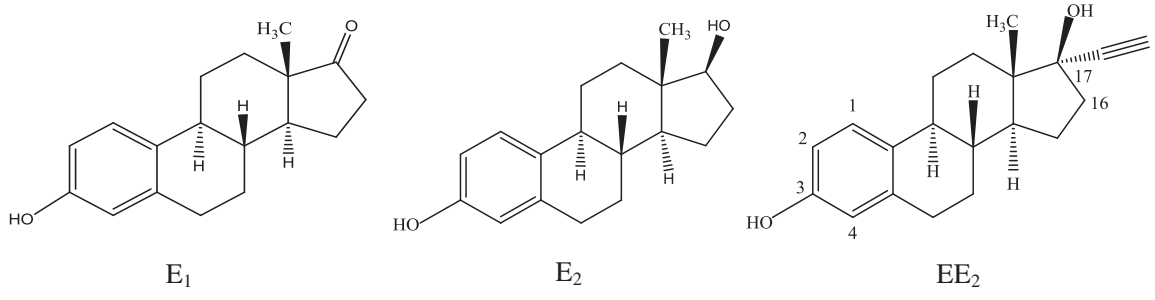


Fig. 1. The structural formulas of the three estrogens.

$MgCl_2{\cdot}6H_2O$ (0.1 g/L), and $KH_2PO_4$ (0.6 g/L). Then, it was boiled for 20 min and allowed to cool down under a gentle stream of nitrogen. After cooling, $NaHCO_3$ (2.52 g/L), $FeCl_2{\cdot}4H_2O$ (0.368 g/L), $Na_2S{\cdot}9H_2O$ (0.5 g/L), and resazurin (0.001 g/L) were added, and the medium pH value was adjusted to approximately 7.0 by using 0.1 mol/L HCl. The serum bottles containing the anaerobic sludge and fresh medium were pre-cultured for a week under dark conditions at 30˚C and 130 rpm in a shaking table for avoiding the introduction of other organic compounds in reactors and keeping the strict anaerobic conditions. Then, the estrogen stock solution was injected into the pre-cultured serum bottles. A volume of 0.5 mL mixed liquor was sampled from the serum bottles at the predetermined time (0, 1, 2, 5, 8, 12, 24, 36, 48, 60, 72, 96, 120, and 168 h) after adding the estrogens. Before sampling, nitrogen was firstly injected into the bottles to prevent the injection of oxygen gas. Methanol was used to elute the estrogen adsorbed by the sludge. In detail, 0.5 mL mixture liquor and 0.5 mL methanol were added into a 1.5 mL centrifuge tube. Then, it was mixed violently on a vortex mixer for 15 min and centrifuged at 4,000 rpm for 5 min. The supernatant was filtered by 0.45 μm organic filtration membrane and estrogen concentration was analyzed by HPLC.

Estrogen concentration in this study was set as high microgram per liter level, due to some industry wastewater, such as contraceptive factory wastewater which contained much higher estrogen than the natural water. Keeping the estrogen concentration as 5 mg/L, sludge concentration as 1,000 mg/L, and agitation at 125 rpm, the degradation characteristics of single estrogen were investigated. Under the condition of three estrogen coexisting, the influence of estrogen concentrations on its degradation was conducted at sludge concentration of 1,000 mg/L and estrogen concentrations of 1, 5, and 10 mg/L, while effects of sludge concentrations were conducted at estrogen concentration of 5 mg/L and sludge concentrations of 500, 1,000, and 2,000 mg/L, respectively.

### 2.3. Analytical methods

Three estrogens were all analyzed by HPLC equipped with a UV detector (SPD-10A VP, SHIMADZU, 280 nm) and an Extend-C18 reversed-phase column (250 mm × 4.6 mm, particle size 5 μm) [13]. The mobile phase consisted of acetonitrile/10 mM phosphoric acid (50:50, *v*/*v*) at a flow of 1.0 mL/min. The injection volume was 20 μl. Retention time of $E_1$, $E_2$, and $EE_2$ were 6.52 min, 4.62 min, and 5.88 min, respectively. A series of injected estrogen in different concentrations (0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, and 5.0 mg/L) were conducted to determine the linear concentration range. Good linearity was obtained with correlation coefficients all above 0.994. The unknown concentration was calculated from the calibration curves. The limit of detection (LOD) and limit of quantification (LOQ) were determined from a signal to noise of three and six, with the LOD of 0.04–0.05 mg/L and LOQ of 0.08–0.1 mg/L for three estrogens. Three replicated standard samples containing 0.1 mg/L of each estrogen were used to determine the standard deviation, which were all less than 5%. The pH was measured with a PHSJ-3F pH-meter (Shanghai Precision & Scientific Instrument Co. Ltd.).

## 3. Results and discussion

### 3.1. Single degradation of three estrogens

The evolution of three estrogen concentrations during the single biodegradation period is shown in Fig. 2. Although the initial conditions were identical, there were great differences in concentration variation during the biodegradation process. Anaerobic biodegradation process of estrogens could be divided into two phases: rapid and slow degradation phase. For $E_2$, it decreased to 2.96 mg/L at 12 h with the removal efficiency of 45.5%, while it reduced very slowly to 0.38 mg/L with a removal efficiency of 92.9% after biodegrading for 168 h. It then reduced to below detection line (BDL). In addition, $E_1$ was produced after $E_2$ degrading for 24 h. $E_1$ concentration increased linearly with time and reached to 1.09 mg/L at 120 h. Then, it had a slightly downward trend, which indicated that $E_1$ was the intermediate product in the process of $E_2$ biodegradation. The accumulation of $E_1$ was due to $E_2$ having larger degradation rate than $E_1$. Kjoholt et al. also found that the half time of $E_1$ and $E_2$ in activated sludge under anoxic conditions was 80 min and 2.01 min [18], which were much faster than that in present study. After finishing the $E_2$ degradation, $E_1$ degradation would predominate. Czajka et al. found the transformation half time of $E_2$ under anaerobic condition was 15 *d*, which was similar with that at rapid degradation phase [19]. Jurgens et al. investigated the biodegradation capacity of $E_2$ in river sediment under the anaerobic condition at 20˚C, and found that $E_2$ could be rapidly transformed to $E_1$ and the process was completed in two days [20]. Joss et al. conducted the batch tests by using the supernatant of activated sludge under anaerobic condition and found that 50% of $E_2$ was transformed to $E_1$ after 7 days. They did not observe the further biodegradation of $E_1$

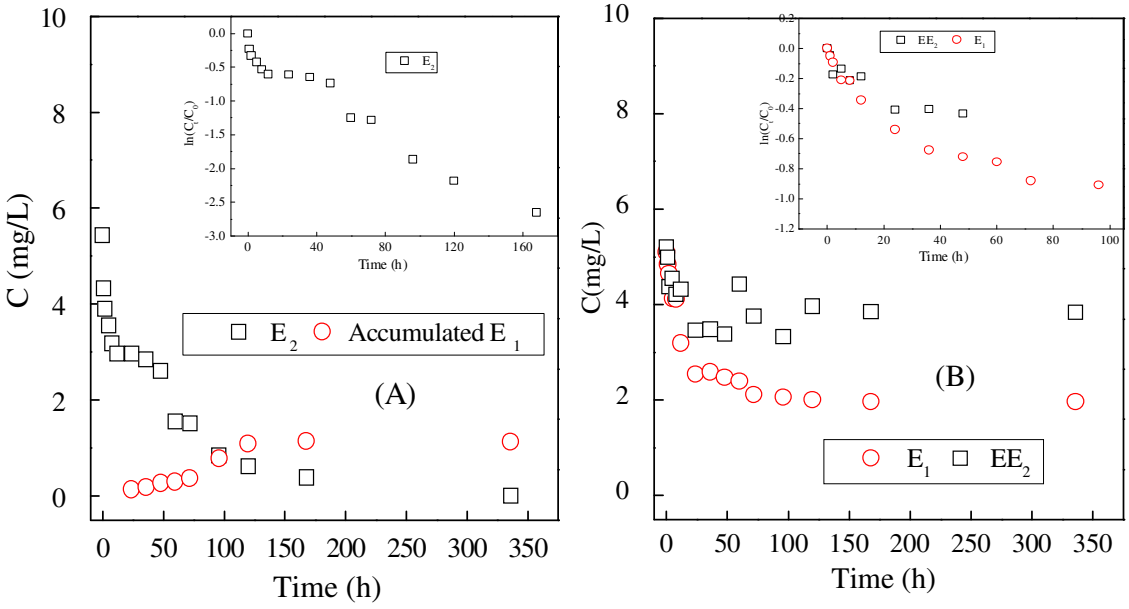


Fig. 2. Single biodegradation characteristics of $E_2$ (A), $E_1$, and $EE_2$ (B) under anaerobic conditions (The experiments were conducted at initial estrogen of 5 mg/L, MLVSS of 1,000 mg/L, temperature at 35°C, and initial pH value of 7.).

and thought that $E_1$ was accumulated as a by-product and there still existed available electron acceptors, such as $Fe^{3+}$ and other organic compounds, to transform $E_2$ under anaerobic conditions [21]. The difference in degradation rate of estrogen at different researches was related to the micro-organism characteristics, environmental conditions, estrogen concentration, etc.

In case of $E_1$ and $EE_2$, their concentrations reduced rapidly from 5 mg/L to 2.97 and 3.46 mg/L with the removal efficiency of 41.7% and 33.5% in the initial 24 h, respectively. In the later 300 h, $E_1$ biodegraded very slowly and the concentration finally stabilized at 1.97 mg/L with the overall removal efficiency of 61.5%. While $EE_2$ concentration fluctuated at approximately 3.5 mg/L and no further biodegradation was found. It indicated that the initial 24 h was crucial to the removal of estrogens in anaerobic sludge system. Mes et al. also found that there was no significant loss for $EE_2$ under strictly anaerobic condition, and adsorption on sludge was the main removal mechanism from water in anaerobic digest process [22], which was similar with the phenomenon in our present study. The initially rapid removal of estrogens was mainly caused by two reasons. Firstly, rapid adsorption of sludge, including the desorbed and non-desorbed part, decreased the estrogen concentrations in aqueous phase. The relatively high octanol-water partition coefficients of $E_1$ ($\log K_{ow} = 3.1$–4.0), $E_2$ ($\log K_{ow} = 3.1$–4.0), and $EE_2$ ($\log K_{ow} = 4.15$) made them have a high partition coefficient between the anaerobic sludge and liquid phase [23]. Secondly, the higher degradation rate was related to the higher estrogen concentration. The lower residual estrogen concentration led to the lower diffusion and lower biodegradation rate during the process. In comparison, the removal efficiency of three estrogens was in the order of $E_2 > E_1 > EE_2$, which was mainly determined by the structure and characteristics of estrogen.

The biodegradation of all three estrogens could be described by the first-order kinetic model and kinetic parameters listed in Table 1. The estrogen biodegradation could be separated into the rapid and slow degradation phase. In the rapid phase, the degradation rate of three estrogens was 0.0217 $h^{-1}$ ($E_1$), 0.0434 $h^{-1}$ ($E_2$), and 0.0084 $h^{-1}$ ($EE_2$), respectively. $E_2$ degraded most rapidly and was 2 and 5.17 times of $E_1$ and $EE_2$. The half-time of three estrogens was 31.94, 15.97, and 82.5 h. In the slow phase, $EE_2$ almost could not be biodegraded, while the degradation rate of $E_1$ and $E_2$ was 0.0039 $h^{-1}$ and 0.0149 $h^{-1}$, respectively, which was only 0.18 and 0.34 times lower than that at rapid degradation phase. However, their half-time increased to 177.7 and 46.51 h.

When the estrogen concentration decreased to a certain value, the degradation rate reduced and the persistence of estrogen increased. The activated sludge was a synchronically functioning, several-component biological system, consisting of different organisms (micro-organisms, micro- and meta-fauna complexes)

Table 1
Biodegradation kinetics parameters of single estrogen under anaerobic conditions

| Compound | Time period (h) | Kinetic equations | Rate constant ($h^{-1}$) | $R^2$ | $t_{1/2}$ (h) |
|---|---|---|---|---|---|
| $E_1$ | 0–24 | $LnC_t/C_0 = -0.0217t - 0.0462$ | 0.0217 | 0.9631 | 31.94 |
| | 24–120 | $LnC_t/C_0 = -0.0039t - 0.5178$ | 0.0039 | 0.8693 | 177.7 |
| $E_2$ | 0–12 | $LnC_t/C_0 = -0.0434t - 0.1528$ | 0.0434 | 0.8286 | 15.97 |
| | 12–168 | $LnC_t/C_0 = -0.0149t - 0.2616$ | 0.0149 | 0.9608 | 46.51 |
| $EE_2$ | 0–48 | $LnC_t/C_0 = -0.0084t - 0.0944$ | 0.0084 | 0.8242 | 82.5 |

and enzymes, and possessing a specific floccular structure. The structure of activated sludge was inhomogeneous and in its inner layers there were a lot of spaces. A diffusion of intermediate products was accomplished in them. Also the sludge flocs were anionic charged and there were large adsorption surface which could be related to some interaction between the activated sludge and the estrogen molecules. Some estrogen molecule adsorbed to the binding sites with low energy could easily desorb from the sludge and be used by the micro-organisms, while the others adsorbed to that with high energy exhibited difficult to desorb and biodegrade, which was called as locking phenomenon and related to the characteristics of the sludge and estrogen [24].

### 3.2. Effect of estrogen concentration on the biodegradation characteristics

The anaerobic biodegradation characteristics and kinetic parameters of three estrogens at different initial concentrations under the combined conditions are shown in Fig. 3 and Table 2. It could be observed that the degradation of three estrogens also divided into two phases, and the kinetic parameters changed with the initial estrogen concentration. For $E_1$, the concentration change was composed of reduced and increased phase. At the reduced phase, the lowest $E_1$ concentration could reach to 0.66, 2.88, and 3.71 mg/L with the removal efficiencies of 37.1, 43.4, and 62.5%, respectively. While the degradation rate constants of $E_1$ had a negative relativity with the initial $E_1$ concentration ($k = -0.007C_0 + 0.0924$, $R^2 = 0.8805$). The half-time increased from 7.36 h (1 mg/L) to 23.96 h (10 mg/L), and increased by 2.26 times. At the increased phase, $E_1$ concentration increased gradually and reached the highest level of 2.65, 10.36, and 13.7 mg/L, which was mainly caused by the production of $E_1$ from the $E_2$ biodegradation process. The produced rate constants of $E_1$ increased from 0.007 $h^{-1}$ (1 mg/L) to 0.0147 $h^{-1}$ (10 mg/L) and had a positive relationship with the initial $E_1$ concentration ($k = 0.008C_0 + 0.0072$, $R^2 = 0.8643$). The $E_1$ concentration decreased at first phase, where the degradation of

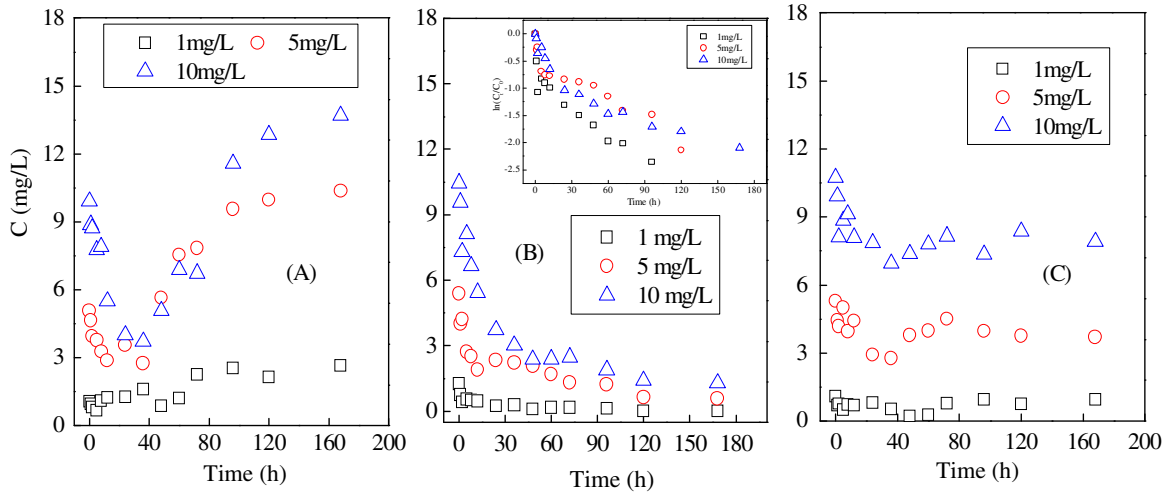


Fig. 3. Effect of estrogen concentrations on the degradation character of $E_1$ (A), $E_2$ (B), and $EE_2$ (C) under the combined conditions (The experiment were conducted at initial estrogen of 1,5 and 10 mg/L, MLVSS of 1,000 mg/L, temperature at 35°C, and initial pH value of 7.).

Table 2
Degradation kinetics parameters of three estrogens at different initial estrogen concentration under anaerobic conditions

| Compound | Estrogen concentration | Time period (h) | Kinetic equations | Rate constant ($h^{-1}$) | $R^2$ | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|
| $E_1$ | 1 | 0–5 | $LnC_t/C_0 = -0.0928t - 0.0243$ | 0.0928 | 0.9681 | 7.36 |
| | | 5–168 | $LnC_t/C_0 = 0.007t - 0.093$ | −0.007 | 0.6434 | −97.57 |
| | 5 | 0–12 | $LnC_t/C_0 = -0.0441t - 0.0685$ | 0.0441 | 0.9309 | 15.48 |
| | | 12–120 | $LnC_t/C_0 = 0.0132t - 0.6811$ | −0.0132 | 0.8208 | −51.74 |
| | 10 | 0–36 | $LnC_t/C_0 = -0.0285t - 0.084$ | 0.0285 | 0.9235 | 23.96 |
| | | 36–120 | $LnC_t/C_0 = 0.0147t - 1.391$ | −0.0147 | 0.9393 | −46.46 |
| $E_2$ | 1 | 0–5 | $LnC_t/C_0 = -0.1506t - 0.1385$ | 0.1506 | 0.8808 | 4.54 |
| | | 5–96 | $LnC_t/C_0 = -0.0171t - 0.8225$ | 0.0171 | 0.9792 | 39.94 |
| | 5 | 0–8 | $LnC_t/C_0 = -0.092t - 0.1041$ | 0.092 | 0.8904 | 7.42 |
| | | 8–168 | $LnC_t/C_0 = -0.0105t - 0.5907$ | 0.0105 | 0.943 | 65.05 |
| | 10 | 0–24 | $LnC_t/C_0 = -0.0403t - 0.1055$ | 0.0403 | 0.9286 | 16.95 |
| | | 24–168 | $LnC_t/C_0 = -0.0073t - 0.9222$ | 0.0073 | 0.964 | 93.56 |
| $EE_2$ | 1 | 0–36 | $LnC_t/C_0 = -0.0043t - 0.3122$ | 0.0043 | 0.2176 | 158.84 |
| | 5 | 0–36 | $LnC_t/C_0 = -0.0061t - 0.1289$ | 0.0061 | 0.4487 | 111.97 |
| | 10 | 0–36 | $LnC_t/C_0 = -0.0097t - 0.0993$ | 0.0097 | 0.8276 | 70.41 |

existing $E_1$ was predominant due to $E_1$ produced from $E_2$ needing a certain time, then increased at the second stage, where the produced rate of $E_1$ from $E_2$ was larger than the $E_1$ degradation rate.

In the case of $E_2$, its degradation process could also be divided into the rapid and slow phase. At rapid degradation phase, $E_2$ concentration reduced from the initial 1, 5, and 10 mg/L to 0.55, 2.52, and 3.72 mg/L, with the removal efficiencies of 45, 50.4, and 62.8%, respectively. The apparent rate constant was calculated as 0.1506, 0.092, and 0.0403 $h^{-1}$, and had a negative relationship with the initial $E_2$ concentration ($k = -0.0122C_0 + 0.1509$, $R^2 = 0.99$). The half-time also increased from 4.54 h (1 mg/L) to 16.95 h (10 mg/L), and increased by 2.73 times. At slow degradation phase, the final $E_2$ concentration decreased to BDL, 0.57 and 1.28 mg/L, with the degradation rate constant of 0.0171, 0.0105, and 0.0073 $h^{-1}$, which was only 0.114, 0.114, and 0.181 times lower than that at rapid degradation phase. It indicated that the rapid degradation stage was crucial to the anaerobic degradation of $E_2$. In addition, the apparent rate constant of $E_2$ at slow stage also had a negative relationship with the initial $E_2$ concentration ($k = -0.0011C_0 + 0.0173$, $R^2 = 0.933$). The half-time of $E_2$ was 39.94, 65.05, and 93.56 h, which was 8.8, 8.77, and 5.52 times higher than that at rapid phase.

For $EE_2$, the degradation process was divided into rapid and slow removal phase. At rapid removal phase (36 h), $EE_2$ concentration reduced from 1, 5, and 10 mg/L to 0.73, 3.84, and 6.97 mg/L, with the removal efficiency of 27, 23.2, and 30.03%, respectively. The apparent rate constant was calculated as 0.0043, 0.0061, and 0.0097 $h^{-1}$, and increased linearly with the $EE_2$ concentration ($k = -0.0006C_0 + 0.0035$, $R^2 = 0.9842$). The half-time decreased from 158.84 h (1 mg/L) to 70.41 h (10 mg/L), and reduced by 55.7%. At the slow removal phase, $EE_2$ concentration stabilized at 0.70, 3.95, and 7.83 mg/L. The $EE_2$ removal efficiency in the whole anaerobic process was 30, 21, and 21.7%, respectively. Due to the addition of methanol in sample pretreatment, which could desorb the reversible adsorbed $EE_2$ on sludge surface, the apparent removal of $EE_2$ was mainly caused by the rapid irreversible adsorption of activated sludge [25]. While the hard biodegradation characteristics of $EE_2$ made the aqueous concentration keeping stabilization after reaching the adsorption equilibrium. Czajka et al. conducted a three year's anaerobic degradation experiment on $EE_2$ and also found that $EE_2$ could not be degraded under anaerobic conditions and the initial removal was caused by the adsorption of anaerobic sludge [19]. It was consistent with the results in our experiment, and also confirmed that the adsorption of anaerobic sludge was the main removal mechanism under anaerobic conditions.

Among the three estrogens, the degradation rate was in the order of $E_2 > E_1 > EE_2$. At the three initial concentrations (1,5, and 10 mg/L), the degradation rate of $E_2$ at rapid phase was the 1.62, 2.09, and 1.41 times higher than that of $E_1$, and 35, 15.1, and 4.2 times higher than that of $EE_2$, which indicated that the anaerobic degradation efficiency was related with the kinds and concentrations of estrogen.

### 3.3. Effect of sludge concentration on the biodegradation characteristics

Concentration change and kinetics parameters of three estrogens at different sludge concentrations are shown in Fig. 4 and Table 3. It could be observed that the estrogen concentration was divided into two phases, and the kinetics parameters were changed with the sludge concentration.

For $E_1$, it was composed of reduced concentration and increased stage, and could be fitted well by the first-order degradation kinetics. At reduced phase (0–12 h), the degradation rate constants increased linearly with the sludge concentration ($k = 0.00005C_{sludge} + 0.0028$, $R^2 = 0.9906$). The half-time reduced from 28.11 h (at 500 mg/L) to 7.11 h (at 2,000 mg/L), and decreased by 2.95 times, which indicated that the higher sludge concentration benefited the $E_1$ removal. The higher sludge concentration not only increased the absolute adsorption amount of estrogen, but also increased the microbial content, which could enhance the degradation rate of estrogen. At increased stage, the higher the sludge concentration, the lower the aqueous $E_1$ concentration. The final $E_1$ under three sludge concentrations stabilized at about 10.2, 9.9, and 7.1 mg/L. The increasing rates of $E_1$ were in the range of 0.0072–0.0163 $h^{-1}$, and the largest value occurred at sludge concentration of 1,000 mg/L, which was the comprehensive result of $E_1$ biodegradation and accumulation from $E_2$. With the increasing sludge concentration, degradation rate of $E_2$ also increased, resulting in the increase of $E_1$ accumulated rate, while the degradation rate of $E_1$ also increased.

In case of $E_2$, the degradation process also contained rapid and slow phase. At rapid phase, the degradation rate constants of $E_2$ increased linearly with the sludge concentration ($k = 0.00007C_{sludge} + 0.0048$, $R^2 = 0.8993$). The half-time reduced from 26.78 h (at 500 mg/L) to 5.19 h (at 2,000 mg/L), and decreased by 4.16 times. Comparing with the rapid phase, the rate constants of slow phase at three sludge concentrations reduced by 63.1, 88.6, and 88.5, which also increased linearly with the sludge concentrations ($k = 0.000004C_{sludge} + 0.007$, $R^2 = 0.9745$). The half-time increased to the 2.7, 8.8, and 8.7 times higher than that at rapid phase, indicating that it was crucial of rapid degradation phase to the $E_2$ removal.

For $EE_2$, at rapid removal phase, its removal rate constant increased from 0.0058 $h^{-1}$ (at 500 mg/L) to 0.0276 $h^{-1}$ (at 2,000 mg/L), and increased by 3.8 times, which also had a positive relationship with sludge concentration ($k = 0.00002C_{sludge} - 0.0033$, $R^2 = 0.9684$). The half-time decreased from 117.76 h to 24.75 h, and reduced by 79%, indicating that the high sludge concentration benefited the $EE_2$ removal. At slow removal phase, $EE_2$ concentration fluctuated at approximate 3.9, 3.86, and 3.04 mg/L with the removal efficiency of 22, 22.8, and 39.2%, which increased with the sludge concentrations.

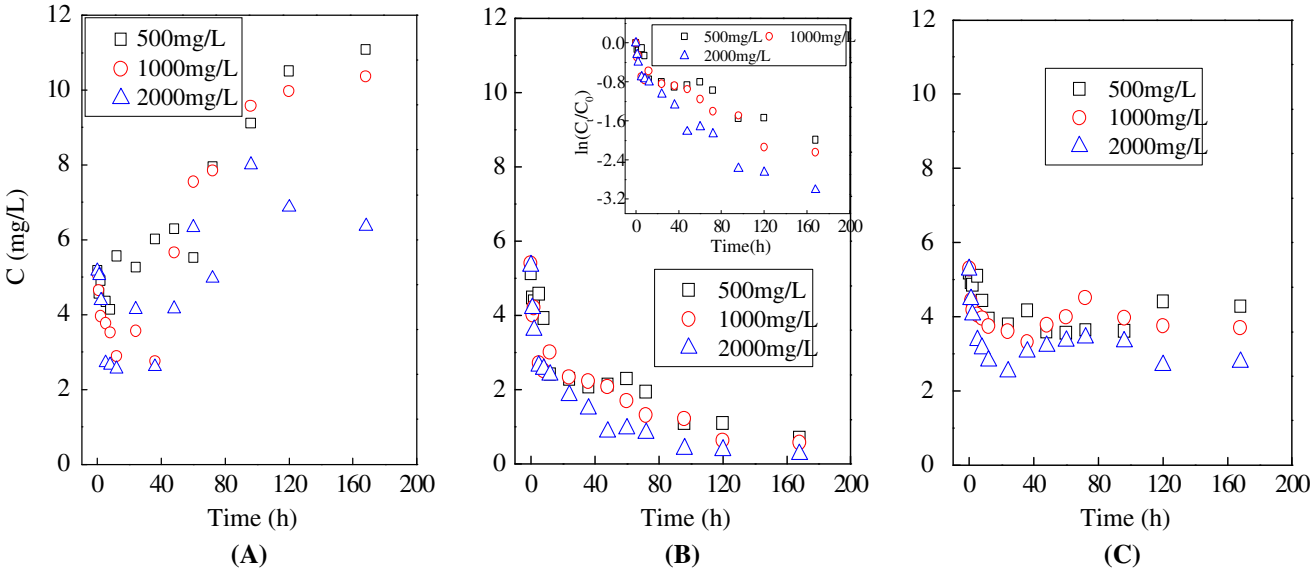


Fig. 4. Effect of sludge concentration on the degradation character of $E_1$ (A), $E_2$ (B), and $EE_2$ (C) under the combined conditions (The experiment were conducted at initial estrogen of 5 mg/L, MLVSS of 500, 1,000 and 2,000 mg/L, temperature at 35 ℃, and initial pH value of 7.).

Table 3
Degradation kinetic parameters of three estrogens at different sludge concentrations under anaerobic conditions

| Compound | Sludge concentration | Time period (h) | Kinetic equations | Rate constant ($h^{-1}$) | $R^2$ | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|
| $E_1$ | 500 | 0–8 | $LnC_t/C_0 = -0.0243t - 0.0367$ | 0.0243 | 0.795 | 28.11 |
| | | 8–120 | $LnC_t/C_0 = 0.0072t - 0.1605$ | −0.0072 | 0.873 | −94.86 |
| | 1,000 | 0–12 | $LnC_t/C_0 = -0.0418t - 0.067$ | 0.0418 | 0.9187 | 16.34 |
| | | 12–96 | $LnC_t/C_0 = 0.0163t - 0.805$ | −0.0163 | 0.8438 | −41.90 |
| | 2,000 | 0–8 | $LnC_t/C_0 = -0.096t - 0.069$ | 0.096 | 0.9024 | 7.11 |
| | | 8–96 | $LnC_t/C_0 = 0.0125t - 0.793$ | −0.0125 | 0.7858 | −54.64 |
| $E_2$ | 500 | 0–36 | $LnC_t/C_0 = -0.0255t - 0.0698$ | 0.0255 | 0.9476 | 26.78 |
| | | 36–168 | $LnC_t/C_0 = -0.0094t - 0.4233$ | 0.0094 | 0.9051 | 72.66 |
| | 1,000 | 0–8 | $LnC_t/C_0 = -0.092t - 0.1041$ | 0.092 | 0.8904 | 7.42 |
| | | 8–168 | $LnC_t/C_0 = -0.0105t - 0.5907$ | 0.0105 | 0.943 | 65.05 |
| | 2,000 | 0–5 | $LnC_t/C_0 = -0.1335t - 0.0673$ | 0.1335 | 0.9598 | 5.19 |
| | | 5–168 | $LnC_t/C_0 = -0.0154t - 0.7481$ | 0.0154 | 0.9414 | 45 |
| $EE_2$ | 500 | 0–60 | $LnC_t/C_0 = -0.0058t - 0.0694$ | 0.0058 | 0.7459 | 117.76 |
| | 1,000 | 0–36 | $LnC_t/C_0 = -0.0094t - 0.1692$ | 0.0094 | 0.7063 | 72.66 |
| | 2,000 | 0–24 | $LnC_t/C_0 = -0.0276t - 0.1891$ | 0.0276 | 0.7846 | 24.75 |

## 4. Conclusions

The anaerobic degradation of estrogens was divided into rapid and slow degradation phase, and could be described well by first-order degradation kinetics. In the single degradation experiment, the rate constant of $E_2$ at rapid phase was 2 and 5.17 times higher than that of $E_1$ and $EE_2$. $EE_2$ almost did not be degraded by the activated sludge at slow phase, and the degradation rate of $E_1$ and $E_2$ also decreased evidently, only 0.18 and 0.34 times lower than that at rapid phase. The initial rapid estrogen removal was related to the irreversible adsorption of estrogen on sludge, which was related with the estrogen kinds and sludge concentration. $E_1$ was the main by-product produced by the degradation of $E_2$ under anaerobic condition, and there was evident accumulation of $E_1$ in the process of $E_2$ degradation.

In the combined degradation experiments, degradation rate had the order of $E_1 > E_2 > EE_2$, and the rapid degradation phase was crucial to the estrogen removal. The degradation rate constant decreased linearly with the initial estrogen concentrations, while increased linearly with the sludge concentrations.

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