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Laboratory Study of Oxytetracycline Degradation Kinetics in Animal Manure and Soil

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Oxytetracycline (OTC) is a major member of tetracyclines, which are widely administered to animals in confined feeding operations. To diminish the contamination of OTC in the environment, which results from the application of OTC-containing manure as fertilizer in agricultural lands, OTC degradation kinetics in manure and soil under laboratory aerobic conditions was investigated. OTC degradation kinetics was found to be described well by the previously developed availability-adjusted first-order model at all moistures and low temperatures (≤25 °C). OTC degradation increased with increasing moisture from 60 to 100%. However, OTC became very persistent in water-saturated manure. Increasing temperature greatly accelerated OTC degradation, and thermal degradation became noticeable at high temperatures (≥35 °C) in manure. At 25 °C, OTC half-life was determined to be 8.1 days in manure with moisture at 80%, 33 days in manure-amended soil (amendment ratio at 5%), and 56 days in non-amended soil with both moistures at 20%, demonstrating that OTC may become persistent in the environment once it is released from manure into soil. No pronounced effect of coexistent antibiotics on OTC degradation in manure was observed.

KEYWORDS: Oxytetracycline; antibiotic; degradation; soil; manure

INTRODUCTION

Because of the economic advantage of size and the development of new technology, traditional small-scale animal farms have been shifted into large concentrated animal feeding operations. Veterinary antibiotics are widely used in these confined animal farms to treat disease, protect the health of animals, and improve growth rate and feed efficiency (*1*). About 21.9 million pounds of antibiotics were administered to farm and companion animals annually from 2002 to 2004 in the United States (*2*). As many veterinary antibiotics are poorly adsorbed in the guts of animals, about 20–90% of the administered parent antibiotic agents are excreted by animals and enter into the environment with the application of manure as fertilizer in agricultural lands (*3, 4*).

The disposal of antibiotic-contaminated manure in the environment has gained increasing attention from both the public and the scientific communities. It was estimated by USDA/ERS that confined livestock and poultry animals in the United States generate about 63.8 million tons (in dry weight) of manure annually (*5*). Because of socio-economic reasons, the major amount of the manure is usually applied to adjoining farmland without long-distance transport after being stockpiled, lagooned, or composted (*6*). The cyclic applications of antibiotic-contaminated manure, especially manure that contain persistent antibiotics, may lead to the development of antibiotic-resistant bacteria in the environment. It has been shown that antibiotic-resistant genes may be transferred to human beings and animals through drinking water and food chains, resulting in diminished success in antibiotic treatment (*7–9*). The pharmaceutical antibiotics may also alter the composition and diversities of indigenous soil microbial communities, thus disturbing the ecosystem functions in nutrient cycling, decomposition, and energy flow (*10, 11*).

Properly treating animal manure before its application in agricultural lands has been proposed to be an effective and feasible means to reduce the amount of veterinary antibiotics ultimately released into the environment (*12, 13*). Degradation studies of several widely used veterinary antibiotics in manure have been reported. Tetracycline was found to degrade faster in liquid pig manure under aerobic conditions than it does under anaerobic conditions (*14*). In cattle, chicken, and swine excreta, tylosin was found to degrade rapidly under aerobic conditions, and its half-life was determined to be 6.2, <7.6, and 7.6 days, respectively (*15*). In calf bedding manure, tylosin degradation was found to follow the first-order kinetics, whereas that of oxytetracycline (OTC) was observed to follow the biexponential kinetic model. OTC was much more persistent than tylosin in manure (*16*). More recently, OTC dissipation in calf manure was further studied in anaerobic digestion and composting. About 59% of OTC with its initial concentration at 9.8 mg L^{-1} was degraded after 64 days of anaerobic digestion at 35 °C. Degradation products, including 4-epi-OTC, α-apo-OTC, and β-apo-OTC, were monitored (*17*). In the composting study,

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manure was mixed with straw and hardwood woodchips, and composting was conducted in self-heating laboratory composters with an initial concentration of extractable OTC at 115 mg kg^{-1} (dry weight). Almost all extractable OTC was removed within 35 days of composting. The half-life of OTC was determined to be approximately 3.2 days (*13*).

Tetracyclines, including tetracycline, OTC, chlortetracycline, and doxycycline, are the most widely used traditional veterinary antibiotics in the United States (*2*). Laboratory and field experiments have demonstrated that tetracyclines are strongly adsorbed and persistent in manure and soil (*18–21*). The biexponential model better fits OTC degradation kinetics than the simple first-order model does (*16, 21*). However, because of the lack of clear physical definition of the parameters in the biexponential model, this approach appears to be a solely mathematical fitting, from which little mechanistic information can be obtained. Additionally, the effect of various environmental factors on the degradation of OTC has not yet been well documented.

In the present study, the degradation kinetics of OTC in manure under laboratory aerobic incubation conditions is investigated. The previously developed availability-adjusted first-order model is used to fit to the experimental data. The effect of manure moisture, temperature, and the presence of other antibiotics is examined. The degradation kinetics in manure and in soil are compared.

MATERIALS AND METHODS

Chemicals, Manure, and Soil. OTC dehydrate (≥98%) and sulfadimethoxine (≥99%) were purchased from Sigma (St. Louis, MO). Penicillin G sodium salt (≥98%), tetracycline (≥98%), and oxalic acid (>99.5%) were purchased from Fluka (Milwaukee, WI). Citric acid anhydrous (ACS certified), methanol (HPLC grade), acetonitrile (optima), and phosphoric acid (85%) were purchased from Fisher Scientific (Fair Lawn, NJ). Water was supplied by a Barnstead E-pure purification system (Dubuque, IA).

Commercially available steer manure (Earthgro, Marysville, OH) was used instead of fresh animal manure in this study to allow the control of manure moisture content and to maintain aerobic conditions. The steer manure was purchased from a garden center in Riverside, CA. The manure sample was sieved to 4 mm and then air-dried in the laboratory for 2 days at 25 °C. Manure moisture was determined to be 51%, and pH was determined to be 8.37 (at manure/water = 1:2 by mass). Organic carbon content and maximum water-holding capacity were 14 and 155%, respectively. The manure sample was analyzed, and no OTC residues were detected.

The soil used in this study was Arlington sandy loam soil, which was sampled from the Riverside Agricultural Experimental Station, University of California, Riverside, CA. The soil organic matter content was 0.92%, and the soil pH was 7.2 (at soil/water = 1:1 by mass).

Degradation Experiments. For the degradation of OTC in manure at different moistures, three portions of 300 g (dry weight) of manure were weighed into three plastic zip bags. A total of 1.5 mL of 1.00 × 10^4 μM OTC acetone solution was spiked into the manure in each bag. After thoroughly mixing the manure in the bag, different amounts of water were added into different bags to obtain three different manure moistures. The three moistures were 60, 80, and 100%, which corresponded to water saturation percentages of 39, 52, and 64%, respectively. The manure in each bag was again thoroughly mixed and then weighed into eight 250 mL glass jars at approximately 35 g (dry weight) per jar. After the weight of each jar was recorded, the jars were loosely covered with aluminum foil and immediately transferred to a constant-temperature room for incubation at 25.0 ± 0.2 °C. Every other day, each jar was weighed, and water was added to compensate any moisture loss. For the experiment of OTC degradation in water-saturated manure, OTC-spiked manure was weighed into 24 40 mL polyethylene centrifuge tubes at 5.56 g (dry weight) per tube. Water was then added into each tube until manure was completely steeped in the water. After being loosely covered with aluminum foil, tubes were transferred to the same constant-temperature room for incubation. At day 0, 2, 5, 10, 20, 35, 50, and 62, one jar/tube from each moisture set was taken out, sealed with a cap, and stored in a freezer at −21 °C until analysis. For the sterilized control, a portion of 300 g (dry weight) of manure was acidified with 22.7 mL of phosphoric acid. After the moisture was adjusted to 80%, the manure was weighed into another set of 24 40 mL polyethylene centrifuge tubes at 5.56 g (dry weight) per tube. The tubes were then sealed with caps and sterilized at 121 °C for a total of three times for 60 min each time. After completely cooling down, 28.0 μL of 1.00 × 10^4 μM OTC acetone solution was added to each tube. Manure samples were then shaken to mix in each sealed tube. Tubes were incubated and stored in the same way as the other samples.

In the experiment of OTC degradation in manure at different temperatures, manure moisture was controlled at 80%, and the incubation temperature was controlled at 15 ± 0.1 (incubator), 25 ± 0.2 (constant-temperature room), 35 ± 0.1 (incubator), and 45 ± 1.0 °C (oven).

When investigating the potential effect of coexistent antibiotics on the degradation of OTC in manure, five portions of 300 g (dry weight) of manure were spiked with different antibiotic solutions. These solutions were (i) 1.50 mL and (ii) 0.75 mL of 1.00 × 10^4 μM OTC acetone solution, (iii) 0.75 mL of 1.00 × 10^4 μM OTC and 0.75 mL of 1.00 × 10^4 μM tetracycline acetone solution, (iv) 0.75 mL of 1.00 × 10^4 μM OTC and 0.75 mL of 1.00 × 10^4 μM sulfadimethoxine acetone solution, and (v) 0.75 mL of 1.00 × 10^4 μM OTC and 0.75 mL of 1.00 × 10^4 μM penicillin G acetone solution. The manure moisture was controlled at 80%, and the incubation temperature was set at 25 ± 0.2 °C.

In the experiment of OTC degradation in soil, OTC was spiked into non-amended and manure-amended soil instead of manure. Manure amendment ratio (manure/soil) was 5:95 by dry weight. The moisture of non-amended and manure-amended soil was controlled at 20%.

Sample Extraction and OTC Concentration Analysis. After thawing at room temperature, the manure/soil sample in each jar was thoroughly mixed and weighed into three 40 mL polyethylene centrifuge tubes at 5.56 g (dry weight) per tube. Then, 2.5 g of citric acid, 1.5 g of oxalic acid, and 15 mL of methanol/water mixture (9:1 in volume) were added into each tube for extraction. After being shaken for 30 min, the tubes were centrifuged at 1.1 × $10^4 g$ for 10 min, and the supernatant in the different tubes was decanted into different 50 mL volumetric flasks. This extraction sequence was repeated for a total of three times, and the supernatants from the same tube were combined in the same volumetric flask. The extract in each flask was diluted to 50 mL with extraction solvent, and a 1.0 mL extract from each flask was then transferred into a 1.5 mL microcentrifuge tube for further centrifugation at 1.7 × $10^4 g$ for 5 min. Supernatants were transferred into 2 mL amber sample vials for OTC concentration analysis.

A Hewlett-Packard series II 1090 HPLC (Wilmington, DE) was used for OTC concentration analysis in the extracts. An Agilent Hypersil ODS column (5 μm, 4.0 × 250 mm) was used for separation. The mobile phase was composed of 80% water (pH adjusted to 3.0 by using phosphoric acid) and 20% acetonitrile, and the flow rate was 1.0 mL min^{-1}. The detection wavelength of the diode array detector was set at 360 ± 20 nm. The injection volume was 10 μL. The retention time of OTC was 6.2 min. A typical HPLC spectrum is shown in **Figure 1**.

The concentration of OTC in manure/soil was calculated with the following equation:

$$C_{\mathrm{OTC}} = 50.0 C_{\mathrm{ext}}/5.56 \quad (1)$$

where C_{OTC} (μmol kg^{-1} dry weight) and C_{ext} (μM) are OTC concentrations in manure/soil and extract, respectively.

Kinetic Model. Because of the desorption hysteresis and irreversible fixation, the percentage of readily available target compound in its total remaining in soil and manure during the degradation process normally decreases as the target compound becomes increasingly unavailable for degradation. In our previous studies, an availability-adjusted first-order kinetic model based on the decreasing availability of the target compound has been developed to fit the degradation kinetics of sulfadimethoxine in manure and manure-amended soil (*12, 22*). Briefly,

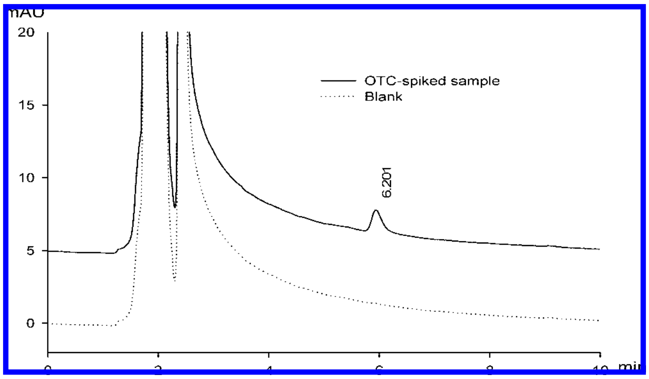

Figure 1. A typical HPLC spectrum of OTC in manure extract.

the degradation rate of the target compound is directly proportional to the concentration of the available target compound in the system, i.e.,

$$-\frac{dC}{dt}=k\lambda C \qquad (2)$$

where C (μmol kg^{-1}) is the total remaining concentration of the target compound at time t (day), and λ is the concentration ratio of the available target compound to the total remaining target compound in the system at time t (day). It is assumed that λ is a function of t and can be expressed as:

$$\lambda=\lambda_0 e^{-at} \qquad (3)$$

where a (day^{-1}) is a positive coefficient called availability coefficient, and λ_0 is the value of λ at $t = 0$. The higher the value of a, the faster the decrease of λ with time will be. After substituting eq 3 into eq 2, we get:

$$\frac{dC}{dt}=-k''Ce^{-at} \qquad (4)$$

where $k'' = k\ \lambda_o$, and is called degradation rate constant. After integration, the availability-adjusted first-order kinetic model is obtained.

$$C_t=C_0 e^{-\frac{k''}{a}(1-e^{-at})} \qquad (5)$$

where C_0 and C_t (μmol kg^{-1}) are the target compound concentrations at times 0 and t (day). The half-life of the target compound can be calculated through the following equation, which is derived from eq 5:

$$t_{1/2}=-\frac{1}{a}\ln\left(1-\frac{0.693a}{k''}\right) \qquad (6)$$

RESULTS AND DISCUSSION

Extraction Recovery. Tetracyclines have been found to chelate with multivalent cations and β-diketones and strongly bind to proteins, which greatly impedes their extraction (*23*). Thus, satisfactory recoveries of OTC from environmental samples have not been always achieved (*8, 9*). In this study, citric acid and oxalic acid are used in the extraction, acting as both acidifying and chelating reagents to overcome the strong buffering capacity of manure and to release OTC from its complexes with multivalent cations. The extraction recoveries of the method used in this study are listed in **Table 1**. All recoveries are above 83%. The detection limit in manure and manure-amended soil is 1.0 μmol kg^{-1} dry weight. In this study, all obtained OTC concentrations in manure and manure-amended soil are presented without recovery adjustments.

The extraction method used in this study was found to be a robust method for the extraction of OTC from manure and soil samples. No distinct differences in recovery are observed between samples with 0−2 days aging times. More than 60% OTC from sterile control (shown in **Figure 3**) is recovered after 62 days of incubation, in which abiotic degradation might have occurred.

Table 1. Extraction Recovery (% mean ± standard deviation) of OTC from Different Matrices

	spiked level (μmol kg^{-1} dry weight)		
matrices (n = 4 for each matrix)	5.0	25.0	12.5
fresh spiked manure	86 ± 5	87 ± 3	88 ± 2
fresh spiked manure-amended soil	83 ± 4	85 ± 6	
1-day aged spiked manure[a]			87 ± 2
2-day aged spiked manure[b]			86 ± 4

[a] Samples were prepared by spiking OTC solution into sterile manure and storing them at room temperature for 1 day. [b] Samples were prepared by spiking OTC solution into sterile manure and storing them at room temperature for 2 days and in a freezer (−21 °C) for 6 days.

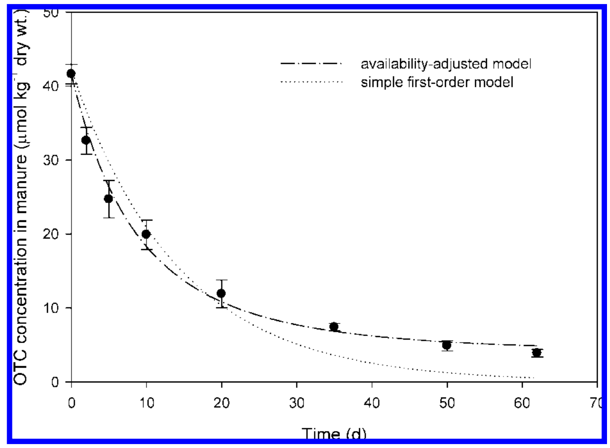

Figure 2. Fitting results of the simple first-order model and the availability-adjusted first-order model.

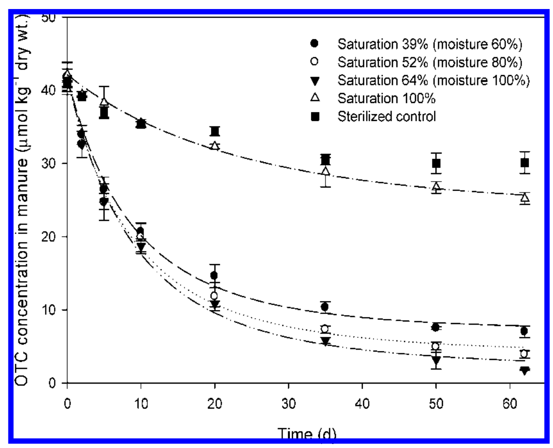

Figure 3. OTC degradation in manure with different moistures. Points are experimental data, and curves are fitting results of the availability-adjusted first-order kinetic model.

Fitting of the Availability-Adjusted Kinetic Model. A set of typical degradation experimental data of OTC in manure is shown in **Figure 2**. Both the simple first-order kinetic model and the availability-adjusted first-order kinetic model are used to fit the experimental data. The regression coefficients, r, of the former and the latter are 0.96 and 0.99, respectively. The availability-adjusted model appears to better fit the experimental data than the simple first-order model does and thus is more accurate to describe OTC degradation kinetics in manure. The successful fitting of the availability-adjusted model to the experimental data also confirms the decreasing availability of OTC in manure during its degradation process. The desorption hysteresis greatly impedes OTC from its degradation in manure.

Effect of Manure Moisture. OTC degradation in manure at different moistures obeys the availability-adjusted model well (**Figure 3**). All values of r are above 0.99 (data not shown). No pronounced degradation was found with the sterilized control. Compared with the sterile control, OTC degradation in nonsterile nonsaturated manure is much faster. When the manure moisture increases from 60 to 100%, the degradation of OTC in manure is slightly enhanced. The obtained values of k'' and a are 0.095 ± 0.006 and 0.0545 ± 0.0061 day^{-1}, 0.102 ± 0.008 and 0.0444 ± 0.0075 day^{-1}, and 0.101 ± 0.007 and 0.0339 ± 0.0068 day^{-1} at a moisture of 60, 80, and 100%, respectively. Based on eq 6, the half-life of OTC is calculated to be 9.3, 8.1, and 7.8 days, respectively. The accelerated degradation might result from the increased λ_0 in eq 3 because more OTC is dissolved in the aqueous phase in a manure system with higher moisture. A higher λ_0 results in a higher k'', which corresponds to a faster degradation. Nevertheless, the enhanced degradation might also be caused by the increased activity of the degrading micro-organisms in manure. It has been found that aerobic microbial activity is strongly related to manure moisture (*24*). In a certain moisture range, the activity of the degrading micro-organisms in manure may increase with the moisture.

With the increase of the moisture, k'' increases; however, a slightly decreases. A lower a indicates a slower decrease in the ratio of the available OTC to the total remaining. The decrease of a with the increase of k'' implies that desorption processes are accelerated with the increasing moisture, and the availability of OTC for degradation is enhanced. Hence, keeping manure at high moistures during its storage or composting treatment may benefit the degradation of OTC in the manure, thus effectively reducing the amount of OTC introduced into the environment with the application of manure in agricultural lands.

However, overly high moisture contents can produce an undesirable effect. Too much water in manure may dramatically reduce the air permeability and even cause anaerobic conditions, under which the target compound might degrade very slowly; hence, water saturation produces an extreme condition. OTC degradation in water-saturated manure appears to be much slower than its degradation under moistures of 60−100% and is only slightly faster than that in the sterile control. The slow degradation of OTC in water-saturated manure and sterile control confirms that aerobic biodegradation is responsible for the major dissipation of OTC in the manure observed in this study.

OTC might be much more persistent in fresh manure than in nonsaturated nonsterile manure as observed in this study. Fresh manure normally is water saturated or contains very high moisture contents, which produces anaerobic conditions in the internal parts of manure piles. Additionally, the steer manure used in this study is commercially available manure, which probably has been composted and aged and may contain a more diverse population of degrading micro-organisms for OTC than fresh manure does.

Unfavorable manure storage management may also cause OTC to persist in manure. In some animal farms, manure is stored in pits, holding ponds, and lagoons, where manure is normally water saturated and is under completely or partially anaerobic conditions (*25*). These kinds of storage management might be helpful in reducing the emission of odors but may prevent OTC and possibly other antibiotics from degradation. If the discharge of veterinary antibiotics to the environment becomes regulated, manure stored in pits, ponds, and lagoons may require further treatments to enhance the degradation of OTC and other antibiotics.

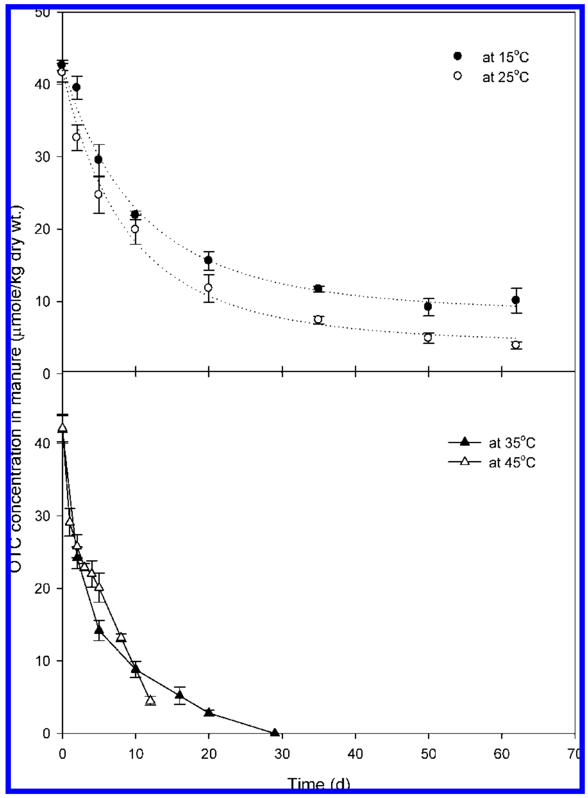

Figure 4. OTC degradation in manure at different temperatures. Points are experimental data. Dotted curves are fitting results of the availability-adjusted first-order kinetic model, and solid straight lines are just the links of points.

OTC degradation in manure at moisture 80% and 25 °C in this study was compared with the degradation of sulfadimethoxine in the same manure under similar conditions; those results are reported in one of our previous studies (*12*). OTC is much more persistent than sulfadimethoxine in manure. The half-life of sulfadimethoxine was reported to be 2.3 days, which is about 2.4 times shorter than that of OTC. This comparative result is consistent with the observations from a field study performed by Blackwell et al. (*26*), in which sulfachloropyridazine, another sulfonamide antibiotic, was shown to degrade rapidly in soil, whereas OTC was found to persist in soil.

Temperature Effect. OTC degradation in manure at different temperatures exhibits different kinetics (in **Figure 4**). Both degradation kinetics at 15 and 25 °C follow the availability-adjusted first-order model well. The obtained values of r are above 0.99 (data not shown). When the temperature is increased from 15 to 25 °C, OTC degradation is enhanced, and k'' markedly increases from 0.079 ± 0.007 to 0.102 ± 0.008 day^{-1}.

The coefficient a is normally dominated by k'' and the desorption rate. A higher k'' results in a faster decrease of the available target compound and thus potentially causes a higher a, that is, a lower ratio of the available target compound to its total remaining. On the other hand, a faster process of desorption may release more target compound from sorption/fixation for degradation during the same period of time, greatly mitigating the unavailability of the target compound. In this study, a slightly decreases from 0.050 ± 0.007 to 0.044 ± 0.008 day^{-1} with the remarkable increase of k'' from 0.079 ± 0.007 day^{-1} at 15 °C to 0.102 ± 0.008 day^{-1} at 25 °C. This phenomenon implies that the increasing temperature accelerates the desorption process of OTC in manure and mitigates the unavailability of OTC during its degradation in manure.

OTC degradation in manure at 35 and 45 °C happens much faster than at 15 and 25 °C. However, the degradation kinetics deviates apparently from the availability-adjusted first-order model as well as from the simple first-order model. At 35 °C, the OTC decrease becomes almost linear after 10 days of incubation. This partial linear degradation kinetics appears more obviously in the degradation at 45 °C, in which OTC declines rapidly and follows linear kinetics after the first two days.

The rapid decrease of OTC degradation kinetics and its deviation from the developed model might be caused by the thermal degradation of OTC at high temperatures, which happens in addition to the biodegradation of OTC in manure. Both tetracycline and OTC have been reported to undergo thermal degradation in aqueous solutions and food (*27, 28*). The half-life of OTC in distilled water at 60 °C was only 2.7 h. Although the thermal degradation of OTC in manure at the same temperature might be much slower than that in pure water because of the sorption/fixation of OTC in manure, thermal degradation may still contribute to the overall degradation of OTC in manure, causing the degradation to happen much faster than it would without thermal degradation. Thermal degradation might also be the major cause of the fast degradation of OTC in the composting study reported by Arikan et al. (*13*). The temperature of the composting blend was raised to 70 °C within the first two days of composting, and more than 90% of OTC was removed within the first six days.

The rapid degradation of OTC at high temperatures might be a useful clue to manure treatment designs. The contamination of OTC and other tetracyclines in animal manure may be greatly diminished if manure is kept at a high temperature for a certain period of time. In areas with strong irradiation of sunlight and relatively high temperatures, such as southern California and Texas, treatments using sunlight to heat manure might be an effective and low-cost way to enhance the degradation of tetracyclines. Further investigations on the thermal degradation kinetics and mechanisms of tetracyclines in manure are expected.

Effect of Coexistent Antibiotics. More than one veterinary antibiotic may be administered to the same group of animals simultaneously or asynchronously. Animal excreta containing different antibiotics are normally mixed together without any identification. Synergetic antibiotic activity may be achieved when two or more antibacterial agents are present in the same medium because different families of antibiotics may inhibit a different spectrum of bacteria. Some degrading micro-organisms for one antibiotic may be inhibited by another coexistent antibiotic, resulting in the persistence of one or more antibiotics in the medium. OTC degradation in manure with the coexistence of other antibiotics has been preliminarily investigated in this study.

OTC degradation in manure at its initial concentration of 25 μmol kg^{-1} (dry weight) with and without coexistent 25 μmol kg^{-1} (dry weight) of OTC (i.e., total initial concentration 50 μmol kg^{-1}), tetracycline, sulfadimethoxine, or penicillin G follows the availability-adjusted model well. All values of r are above 0.99 (data not shown). Values of k'' and a are listed in **Table 2**. The value of k'' for OTC degradation in the presence of any one of the investigated coexistent antibiotics is slightly lower than that for pure OTC at 25 μmol kg^{-1}. However, no distinct difference between any two obtained values of k'' is observed, implying that the coexistence of any one of these antibiotics at the investigated concentration level has no pronounced effect on the degradation of OTC in manure. Separation of manure with different antibiotics is not needed in manure storage management.

Table 2. Values of K'' and a of OTC Degradation in Manure with and without a Coexistent Antibiotic

initial concentration (μmol kg^{-1} dry weight)			
OTC	coexistent antibiotic and concentration	K'' (day^{-1})	a (day^{-1})
25	none	0.108 ± 0.013	0.049 ± 0.011
	OTC, 25	0.102 ± 0.008	0.044 ± 0.008
	tetracycline, 25	0.103 ± 0.009	0.058 ± 0.009
	sulfadimethoxine, 25	0.090 ± 0.009	0.038 ± 0.008
	penicillin G, 25	0.096 ± 0.011	0.046 ± 0.012

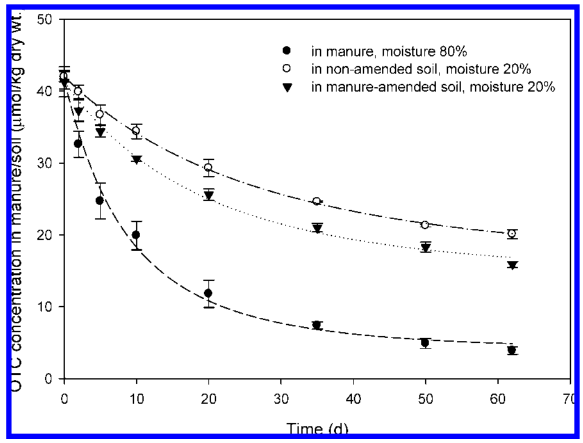

Figure 5. OTC degradation in manure, manure-amended, and non-amended soil. Points are experimental data, and curves are fitting results of the availability-adjusted first-order kinetic model.

OTC Degradation in Soil. The degradation kinetics of OTC in both non-amended and manure-amended soil obeys the availability-adjusted model. The obtained values of r are above 0.99 (data not shown). OTC degradation in manure appears much faster than in non-amended and manure-amended soil (shown in **Figure 5**). The obtained values of k'' for the degradation in manure, manure-amended, and non-amended soil are 0.102 ± 0.008, 0.035 ± 0.003, and 0.023 ± 0.001 day^{-1}, respectively, and the values of a are 0.044 ± 0.008, 0.034 ± 0.005, and 0.025 ± 0.002 day^{-1}, respectively. The calculated half-lives of OTC in manure, manure-amended soil, and non-amended soil are 8.1, 33, and 56 day, respectively.

On the basis of what is observed in **Figure 3**, the higher moisture content in manure (80%) is likely to explain the faster degradation of OTC in manure than that in amended and non-amended soil (moisture at 20%). However, higher organic content in manure than in soil may also result in stronger adsorption of OTC and sharply reduce the OTC degradation rate in manure. As indicated by the faster degradation of OTC in manure-amended soil than in non-amended soil at the same moisture, the much faster degradation of OTC in manure than that in soil may mainly result from the richer degrading micro-organisms in manure. A similar phenomenon has been observed in the degradation of sulfadimethoxine in manure-amended soil (*22*).

This result implies that OTC may become much more persistent in the environment once it is released from contaminated manure into soil. With the cyclic application of contaminated manure, OTC may accumulate in soil. Hence, proper storage management and appropriate treatments for OTC-contaminated manure may effectively diminish OTC contamination in the environment. High temperatures and moderately high moisture under aerobic conditions during manure storage and composing treatment might be critical.

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