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# Degradation of Estrogenic Hormones in a Silt Loam Soil

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Estrogenic hormones are endocrine-disrupting compounds, which disrupt the endocrine system function of animals and humans by mimicking and/or antagonizing endogenous hormones. With the application of sludge biosolid and animal manure as alternative fertilizers in agricultural lands, estrogens enter the soil and become an environmental concern. The degradation kinetics of 17$\beta$-estradiol, an estrogenic hormone of major concern, in a silt loam soil were investigated in this study. It was found that 17$\beta$-estradiol degraded rapidly in nonsterilized soil with a half-life of 0.17 day. The degradation rate constant was proportional to the percentage of nonsterilized soil, indicating that microorganisms are directly responsible for the rapid degradation of 17$\beta$-estradiol in soil. The half-life of 17$\beta$-estradiol in 20% nonsterilized soil was slightly shortened from 1.3 to 0.69 day with the increase of soil moisture from 10 to 20% and was greatly decreased from 4.9 to 0.92 day with the increase of temperature from 15 to 25 °C. The coexistence of 40 $\mu$mol kg$^{-1}$ sulfadimethoxine, a veterinary antibiotic, decreased the degradation rate constant of 17$\beta$-estradiol from 0.750 ± 0.038 to 0.492 ± 0.016 day$^{-1}$. The degradation kinetics of another three estrogenic hormones, including 17α-estradiol, estrone, and estriol, were also investigated and compared. Estrone was identified as a degradation product of 17$\beta$-estradiol and the most persistent hormone among the four investigated estrogens. Estriol was observed in the degradation of estrone and 17α-estradiol.

**KEYWORDS: 17$\beta$-Estradiol; estrogenic hormones; degradation; kinetics; soil; 17α-estradiol; estrone; estriol**

## INTRODUCTION

Estrogenic steroidal hormones are endocrine-disrupting compounds, which disrupt the endocrine system function of animals and humans by mimicking and/or antagonizing endogenous hormones. The occurrence of steroidal hormones at detectable concentrations in the environment has gained increasing attention from both the public and the scientific communities (*1−3*). Two estrogenic hormones, 17$\beta$-estradiol and estrone, are of major concern, because they exert their physiological effects at lower concentrations than other steroids and are detected at concentrations above their lowest observable effect level (*4−6*). It has been reported that exposure to estrogen levels as low as 1 ng L$^{-1}$ is sufficient to cause the feminization of male trout (*7*) and the development of intersex roach in rivers (*8*). On the basis of its link to human breast cancer (*9*), 17$\beta$-estradiol has been classified as a carcinogen (*10*).

In a national reconnaissance, estrogenic steroidal hormones have been detected in 20% of the streams in the United States (*1*). The most likely contamination sources of estrogenic hormones in the environment are the disposals from municipal wastewater treatment plants (WWTPs) and concentrated animal-feeding operations (CAFOs) (*4*). Human-excreted estrogens are treated in WWTPs and then released into the environment. Detectable levels of estrogens are often reported in the effluent (*3, 11, 12*) and the biosolids (*13*) of WWTPs. As a result, raised vitellogenin (an egg yolk precursor protein that is normally produced only by adult females) levels have been detected in fish exposed to WWTPs outfalls (*14, 15*). Besides human waste, animal waste from CAFOs also contains considerable amounts of estrogenic hormones (*16−18*). The incomplete degradation of hormones may occur during manure storage (*19, 20*).

Applying animal manure or sludge biosolids into agricultural lands as alternative fertilizers is a widely adopted practice in modern agriculture (*21*). Through the land application of these solid wastes, estrogenic hormones enter the soil. The aquatic contamination of estrogenic hormones resulting from manure fertilization is frequently reported (*4, 22−25*). Thus, the environmental behaviors of hormones in soil have become of concern. It was reported that the sorption of estrogenic hormones in soil is correlated with soil organic matter and clay mineral content, but not with the $K_{ow}$ of hormones (*26, 27*). Natural and synthetic estrogenic hormones, including 17$\beta$-estradiol, estrone (*28*), and 17α-ethynylestradiol (*29*), were found to degrade rapidly in soil. A biexponential model was found to better fit the degradation kinetics of 17$\beta$-estradiol and estrone in soil than the simple first-order model (*30*). Due to the lack

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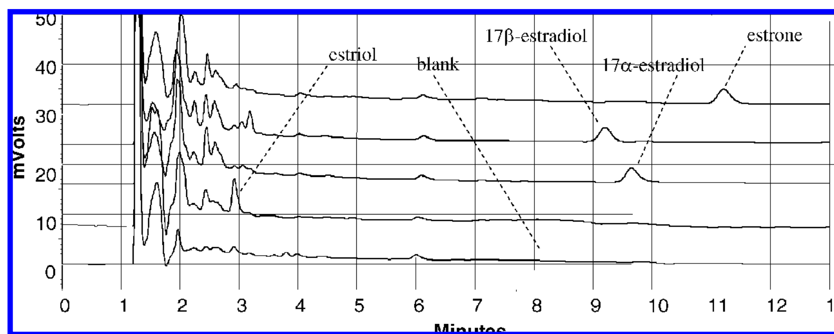


**Figure 1.** HPLC spectra of 17α- and 17$\beta$-estradiol, estrone, and estriol in soil extract.

of clear physical definition of parameters, little mechanistic information from the model fitting is obtained. Soil temperature and moisture appeared to greatly affect hormone degradation (*28*). The addition of municipal biosolids and swine manure slurry was found to hasten the conversion of 17$\beta$-estradiol to estrone and the mineralization of 17$\beta$-estradiol in soil (*31*). However, the role of microorganisms in the degradation process of estrogenic hormones in soil and the degradation kinetics are not well documented. Both 17α-estradiol and estriol are hormonally active metabolites of 17$\beta$-estradiol, but little is known about their degradation in soil.

In this study, the degradation of 17$\beta$-estradiol in soil with different percentages of sterilized soil was investigated, and different kinetic models were used to fit the degradation data. The effects of soil temperature and moisture and the presence of different veterinary antibiotics were quantitatively examined. The degradation rates of 17α- and 17$\beta$-estradiol, estrone, and estriol were compared.

## MATERIALS AND METHODS

**Chemicals and Soil.** Estrone (99%), 17α- (98%) and 17$\beta$-estradiol (97%), estradiol (98%), oxytetracycline (≥98%), sulfadimethoxine (99%), and tylosin (95%) were purchased from Sigma-Aldrich (St. Louis, MO). Water (HPLC grade), acetonitrile (HPLC grade), and acetone (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals were used without further purification.

Soil used in this study was obtained from the top layer (0−10 cm) of a grassland in University Park, PA. On the same day of collection, soil was transported to the laboratory at Delaware State University, Dover, DE, and air-dried. After manual sorting to remove gravels and plant residues, the soil was sieved to 1 mm. The contents of clay, silt, and sand were determined to be 26.9, 65.1, and 8.0%, respectively, and the soil was classified as a silt loam soil on the basis of USDA soil texture classification. Soil pH (at soil/water = 1:2 by weight), organic carbon content, and moisture were determined to be 5.54, 1.44%, and 4.77%, respectively. Sterile soil was prepared by sterilizing the soil at 121 °C in an autoclave three times for 40 min each time.

**Degradation Experiments.** To investigate 17$\beta$-estradiol degradation in soil with different percentages of nonsterilized soil, five portions of soil were prepared by mixing nonsterilized and sterilized soil at five different ratios. The total weight of each portion of soil was 240 g (dry wt). The percentage of nonsterilized soil in the total weight of soil was 10, 20, 25, 33, and 100% by dry weight. In each portion of soil, 0.96 mL of $1.0 \times 10^3$ $\mu$M 17$\beta$-estradiol acetone solution was added to achieve a spiking concentration at 4.0 $\mu$mol kg$^{-1}$ dry wt, and 24.5 g of sterile water was added to adjust soil moisture to 15%. Spiked soils were then thoroughly mixed in a Hamilton Beach chopper. After spiking and mixing, each portion of soil was weighed into seven 250-mL glass jars at approximately 34 g per jar. The weight of each jar was recorded. Jars were then loosely covered with plastic caps and placed in an incubator at 25 ± 0.1 °C. Each jar was weighed, and water was added to compensate for any moisture loss every day. At different durations of incubation within 6 days, one jar from each of the five portions of soil was taken from the incubator and then stored in a freezer at −21 °C until analysis.

For the degradation of 17$\beta$-estradiol in sterilized soil, soil was weighed into 21 50-mL polyethylene centrifuge tubes at 5.00 g (dry wt) per tube after the soil moisture was adjusted to 15%. Tubes were then loosely capped and transferred into the autoclave for sterilization. After the tubes had completely cooled, 20 $\mu$L of $1.0 \times 10^3$ $\mu$M 17$\beta$-estradiol acetone solution was spiked into the soil in each tube. Tubes were then immediately sealed with caps and transferred into the incubator for incubation at 25 ± 0.1 °C. At different durations within 6 days, three tubes were taken out and stored in a freezer at −21 °C until analysis.

For all other experiments in this study, the percentage of nonsterilized soil in each portion of soil (240 g dry wt each) was 20%, the soil moisture was 15%, the incubation temperature was 25 ± 0.1 °C, and the initial concentration of spiked hormone was 4.0 $\mu$mol kg$^{-1}$ dry wt. All other experimental conditions were the same as above unless otherwise stated.

In the investigation of moisture effect, different amounts of water were added to control the soil moisture at 10, 15, and 20%. Regarding the degradation in water-saturated soil, soil was weighed into 21 50-mL centrifuge tubes at 5.00 g (dry wt) per tube. After 20 $\mu$L of $1.0 \times 10^3$ $\mu$M 17$\beta$-estradiol acetone solution was spiked into the soil in each tube, a sufficient amount of water was added to make the soil completely steeped in water.

In the experiment of temperature dependence, incubations were conducted at 15 ± 0.1, 25 ± 0.1, and 35 ± 0.2 °C, respectively. In the investigation of antibiotic effect, 0.19, 0.96, and 4.80 mL of $1.0 \times 10^4$ $\mu$M sulfadimethoxine acetone solution, 0.96 mL of $1.0 \times 10^4$ $\mu$M oxytetracycline acetone solution, and 0.96 mL of $1.0 \times 10^4$ $\mu$M tylosin acetone solution were added to soil with 17$\beta$-estradiol.

**Sample Extraction and Hormone Concentration Analysis.** After removal from the freezer and thawing at room temperature, the soil in each jar was thoroughly mixed and then weighed into three 50-mL polyethylene centrifuge tubes at 5.00 g (dry wt) per tube. Five grams of anhydrous $Na_2SO_4$ and 10 mL of acetone were added into each tube. Tubes were vigorously shaken in a reciprocating shaker for 30 min and then centrifuged at $1.1 \times 10^4 g$ for 10 min. The supernatant from each tube was decanted into another centrifuge tube. A second portion of 10 mL of acetone was then added to each tube, and the soil was extracted again. Supernatants from the same extraction tube were collected in the same collection tube. After the collection tubes were centrifuged at $1.1 \times 10^4 g$ for 10 min, supernatants were decanted into 50-mL serum bottles and dried under gentle $N_2$ streams. The dried extract in each serum bottle was then reconstituted with 1.00 mL of methanol. After being thoroughly mixed in a sonicator for 5 min, the suspension in each serum bottle was transferred into a 1.5-mL microcentrifuge tube for centrifugation at $1.5 \times 10^4 g$ for 5 min. The supernatants were then transferred into 1.5-mL sample vials for high-performance liquid chromatograph (HPLC) analysis.

Hormone concentration analysis was performed using a Shimadzu 2010A HPLC. The column was a 4.6 mm × 25 cm Allsphere ODS-2 5 $\mu$m analytical column, and the column temperature was set at 40 °C. The mobile phase was composed of 48% acetonitrile and 52% water (pH was adjusted to 3 with $H_3PO_4$), and the flow rate was 1.5 mL

min$^{-1}$. Sample injection volume was 10 $\mu$L, and the detector wavelength was set at 225 nm. The HPLC spectra of 17α- and 17$\beta$-estradiol, estrone, and estriol in soil extract are shown in **Figure 1**.

The concentration of hormone in soil was calculated on the basis of the equation

$$C_{\text{soil}} = \frac{C_{\text{ext}} \times 1.00}{5.00} \tag{1}$$

where $C_{\text{soil}}$ ($\mu$mol kg$^{-1}$ dry wt) and $C_{\text{ext}}$ ($\mu$M) are the concentrations of 17$\beta$-estradiol or other estrogenic hormone in the soil and soil extract, respectively; 1.00 (mL) and 5.00 (g) are the final volume of soil extract and the mass of extracted soil, respectively.

A recovery test with four replicates at 2.0 $\mu$mol kg$^{-1}$ dry wt was performed. The extraction recoveries of estriol, 17$\beta$- and 17α-estradiol, and estrone from the silt loam soil used in this study were determined to be 96.4 ± 8.2, 86.7 ± 5.6, 89.7 ± 6.7, and 98.2 ± 8.2%, respectively. The detection limits for these four hormones in soil were not higher than 0.20 $\mu$mol kg$^{-1}$ dry wt.

**Kinetic Model.** The degradation kinetics of many organic contaminants in soil can be expressed using eq 2, in which the degradation rate of the target compound is directly proportional to the concentration of nonadsorbed target compound in soil

$$-\frac{dC}{dt} = k\lambda C \tag{2}$$

where $C$ ($\mu$mol kg$^{-1}$) is the total remaining concentration of the target compound at time $t$ (days), $\lambda$ is the molar ratio of the nonadsorbed target compound to the total remaining target compound in soil at time $t$ (days), and $k$ (day$^{-1}$) is the first-order rate constant. When $\lambda$ remains constant during the degradation process, eq 2 can be written as eq 3 and the degradation kinetics follow the simple first-order model

$$\frac{dC}{dt} = -k'C \tag{3}$$

where $k' = \lambda k$. The half-life can be calculated on the basis of eq 4.

$$t_{1/2} = \frac{0.693}{k'} \tag{4}$$

When a slow desorption process occurs, however, the percentage of nonadsorbed target compound, which is readily available for degradation, in its total remaining in soil decreases with time and the target compound becomes increasingly unavailable for degradation. Thus, $\lambda$ decreases with time and the degradation kinetics deviate from the simple first-order model. In our previous studies, an availability-adjusted first-order kinetics model based on the decreasing availability of the target compound was developed (*32−34*). Briefly, it is assumed that $\lambda$ is a function of $t$ and can be expressed as

$$\lambda = \lambda_0 \, e^{-at} \tag{5}$$

where $a$ (day$^{-1}$) is a positive coefficient called the availability coefficient and $\lambda_0$ is the value of $\lambda$ at $t = 0$. The higher the value of $a$, the faster the decrease of $\lambda$ with time will be. After substituting eq 5 into eq 2, we get

$$\frac{dC}{dt} = -k''C \, e^{-at} \tag{6}$$

where $k'' = k\lambda_0$. After integration, the availability-adjusted first-order kinetic model is obtained

$$C_t = C_0 \, e^{-\frac{k''}{a}(1-e^{-at})} \tag{7}$$

where $C_0$ and $C_t$ ($\mu$mol kg$^{-1}$) are the target compound concentrations at time 0 and $t$ (days). The half-life of the target compound can be calculated using eq 8, which is derived from eq 7.

$$t_{1/2} = -\frac{1}{a} \ln\left(1 - \frac{0.693a}{k''}\right) \tag{8}$$

Both the simple first-order model and the availability-adjusted first-order model were used to fit the degradation kinetic data. The simple first-order model was always used to describe the degradation kinetics unless the availability-adjusted first-order model was found to better fit the kinetics than the simple first-order model with a higher regression coefficient.

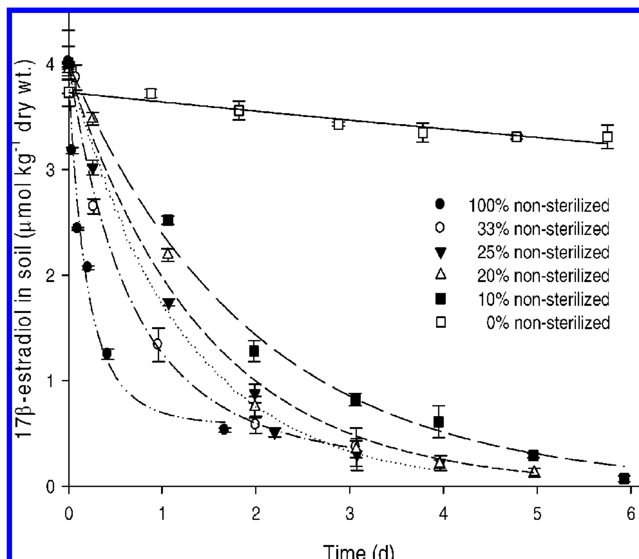


**Figure 2.** Degradation of 17$\beta$-estradiol in soil with different percentages of nonsterilized soil. Points are experimental data, and lines are model-fitting results. Each point and error bar represents the mean value of three samples for analysis (three replicates in 100% nonsterilized) and the corresponding standard deviation.

## RESULTS AND DISCUSSION

**Degradation of 17$\beta$-Estradiol in Soil with Different Percentages of Nonsterilized Soil.** The degradation kinetics of 17$\beta$-estradiol in soil obey the simple first-order model at low percentages of nonsterilized soil (0, 10, 20, and 25%) and follow the availability-adjusted first-order model at high percentages of nonsterilized soil (33 and 100%). Fitting results are shown in **Figure 2**, and the values of model-fitting parameters are shown in **Table 1**.

As shown in **Figure 2**, 17$\beta$-estradiol degraded rapidly in partially and nonsterilized soil. The half-life of 17$\beta$-estradiol in nonsterilized soil is only 0.17 day. On the basis of the study of Colucci et al. (*28*), the half-life of 17$\beta$-estradiol in three different soils at 30 °C ranged from 0.22 to 0.47 day. The obtained half-life in this study is quite consistent with the reported values.

With the decreasing percentage of nonsterilized soil, 17$\beta$-estradiol degradation was slowed. When the percentage of nonsterilized soil reached 0%, no significant degradation was observed. Similarly, 17α-ethynylestradiol, a synthetic estrogenic hormone with a structure similar to that of 17$\beta$-estradiol, was reported to be also very stable in sterile soil (*29*). The persistence of 17$\beta$-estradiol in sterile soil indicates that the degradation of 17$\beta$-estradiol in soil is mainly a biodegradation process. Due to the rapid biodegradation, pretreatments might be needed upon sampling to stabilize 17$\beta$-estradiol in environmental samples. It was reported that acidification (pH ≤2) helps to preserve estrogenic hormones in manure samples (*16, 35*). On the basis of the observation in this study, the authors believe that the acidification sterilized the manure samples; thus, no significant degradation was found. Adding sterilizing agents may preserve estrogenic hormones as well.

Furthermore, a linear correlation was obtained between the degradation rate constant ($k'$ or $k''$) and the percentage of nonsterilized soil (shown in **Figure 3**). The intercept is almost

**Table 1.** Model Fitting Results of 17$\beta$-Estradiol in Soil with Different Percentages of Nonsterilized Soil[a]

| % of nonsterilized soil | rate constant (day$^{-1}$) | availability coefficient (day$^{-1}$) | half-life $t_{1/2}$ (days) | regression coefficient, $r$ |
|---|---|---|---|---|
| 0[b] | $K = 0.024 \pm 0.002$ | | 29 | 0.96 |
| 10[b] | $K = 0.515 \pm 0.028$ | | 1.3 | 0.99 |
| 20[b] | $K = 0.750 \pm 0.038$ | | 0.92 | 0.99 |
| 25[b] | $K' = 0.828 \pm 0.051$ | | 0.84 | 0.99 |
| 33[c] | $K' = 1.43 \pm 0.18$ | $a = 0.44 \pm 0.19$ | 0.54 | 0.99 |
| 100[c] | $K' = 4.55 \pm 0.50$ | $a = 2.38 \pm 0.54$ | 0.17 | 0.99 |

[a] Soil moisture was 15%, and incubation temperature was 25 °C. [b] Fitted using the simple first-order model. [c] Fitted using the availability-adjusted first-order model.

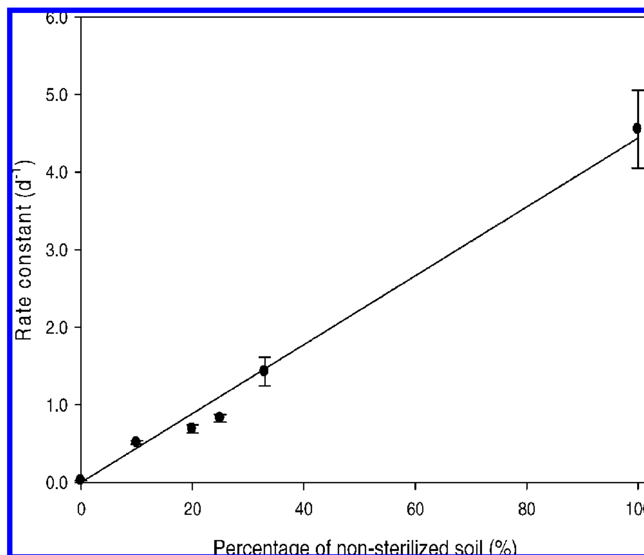


**Figure 3.** Linear correlation between 17$\beta$-estradiol degradation rate constant ($K$ or $K'$) and the percentage of nonsterilized soil.

0, implying that the 17$\beta$-estradiol degradation rate constant in soil is directly proportional to the percentage of nonsterilized soil. This result further confirms that microorganisms are directly responsible for the rapid degradation of 17$\beta$-estradiol in soil. The higher the concentration of degrading microorganisms, the faster the degradation process will be. Even faster degradations could be obtained if fresh soil were used instead of air-dried soil for this study. However, the fast degradation of 17$\beta$-estradiol in sterilized soil was also reported (*28*). We agree with Hanselman et al. (*2*) that the reported fast degradation in autoclaved soil might result from incomplete sterilization in the experiments.

The observation that the degradation rate constant was directly proportional to the percentage of nonsterilized soil also suggests that using a mixture of sterilized and nonsterilized soil might be an acceptable technique to obtain more accurate data in the investigations of the biodegradation of organic contaminants that are stable in sterile soil but degrade rapidly in nonsterilized soil. In this case, the degradation rate constant in nonsterilized soil can be simply calculated on the basis of the equation

$$k_{ns} = k_{ps} \times \frac{100\%}{A\%} \quad (9)$$

where $k_{ns}$ and $k_{ps}$ are the rate constants in nonsterilized and partially sterilized soil, respectively, and $A\%$ is the percentage of nonsterilized soil in the mixture.

The slow desorption process of 17$\beta$-estradiol in soil may be the major factor that caused the degradation kinetics to deviate from the simple first-order model to the availability-adjusted first-order model with the increasing percentage of nonsterilized soil. As stated above, 17$\beta$-estradiol degraded more slowly in soil with low percentages of nonsterilized soil than in soil with high percentages of nonsterilized soil. In samples with low percentages of nonsterilized soil, the decrease of nonsorbed 17$\beta$-estradiol was slow, so the slow desorption process might not limit 17$\beta$-estradiol concentration in solution, keeping $\lambda$, that is, the ratio of nonadsorbed 17$\beta$-estradiol in its total remaining, constant or nearly constant. Hence, the degradation kinetics followed the simple first-order model. However, at high percentages of nonsterilized soil, the decrease of nonsorbed 17$\beta$-estradiol concentration was fast and the slow desorption process might limit 17$\beta$-estradiol concentration in solution. In that case, the ratio of nonsorbed 17$\beta$-estradiol to the total remaining in soil, $\lambda$, would decrease with time, and the degradation kinetics would follow the availability-adjusted first-order model.

**Table 2.** Model-Fitting Results of 17$\beta$-Estradiol Degradation in 20% Nonsterilized Soil at Different Moistures[a]

| soil moisture (%) | rate constant, $K$ (day$^{-1}$) | half-life (days) | regression coefficient, $r$ |
|---|---|---|---|
| 10[b] | $0.548 \pm 0.023$ | 1.3 | 0.99 |
| 15[b] | $0.750 \pm 0.038$ | 0.92 | 0.99 |
| 20[b] | $1.00 \pm 0.034$ | 0.69 | 0.99 |
| saturated[b] | $0.574 \pm 0.034$ | 1.2 | 0.99 |

[a] Incubation temperature was 25 °C. [b] Fitted using the simple first-order model.

**Degradation in Soil with Different Moistures.** The degradation kinetics of 17$\beta$-estradiol in 20% nonsterilized soil with different moistures follow the simple first-order model. Values of model-fitting parameters are listed in **Table 2**. The rate of 17$\beta$-estradiol degradation in soil increased with increasing soil moisture. The accelerated degradation may result from the increased $\lambda$ in eq 2, because the increasing amount of 17$\beta$-estradiol was dissolved in the aqueous phase in soil with the increase of moisture. However, when the soil was saturated by water, although more 17$\beta$-estradiol was dissolved in the water phase and readily available for degradation, the degradation was slowed and the rate constant was reduced to almost the same as that at moisture 10%. This implies that anaerobic conditions hinder the degradation of 17$\beta$-estradiol. This observation is consistent with the results reported by Ying and Kookana (*36*). Keeping soil moderately moist and aerobic may effectively prompt the degradation of 17$\beta$-estradiol in soil.

**Temperature Dependence.** The degradation kinetics of 17$\beta$-estradiol in 20% nonsterilized soil at 15, 25, and 35 °C follow the simple first-order model, and fitting results are shown in **Figure 4**. The degradation greatly depended on soil temperature. At 15 °C, the rate constant ($k'$) and the half-life were 0.141 ± 0.008 day$^{-1}$ and 4.9 days, respectively. When the temperature was increased to 25 °C, the rate constant increased to 0.750 day$^{-1}$, which is 4.3 times higher than that at 15 °C. Correspondingly, the half-life was reduced to 0.92 day. However, further increase of temperature from 25 to 35 °C did not show pronounced enhancement in 17$\beta$-estradiol degradation. The rate constant was increased to 0.894 day$^{-1}$, which is only 0.19 times

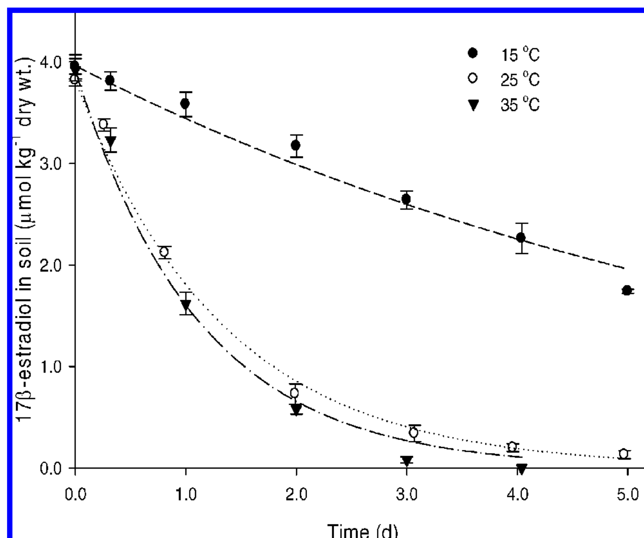


**Figure 4.** Degradation of 17$\beta$-estradiol in 20% nonsterilized soil at different temperatures. Points are experimental data, and lines are fitting results using a simple first-order model. Each point and error bar represents the mean value of three samples for analysis and the corresponding standard deviation.

higher than that at 25 °C, and the half-life was reduced to 0.78 day. No consistent increase of rate constant with increasing temperature was observed. A similar term of temperature dependence was also observed in the degradation of 17α-ethynylestradiol (*29*).

The specific term of temperature dependence of 17$\beta$-estradiol degradation may result from the bioactivity changes of degrading microorganisms in soil. As stated above, the rapid degradation of 17$\beta$-estradiol in soil is a biodegradation process. The degradation rate is directly determined by the bioactivity of degrading microorganisms. At 15 °C, the temperature might be still too low for the degrading microorganisms; thus, the bioactivity remained low and the degradation of 17$\beta$-estradiol was slow. When the temperature increased to 25 °C, the degrading microorganisms became much more active and rapid biodegradation was observed. With the increasing temperature, the bioactivity of degrading microorganisms might continue to increase. However, when the temperature reaches 35 °C, the bioactivity of some degrading microorganisms might be inhibited and the degradation slowed, becoming similar to that at 25 °C.

**Effect of Coexistent Antibiotic.** Veterinary antibiotics may coexist with estrogenic hormones in animal waste and animal manure-fertilized agricultural soil. Antibiotics are routinely administered to farm animals for purposes of therapeutical treatment and health protection (*37*). Up to 90% of the applied antibiotics are excreted by animals in urine and feces. The coexistent antibiotics may greatly reduce the biodegradation of organic contaminants in the environment because pharmaceutical antibiotics are designed to affect mainly microorganisms and the toxic dose for microorganisms is often very low. Thus, it has been suggested that evaluations of the fate and transport of estrogenic hormones in the environment should include the effect of coexisting veterinary antibiotics (*38*).

The degradation of 17$\beta$-estradiol in 20% nonsterilized soil with the presence of sulfadimethoxine, a widely used sulfonamide veterinary antibiotic, at different concentrations obeys the simple first-order model (fitting results shown in **Figure 5a**). With the increase of sulfadimethoxine concentration in soil from 0 to 40 and 200 $\mu$mol kg$^{-1}$, the degradation rate constant of

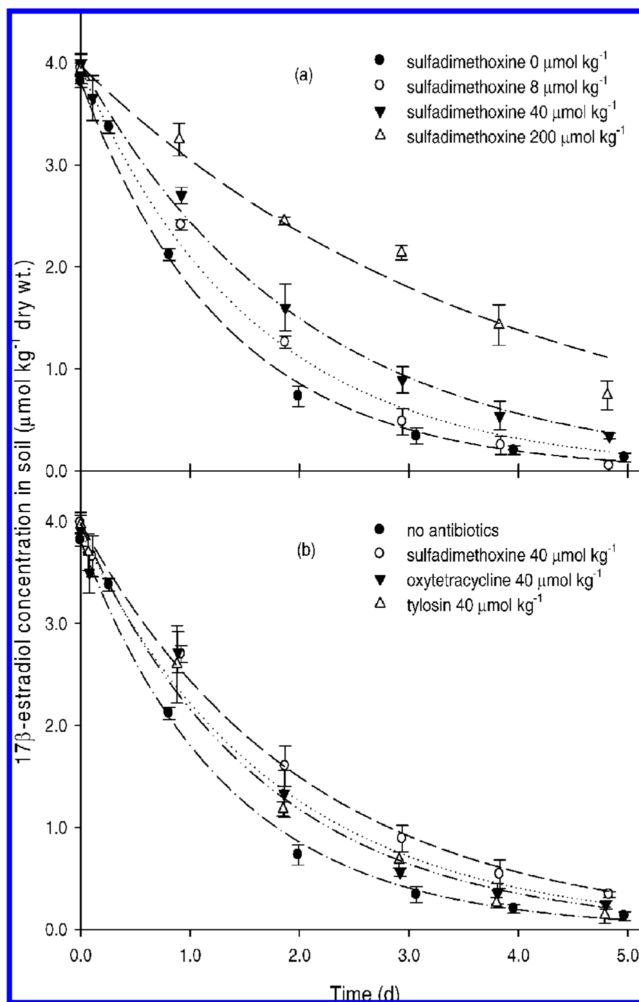


**Figure 5.** Degradation of 17$\beta$-estradiol in 20% nonsterilized soil with the presence of sulfadimethoxine at different concentrations (**a**) and with different antibiotics (**b**). Points are experimental data, and lines are model-fitting results. Each point and error bar represents the mean value of three samples for analysis and the corresponding standard deviation.

17$\beta$-estradiol was significantly decreased from 0.750 ± 0.038 to 0.492 ± 0.016 and 0.264 ± 0.021 day$^{-1}$, and the half-life was extended from 0.92 to 1.4 and 2.6 days, respectively. No significant difference ($P$ = 0.05) was found between the degradation rates of those with 0 and 8 $\mu$mol kg$^{-1}$ sulfadimethoxine. Compared with the sulfadimethoxine concentration effect on the degradation of sulfadimethoxine itself (*32*), 17$\beta$-estradiol degradation was less inhibited, implying that sulfadimethoxine has less impact on 17$\beta$-estradiol-degrading microorganisms than on sulfadimethoxine-degrading microorganisms.

The degradation of 17$\beta$-estradiol in 20% nonsterilized soil with the presence of 40 $\mu$mol kg$^{-1}$ of oxytetracycline (a tetracycline antibiotic) or tylosin (a macrolide antibiotic) also follows the simple first-order model (fitting results shown in **Figure 5b**). No significant decrease in the degradation rate of 17$\beta$-estradiol was observed ($P$ = 0.05) with the presence of oxytetracycline or tylosin at 40 $\mu$mol kg$^{-1}$, whereas a significant reduction was observed with the presence of sulfadimethoxine at the same concentration. Sulfadimethoxine appeared to have a stronger impact in reducing the degradation rate of 17$\beta$-estradiol than did oxytetracycline and tylosin.

Compared with the reported concentrations of veterinary antibiotics in agricultural soil (*37, 39*), the concentrations of the three selected antibiotics added in the soil in this study were

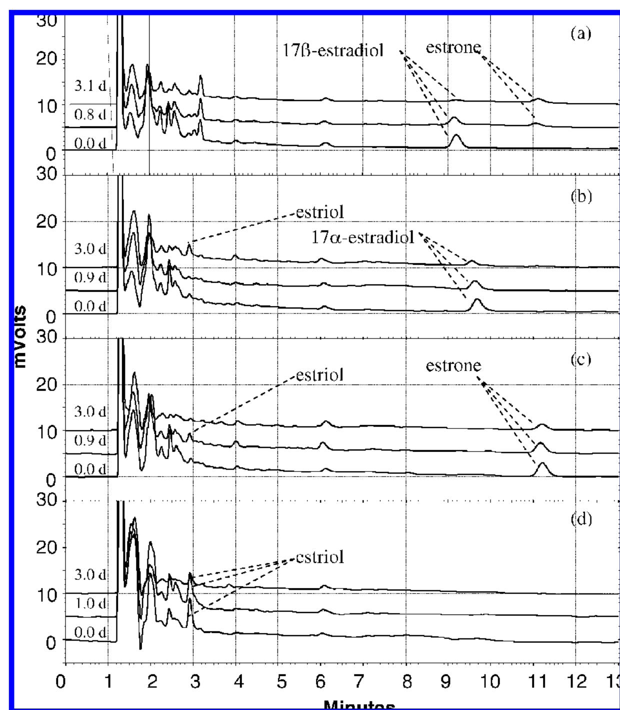


**Figure 6.** HPLC spectra of the degradation products of 17$\beta$-estradiol (**a**), 17α-estradiol (**b**), estrone (**c**), and estriol (**d**) in 20% nonsterilized soil.

about 1000 times higher. The observed nonsignificant reduction in the degradation rate of 17$\beta$-estradiol might imply that the occurrence of veterinary antibiotics in agricultural soil has no obvious effect on the fate and transport of estrogens in the environment.

**Degradation of Four Estrogenic Hormones in Soil.** The HPLC spectra of 17$\beta$- and 17α-estradiol, estrone, and estriol and their degradation products in 20% nonsterilized soil are shown in **Figure 6**. During the degradation of 17$\beta$-estradiol in soil (in **Figure 6a**), estrone was observed as the degradation product and was found to be slightly persistent in the degradation process. This observation is consistent with those reported by several other studies that estrone is the major degradation product of 17$\beta$-estradiol (*18, 35, 38, 40*). However, 17α-estradiol was not observed as a degradation product, which was detected in the degradation process of 17$\beta$-estradiol in dairy manure (*35*). In the degradation of 17α-estradiol in soil (in **Figure 6b**), estriol was observed as a degradation product. However, it did not accumulate and presented only for a short period in the degradation process. Similar to the degradation of 17α-estradiol, estriol was also observed to present for a short period in the degradation process of estrone (in **Figure 6c**). In the degradation of estriol, no new peaks were observed in the HPLC spectra.

The degradation kinetics of four estrogenic hormones in 20% nonsterilized soil all obey the simple first-order model. Values of model-fitting parameters are listed in **Table 3**. The degradation rate constant follows the order 17$\beta$-estradiol > estriol > 17α-estradiol > estrone. Among these four estrogenic hormones, estrone has the lowest degradation rate. This might be why estrone is always observed in the degradation process of 17$\beta$-estradiol. On the basis of sorption study results (*26, 27*), estrone is about 10 times more sorptive than 17$\beta$-estradiol in soil, which implies that the value of $\lambda$ in eq 2 for estrone could be much lower than that of 17$\beta$-estradiol. This might be why estrone appears to be markedly more persistent than 17$\beta$-estradiol in soil at the same condition.

**Table 3.** Model-Fitting Results of the Degradation of Four Estrogenic Hormones in 20% Nonsterilized Soil[a]

| hormone | rate constant, $K$ (day$^{-1}$) | half-life (days) | regression coefficient, $r$ |
|---|---|---|---|
| 17$\beta$-estradiol[b] | 0.750 ± 0.038 | 0.92 | 0.99 |
| estriol[b] | 0.458 ± 0.030 | 1.5 | 0.99 |
| 17α-estradiol[b] | 0.360 ± 0.017 | 1.9 | 0.99 |
| estrone[b] | 0.260 ± 0.013 | 2.7 | 0.99 |

[a] Soil moisture was 15% and incubation temperature was 25 °C. [b] Fitted using the simple first-order model.

The half-life of each of the four investigated estrogenic hormones is below 3 days in 20% nonsterilized soil. It is reasonable to believe that their half-lives are remarkably lower than 3 days in 100% nonsterilized soil. The rapid degradation of these estrogenic hormones implies that minimizing runoff and leaching may greatly help reduce the contamination of estrogenic hormones in the water environment resulting from manure and biosolid application in soil (*22*).

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