Published online October 27, 2006

Sulfadimethoxine Degradation Kinetics in Manure as Affected by Initial Concentration, Moisture, and Temperature

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ABSTRACT

Sulfadimethoxine is a widely used sulfonamide veterinary antibiotic and could be a source of agricultural contamination. Therefore, information is needed about its degradation kinetics in manure under aerobic conditions. Based on the analysis of first-order kinetics and the assumption that sulfadimethoxine availability for degradation in manure could be limiting, a new kinetic model was developed and was found to fit the degradation kinetics well. The degradation rate in sterile manure was found to be much lower than in nonsterile manure, indicating that biodegradation was significant. In biologically active manure, the degradation rate constant decreased with increasing initial concentration of sulfadimethoxine, implying that the activity of the degrading microorganisms was inhibited. Increasing moisture or temperature was found to increase sulfadimethoxine degradation in manure. Mixing manure containing high levels of sulfadimethoxine with manure containing lower levels may result in more rapid degradation, thus greatly diminishing sulfadimethoxine contamination in manure and significantly reducing sulfadimethoxine inputs into the environment. During treatment, keeping the manure moist and storing in a moderately warm place under aerobic conditions may also help to diminish sulfadimethoxine contamination.

FOR PURPOSES OF THERAPEUTICAL TREATMENT AND GROWTH PROMOTION, veterinary antibiotics are widely administered to animals in the agricultural industry. It was estimated that more than 22 million pounds of antibiotics were used to treat farm animals and pets in the USA during 2002 [Animal Health Institute (AHI), 2003]. Another study reports that nontherapeutical use of antibiotics for livestock production in the USA has exceeded 24.6 million pounds per year (Mellon et al., 2001). In Europe, the total consumption of veterinary antibiotics in 1999 was estimated to be 10.3 million pounds [FEDESA (European Federation of Animal Health), 2001]. The application of antibiotics as growth promoters in feed additives has been forbidden in Switzerland since 1999. To date, the nontherapeutical use of antibiotics as growth promoters in Europe is almost completely restricted and the relevant consumption is quickly declining (Thiele-Bruhn, 2003). However in Europe, a new trend has developed with increased therapeutic use of antibiotics (Alder et al., 2001).

After the application to animals, antibiotics will eventually enter the environment. In fish farming, antibiotics are given as feed additives, resulting in a direct release of antibiotics into the aquatic environment (Thurman et al., 2002). It was estimated that ~70 to 80% of drugs administered to fish enters the environment and antibiotic residues with significant antibacterial activity were found in the sediment of fish hatcheries (Samuelsen et al., 1992). However, the major route through which veterinary antibiotics enter the environment is the excretion of feces and urine from medicated animals in livestock and poultry farming, and the subsequent application of contaminated manure as fertilizer into agricultural land (Ariese et al., 2001; Boxall et al., 2003). It was found that as much as ~40 to 90% of the administrated antibiotics are excreted as parent compounds by animals after medication (Halling-Sorensen et al., 2001; Winckler and Grafe, 2001). The concentration of antibiotics in manure excreted from treated animals can be as high as several hundreds of mg kg^{-1} (Migliore et al., 1997; De Liguoro et al., 2003). Soil and water contamination from manure fertilization and at concentrated animal operations has been frequently reported (Rabolle and Spliid, 2000; Hamscher et al., 2002; Boxall et al., 2002). There is concern that residual concentrations of antibiotics in agricultural soil can easily reach levels similar to pesticides if the manure loading for fertilization increases to the kilograms per hectare level (Thiele-Bruhn, 2003).

The widespread contamination of antibiotics in the environment may put human health and sensitive ecosystems at risk. First, exotic antibiotics may alter the composition and diversity of indigenous soil microbial communities, which are of fundamental importance for ecosystem function in nutrient cycling, decomposition, and energy flow (McCracken and Foster, 1993; Schmitt et al., 2004). Second, exotic antibiotics may cause the formation of resistance, even cross and multiple resistances, in organisms in the environment (Al-Ahmad et al., 1999; Sengelov et al., 2003) and possibly threaten human and animal health by diminishing the success of antibiotic treatment. Evidence has been presented that antibiotic resistant genes from microorganisms in the environment can be transferred directly to humans (Rhodes et al., 2000). Third, widespread contamination of veterinary antibiotics exposes humans and animals to a constant low concentration of antibiotics. Leaching and runoff of antibiotics from manure-fertilized lands is threatening the quality of drinking water (Hirsch et al., 1999; Kolpin et al., 2002). Though the effects of long-term exposure to low concentrations of antibiotics are not yet clear, the potential danger resulting from veterinary antibiotic contamination to human and animal health cannot be neglected.

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Published in J. Environ. Qual. 35:2162–2169 (2006).
Technical Reports: Organic Compounds in the Environment
doi:10.2134/jeq2006.0178

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Focusing on eliminating the contamination caused by veterinary antibiotics, numerous studies have been performed to investigate the degradation of antibiotics in manure (Kuhne et al., 2000; Teeter and Meyerhoff, 2003), soil (Gilbertson et al., 1990; Marengo et al., 1997), and water (Alexy et al., 2004; Boreen et al., 2004). Aerobic conditions were always found to be beneficial to the degradation of antibiotics in manure and water (Kuhne et al., 2000; Ingerslev et al., 2001). Along with degradation and transport of veterinary antibiotics in the subsurface, adsorption of antibiotics in soil with and without manure has also been investigated (Rabolle and Spliid, 2000; Thiele-Bruhn and Aust, 2004).

Rather than developing technologies to enhance the degradation of antibiotics in soil and to prevent them from contaminating surface and ground water, a more effective and practical way to reduce contamination from veterinary antibiotics would be eliminating the antibiotics in the manure before its application to agricultural land as fertilizer (Wang et al., 2006). However, the degradation kinetics of antibiotics in manure, as well as the effects of various factors on degradation kinetics, has rarely been investigated. A simple first-order model has generally been used to fit the degradation kinetics of antibiotics even though the fitting results were poor in almost every case (Gilbertson et al., 1990; Ingerslev and Halling-Sorensen, 2001; De Liguoro et al., 2003). In some studies, the values of the correlation coefficient, *r*, were even not presented. A bi-exponential curve was found to provide a better fit than the simple first-order model for the antibiotic degradation in manure (De Liguoro et al., 2003; Blackwell et al., 2005). However, due to the lack of clear physical definition of each parameter in the bi-exponential model, this approach can be viewed as merely a mathematic regression, from which little mechanistic information can be obtained.

As a widely used sulfonamide antibiotic, sulfadimethoxine [4-amino-N-(2,6-dimethoxy-4-pyrimidinyl) benzenesulfonamide] has been recently studied, including sorption in soil (Thiele-Bruhn and Aust, 2004), photostability in water (Lunestad et al., 1995), remediation (Forni et al., 2002; Calza et al., 2004), and occurrence in the environment (Kolpin et al., 2002; Thurman et al., 2002). However, little information is available on sulfadimethoxine degradation kinetics in manure and the effects of various environmental factors. In the present study, sulfadimethoxine was chosen as a target antibiotic because of its widespread use. The degradation kinetics of sulfadimethoxine in manure was studied and a model based on the first-order kinetics was developed to fit the observed degradation. The effects of the initial concentration of sulfadimethoxine, manure moisture, and temperature on the degradation kinetics were investigated.

MATERIALS AND METHODS

Chemicals and Manure

Sulfadimethoxine (>99%) was purchased from Sigma (St. Louis, MO). Acetonitrile (optima grade), acetic acid [high performance liquid chromatography (HPLC) grade], methanol (HPLC grade), phosphoric acid (certified), sodium chloride (certified), and sodium azide (certified) were purchased from Fisher (Fair Lawn, NJ). Acetone (HPLC grade) was purchased from Burdick & Jackson (Muskegon, MI).

Steer manure (Earthgro, Marysville, OH 43041) was purchased from K-Mart at Riverside, CA. Manure was sieved using a 4-mm sieve and then air-dried in the laboratory at 25°C for 2 d. The moisture content was determined to be 39%. Manure pH measured at manure/water ratio = 1:2 (in wet weight) was 8.37. Organic carbon content and maximum water-holding capacity were 14 and 155%, respectively.

Sorption Experiments

Sulfadimethoxine sorption in manure was determined using the batch equilibration procedure. All experiments were performed in triplicates. Sulfadimethoxine solutions at 5 concentrations ranging from 35.3 to 158.6 μM were prepared in water with 0.01 *M* NaCl and 0.2% NaN_3. Ten mL of sulfadimethoxine solutions were added into 20-mL serum bottles with 5.00 g (wet weight) sterile manure. The bottles were sealed with aluminum caps with Teflon-coated butyl rubber septa, and were shaken horizontally in a reciprocating shaker (Eberbach, Ann Arbor, MI) at a vigorous rate for 3 h at 25 ± 0.5°C. A portion of slurry from each bottle was transferred into 1.5-mL polypropylene centrifuge tube and centrifuged at 14 000 rpm for 3 min in a microcentrifuge (Spintron, Meuchen, NJ). Supernatant from each tube and the original sulfadimethoxine solutions were analyzed using HPLC to obtain the sulfadimethoxine concentration. Blank controls were also analyzed. A preliminary study showed that sorption reached more than 95% of equilibrium after 3 h of shaking under these conditions and no significant degradation of sulfadimethoxine was observed. Adsorption of sulfadimethoxine in manure was calculated using Eq. [1]:

$$Q = \frac{C_{\text{ini}} \times 10.0 - C_{\text{e}} \times (10.0 + 5.0 \times 28\%)}{5.0 \times (100\% - 28\%)} \qquad [1]$$

where Q (μmol kg^{-1} dry weight) is the adsorbed quantity of sulfadimethoxine in manure, C_{ini} (μM) and C_{e} (μM), respectively, are the initial and equilibrium concentrations in the aqueous phase, and 28% is the weight percentage of water in the wet manure with moisture at 39%.

Degradation Experiments

For the experiments of sulfadimethoxine degradation in manure with different initial concentrations, five portions of 328 g (dry weight) manure were weighed and placed into 5 plastic ziplock bags. Then 50 g of manure from each bag was transferred into 5 250-mL beakers and 6.0 mL acetone solution containing 1.0×10^3, 2.0×10^3, 5.0×10^3, 1.0×10^4, and 1.5×10^4 μM sulfadimethoxine was spiked, respectively, into the manure in each beaker. After evaporation of acetone in a vacuum hood, sulfadimethoxine-spiked manure was put back into the original zip bags and then the manure was thoroughly mixed manually. After addition of 264 g water, the manure in each bag was thoroughly mixed again. The moisture of manure in each bag was 83% and the initial concentrations of sulfadimethoxine in manure were 1.8×10^1, 3.6×10^1, 9.1×10^1, 1.8×10^2, and 2.7×10^2 μmol kg^{-1} (dry weight), respectively. The spiked manure in each bag was then weighed into eight 150-mL glass jars at 7×10^1 g per jar. After the weight of each jar was recorded, jars were loosely covered with aluminum foil and subsequently put into a 25 ± 0.5°C constant-temperature room for incubation. Every 2 d, jars were opened and water was added to compensate for any moisture loss. At Day 0, 2, 5, 9, 14, 19, 24, and 30, one jar from each initial sulfadimethoxine concentration was taken out,

sealed with a cap, and then put into a −21°C freezer until sample extraction for sulfadimethoxine analysis.

To investigate the trend of sulfadimethoxine partition between solid and aqueous phase in manure during the degradation process, an additional set of spiked manure with initial concentration at 1.8×10^2 μmol kg^{-1} (dry weight) was incubated under identical conditions. At Day 0, 0.3, 1, 1.2, 1.9, 3.0, 4.2, and 4.9, one jar was taken out and manure was immediately weighed into six 40-mL polyethylene centrifuge tubes at 10.0 g per tube. For three of the tubes, 10.0 mL water was immediately added. After gentle upside-down shaking for 3 times, manure was centrifuged at 11 000 rpm (International Equipment, Needham, MA) with temperature controlled at 25°C for 10 min. A portion of supernatant from each tube was further centrifuged at 14000 rpm in a microcentrifuge for 3 min. Supernatants were transferred into GC-vials for HPLC analysis. The detected sulfadimethoxine in aqueous solution was assumed to be that partitioned in aqueous phase in manure at the corresponding incubation time. For the other 3 tubes, manure was immediately extracted for the analysis of total remaining sulfadimethoxine. The percentage of sulfadimethoxine in the aqueous phase compared to the total remaining was calculated using Eq. [2]:

$$P = \frac{\left[\dfrac{C_{aq} \times (10.0 + 10.0 \times 28\%)}{1000}\right]}{M} \times 100\% \quad [2]$$

where P (%) is the percentage of aqueous phase sulfadimethoxine in the total remaining, C_{aq} (μM) is detected aqueous concentration of sulfadimethoxine, and M (μmol) is the total remaining amount of sulfadimethoxine in manure.

For the degradation of sulfadimethoxine in sterile manure, 2 portions of 328 g (dry weight) manure were weighed into 2 Fisher autoclave bags. In one of the bags, 100 mL aqueous solution containing 15 mL 85% H_3PO_4 was added and the manure was thoroughly mixed. The pH of the acidified manure was measured to be 4.70 at manure/water ratio = 1:2 (wet weight). Both bags of manure were then sterilized at 121°C for 40 min. Sterilization was repeated a total of 3 times. After cooling completely, a sulfadimethoxine acetone solution at 1.5×10^4 μM was spiked into the sterile manure in a laminar flow hood. Sterile deionized water was used to adjust the manure moisture content to 83%. All jars and metal caps were sterilized in an oven at 150°C for 6 h. After thorough mixing, the manure samples were weighed and placed into jars inside a laminar–flow hood, the jars were immediately sealed with caps, and were put into a 25 ± 0.5°C temperature-constant room. No moisture compensation was performed during incubation. The degradation of sulfadimethoxine in sterile acidified manure was performed as a blank control.

To investigate the effect of manure moisture content, the degradation of sulfadimethoxine in manure at moisture contents of 39, 60, and 83% was studied. The initial concentration was 9.1×10^1 μmol kg^{-1} (dry weight) and all the other experimental procedures were the same as those described above.

Degradation of sulfadimethoxine in nonsterile manure at different temperatures was also performed. Manure moisture content was kept at 83% and the initial concentration of sulfadimethoxine was controlled at 9.1×10^1 μmol kg^{-1} (dry weight). Incubation temperature was set at 25 ± 0.5°C and 40 ± 0.2°C, respectively.

The samples were stored for a period less than 31 d in a −21°C freezer.

Sample Extraction and Concentration Analysis

After thawing at room temperature, the manure in each jar was thoroughly mixed and 12-g samples were weighed and placed into three 40-mL polyethylene centrifuge tubes. Fifteen mL of methanol/acetic acid mixture (10:1 in volume) was added into each tube. After sealing with caps, the tubes were vigorously shaken for 30 min in a reciprocating shaker. Tubes were then centrifuged at 11 000 rpm for 10 min and supernatants were transferred into 50-mL volumetric flasks. After the extraction was repeated 3 times, each flask was filled to the volumetric line with methanol/acetic acid mixture, sealed with a stopper, and then gently shaken for 30 s. One mL of aliquot from each flask was subsequently placed into a 1.5-mL polypropylene microcentrifuge tube and was centrifuged at 14000 rpm for 3 min. Supernatant in each tube was then transferred into a GC-vial for sulfadimethoxine concentration analysis.

A Hewlett-Packard Series II 1090 High Performance Liquid Chromatography (HPLC) was used for concentration analysis. An Agilent Hypersil ODS 5-μm, 4.0- × 250-mm column was used for separation. Mobile phase was composed of 75% water containing 10 mM ammonium acetate, 10 mM acetic acid, and 25% acetonitrile. The operation wavelength of diode array detector was set at 270 ± 20 nm with reference of 450 ± 80 nm. The retention time of sulfadimethoxine under these operation conditions was 8.5 min.

The extraction recovery of sulfadimethoxine from manure was determined to be 89.5 ± 0.7 and 90.9 ± 0.8% at spiking concentrations of 15.2 and 152 μmol kg^{-1} (dry weight), respectively. With an eight-replicate test, limit of detection (LOD) and limit of quantification (LOQ) of this analytical method was determined to be 1.1 and 3.6 μmol kg^{-1} (dry weight), respectively. Repeated HPLC analysis showed that sulfadimethoxine was very stable in both aqueous and organic extracts. Less than 5% concentration changes were observed for a period of 2 d at room temperature.

All concentration values presented in this study have been adjusted based on the extraction recovery.

Kinetic Model

The degradation kinetics for many pesticides (Beulke and Brown, 2001; Guo et al., 2004) and other organic contaminants (De Liguoro et al., 2003; Xu and Obbard, 2004) in the environment obey the simple first-order model, which can be expressed as below.

$$\frac{dC}{dt} = -kC \quad [3]$$

where, C (μmol kg^{-1} or μmol L^{-1}) is the concentration of the target compound at time, t is time (d), and k (d^{-1}) is the rate constant.

Sorption and fixation may greatly reduce the degradation rate of organic contaminants in environmental media since adsorbed target compounds are generally unavailable to bacteria (Thiele-Bruhn, 2003). For materials high in organic matter, a significant amount of target compounds may be adsorbed. If the ratio of aqueous phase to the total remaining target compound in the manure at time t is λ (i.e., λ = P/100%), then Eq. [3] can be written as:

$$\frac{dC}{dt} = -k\lambda C \quad [4]$$

Situations where λ is a constant result in an effective rate constant, k', and Eq. [4] can be written as:

$$\frac{dC}{dt} = -k'C \quad [5]$$

and the overall kinetics would obey the simple first-order model.

However, if λ is variable with time (i.e., λ(t)), the degradation kinetics in the manure will not follow the simple first-order model. It is assumed that the ratio can be expressed as

$$\lambda(t) = \lambda_o e^{-at} \quad [6]$$

where a is a positive constant called the availability coefficient and λ_o is the fraction of nonadsorbed amount in the total amount of the target compound at $t = 0$. A higher value of a indicates that the ratio λ decreases from its initial value faster with time during the degradation process. Substituting Eq. [6] into Eq. [4], we get:

$$\frac{dC}{dt} = -k''Ce^{-at} \quad [7]$$

where, $k'' = k\lambda_o$. Integrating Eq. [7] gives a kinetic model that accounts for the availability of sulfadimethoxine for degradation.

$$C_t = C_0 e^{-\frac{k''}{a}(1-e^{-at})} \quad [8]$$

where, C_0 (μmol kg^{-1} or μmol L^{-1}) and C_t (μmol kg^{-1} or μmol L^{-1}) are the target compound concentration at time 0 and t, respectively. Equation [8] is called the availability-adjusted first-order model.

RESULTS AND DISCUSSION

Decreasing Availability of Sulfadimethoxine in Manure during Degradation

The percentage of aqueous sulfadimethoxine relative to the total remaining, P, decreased with degradation time (Fig. 1). Significance analysis was performed to identify any existence of significant difference between the obtained values of P during the degradation. Results of the t-test at a probability of 0.05 indicated that the differences between all obtained values of P are significant except for those between column 3 and 4, 4 and 5, 5 and 7, 5 and 8, and all those between the last 3 columns in Fig. 1. Although the sampling process might cause some sorbed sulfadimethoxine to partition into the aqueous phase and no significant difference was identified between some of the P values, the trend of decreasing availability of sulfadimethoxine with degradation time cannot be disputed. During the 5-day degradation experiment, the percentage of aqueous sulfadimethoxine decreased from 48.0 ± 1.2 to 15.0 ± 2.6%. This evidence supports the use of Eq. [6] to describe degradation kinetics for compounds like sulfadimethoxine, where the ratio λ(t) decreases with time.

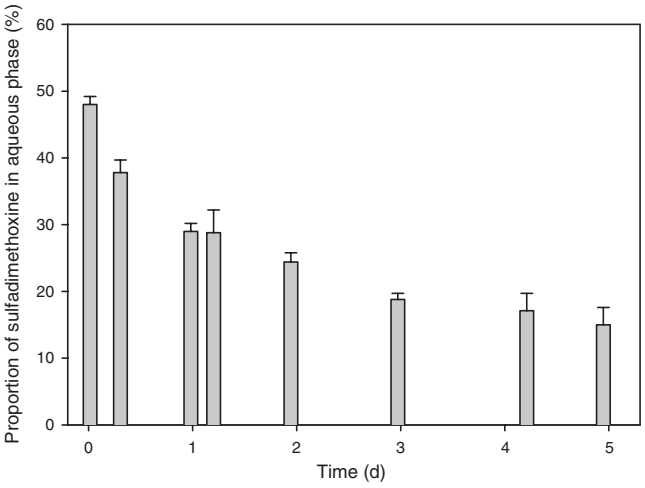

Fig. 1. The percentage of aqueous-phase sulfadimethoxine relative to the total remaining in the manure during the degradation period.

There are at least two possible reasons for the observed decrease in the availability of sulfadimethoxine in the manure during the degradation experiment. One is that the sorption of sulfadimethoxine in manure follows an L-shaped isotherm, where the slope of the adsorption isotherm decreases with the increasing sulfadimethoxine concentration (Sparks, 2003). In this case, the ratio of the adsorbed amount to the total remaining amount in manure increases with the decreasing total amount of sulfadimethoxine. Hence, during the degradation process, and if no desorption hysteresis occurs, the availability of sulfadimethoxine is controlled by the sorption isotherm and thus decreases with the degradation. However, the sorption experiments showed that sulfadimethoxine sorption isotherm in manure was almost linear (Fig. 2). No significant decrease in the slope of the sorption isotherm with the increasing sulfadimethoxine concentration was observed. Therefore, the shape of the sulfadimethoxine sorption isotherm was not the cause of the decreasing availability of sulfadimethoxine during its degradation in manure.

Another possible explanation is that desorption hysteresis occurred during the sulfadimethoxine degradation process. If desorption hysteresis did not occur, adsorbed sulfadimethoxine would always be in equilibrium with the nonadsorbed sulfadimethoxine and would follow the sorption isotherm during the degradation process. As shown in Fig. 2, the sorption isotherm of sulfadimethoxine in manure was almost linear. If desorption hysteresis did not occur, no significant decrease in the percentage of aqueous sulfadimethoxine would have been observed during degradation in manure. The observed phenomena shown in Fig. 1 and 2 are indicative of desorption hysteresis and provides an explanation for the decreasing availability of sulfadimethoxine in manure during the degradation process.

The decreasing availability of sulfadimethoxine in manure during degradation was further confirmed by the

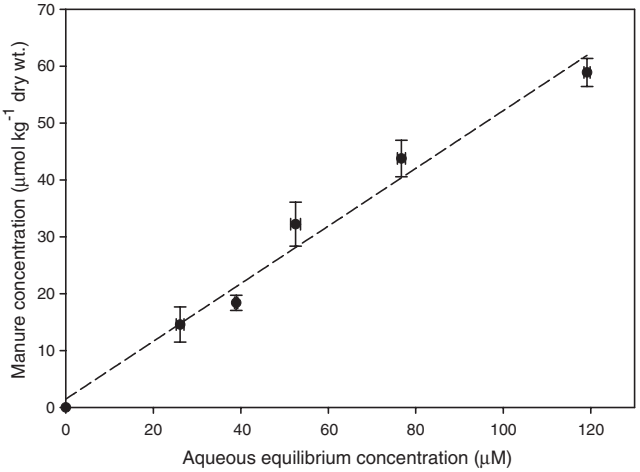

Fig. 2. Sulfadimethoxine sorption isotherm in manure at 25°C.

fitting results of sulfadimethoxine degradation kinetics using a simple first-order model and the availability-adjusted first-order model. A comparison of the fit of each model to the experimental measurements for an initial concentration of 17.8 μmol kg^{-1} is shown in Fig. 3. At the beginning of the degradation process, the simple first-order model overpredicts the measured concentration values. After 5 d, the predicted concentrations approached zero, while the experimental values approached a nearly constant value of 5 μmol kg^{-1}. The regression correlation coefficient, *r*, was 0.87. The simple first-order model provides an inaccurate description of sulfadimethoxine degradation kinetics in manure. The fit of the availability-adjusted first-order model is also shown in Fig. 3. This kinetic model fits the sulfadimethoxine degradation measurements very well and had a correlation coefficient, *r*, that was >0.99. Compared to the simple first-order model, the improved fit of the availability-adjusted first-order model indicates that the availability of sulfadimethoxine in manure is one of the factors affecting the degradation kinetics.

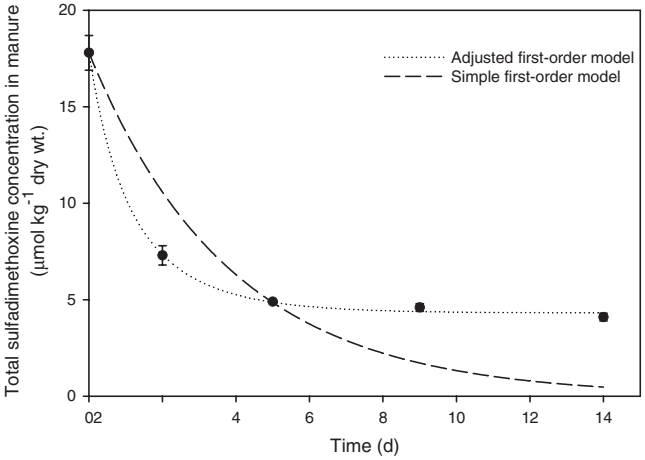

Fig. 3. Comparison of the fit of the simple first-order model and the availability-adjusted first-order model for sulfadimethoxine degradation in manure. Points are experimental data and lines are the fitting results.

Degradation of Sulfadimethoxine in Manure with Different Initial Concentrations

Sulfadimethoxine degraded very fast in nonsterile manure (Fig. 4a). Within 3 d, half of the sulfadimethoxine added to the manure was degraded. This indicates that storage of manure under aerobic conditions for a period of time could be very effective in eliminating sulfadimethoxine from manure and provides a method to mitigate sulfadimethoxine contamination in the environment. Conversely, immediate application of fresh manure from animals treated with sulfadimethoxine may result in unnecessary contamination.

Sulfadimethoxine degradation kinetics in sterile and nonsterile manure obeys the adjusted first-order model (Fig. 4a and b). All values of correlation coefficient, *r*, were >0.99 (data not shown). The values of the first-order rate constant, *k″*, and the availability coefficient, *a*, are listed in Table 1. Half-lives of sulfadimethoxine in

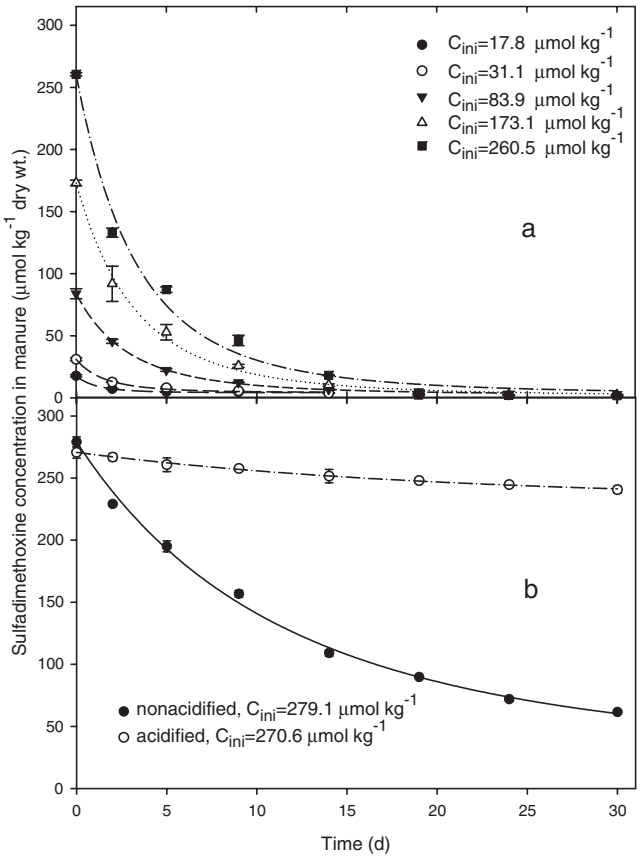

Fig. 4. The degradation kinetics of sulfadimethoxine in (a) nonsterile and (b) sterile manure. Points are experimental data. Lines are fitting results using the adjusted first-order model.

the manure at different initial concentrations were calculated from using Eq. [9]:

$$t_{1/2} = -\frac{1}{a}\ln\left(1 - \frac{0.693a}{k''}\right) \quad [9]$$

which was derived from Eq. [8]. Sulfadimethoxine half-lives in the manure are also listed in Table 1.

Sulfadimethoxine degradation in sterile manure appeared to be much slower than that in nonsterile manure. The rate constant, *k″*, at an initial concentration of 279.1 μmol kg^{-1} in sterile nonacidified manure was 0.080 ± 0.005 d^{-1}, which was less than one-third of that in nonsterile manure with a similar initial concentration. It is well known that the degradation of organic contam-

Table 1. Values of the rate constant, *k″*, the availability coefficient, *a*, and the half-life, $t_{1/2}$, for sulfadimethoxine degradation in sterile and nonsterile manure at different initial concentrations.

Initial concentration	First-order rate constant, *k″*	Availability coefficient, *a*	Half-life, $t_{1/2}$
μmol kg^{-1} (dry wt.)	d^{-1}		d
Nonsterile manure			
17.8	0.699 ± 0.032	0.495 ± 0.028	1.36
31.1	0.560 ± 0.061	0.290 ± 0.049	1.53
83.9	0.332 ± 0.008	0.098 ± 0.007	2.34
173.1	0.303 ± 0.020	0.066 ± 0.018	2.48
260.5	0.294 ± 0.034	0.067 ± 0.030	2.56
Sterile manure†			
279.1	0.080 ± 0.005	0.033 ± 0.007	10.2

† Nonacidified.

inants in sterile media results only from chemical processes, whereas in nonsterile media degradation results from both chemical and biological processes (Ma et al., 2001; Guo et al., 2004). The higher degradation rate in nonsterile manure indicated that microorganisms were responsible for the significant part of sulfadimethoxine degradation in manure. Hence, optimizing conditions to promote the bioactivity and enriching microorganisms may be an effective means to eliminate antibiotic contamination in manure. Amending active sludge into manure to enhance the degradation of antibiotics might be worthy of further investigation. As a blank control, sulfadimethoxine in sterile acidified manure did not exhibit a significant degradation during the incubation period.

It is well known that the rate constant of a chemical reaction is determined by temperature and reaction activation energy (Cotton et al., 1995). It could be taken for granted that the first-order rate constants for the degradation of organic contaminants in soil and other environmental media are independent on the initial concentration of the contaminants. However, in nonsterile manure, the degradation rate constant varied with the changes of sulfadimethoxine initial concentration. When the initial concentration increased from 17.8 to 260.5 μmol kg^{-1}, the degradation rate constant decreased from 0.699 to 0.294. The higher the initial concentration of sulfadimethoxine in manure, the lower the degradation rate constant. This suggested a conflict between the theory of reaction kinetics and the values of rate constant obtained in this study. In fact, the degradation kinetics of sulfadimethoxine observed in this study was an integration of all the kinetic processes, such as sorption and desorption, biological degradation, and chemical degradation. As discussed above, the microorganisms were responsible for the significant part of sulfadimethoxine degradation in manure. Often, the bioactivity of the degrading microorganisms in manure or soil is assumed to be constant at different initial concentrations of target compound. However, it may not be true if the bioactivity of the degrading and/or nondegrading microorganisms is inhibited to some extent with the addition of a toxic (e.g., antibiotic) chemical. At the higher concentrations, the target compound would be more lethal, which would lower the bioactivity of the indigenous microorganisms. Pharmaceutical antibiotics are designed to primarily affect microorganisms; so even at very low concentrations, the activity of certain species of microorganisms would be inhibited (Halling-Sorensen et al., 2002). This would lead to a reduction in the degradation rate constant with increasing initial concentration of antibiotic.

A similar phenomenon was observed in the degradation of 1,3-D and MITC in soil (Ma et al., 2001). The degradation kinetics of these 2 fumigants was found to obey the simple first-order model. However, the observed rate constants decreased with increasing initial concentration. It was concluded that the decrease of 1,3-D degradation rate constant was caused by a reduction of the bioactivity of the degrading microorganisms, since these two fumigants are toxic to bacteria.

In nonsterile manure, the availability coefficient a, decreased with decreasing rate constant, k''. Since larger a signifies a more rapid reduction of aqueous sulfadimethoxine percentage relative to the total remaining in the manure, the decrease of a with decreasing k'' implied that the availability of sulfadimethoxine for conditions with a high degradation rate constant was lower than for conditions with a low degradation rate constant, given the same incubation time. Thus a trend was found where sulfadimethoxine would be continually available (i.e., a would be 0) conditions with a low degradation rate. For this case, desorption would have sufficient time to maintain equilibrium while the degradation process commences, and the overall degradation kinetics would follow the simple first-order model. The decrease of a with the decreasing k'' confirmed the existence of sulfadimethoxine desorption hysteresis, which impeded sulfadimethoxine from rapid degradation in manure.

As a result of the decreasing degradation rate constant, the half-life increased with the increasing initial concentration of sulfadimethoxine in nonsterile manure (in Table 1). This indicates that for a given incubation time a higher percentage of sulfadimethoxine could be degraded in manure at a lower initial concentration than at a higher initial concentration. Thus, an approach to manage manure would include mixing highly contaminated manure with slightly contaminated or noncontaminated manure. Isolating animals that are receiving therapeutic treatment with high doses of sulfadimethoxine would allow easy identification of the excreta that needs to be mixed with that from untreated animals. Lowering the initial concentration of sulfadimethoxine in the manure helps reduce sulfadimethoxine contamination in the environment.

Effect of Manure Moisture

Sulfadimethoxine degradation in manure was enhanced with the increase of manure moisture (in Fig. 5). At all moistures, the degradation kinetics obeyed the adjusted first-order model. The values of the correlation coefficient, r, were all above 0.99 (data not shown). When manure moisture is increased from 39 to 60 and 83%, the rate constant, k'', increases from 0.183 ± 0.018

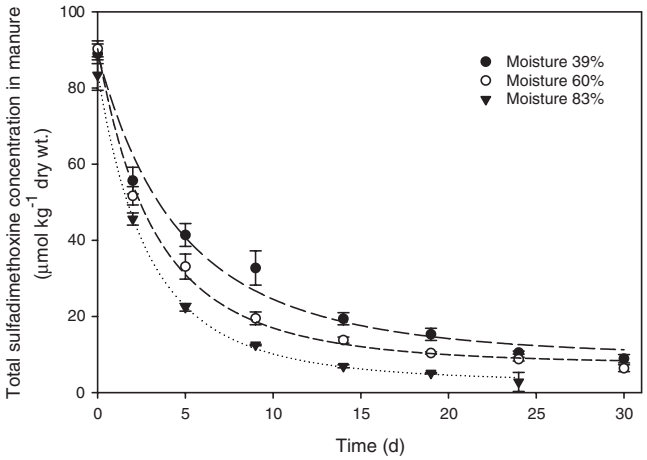

Fig. 5. The degradation of sulfadimethoxine in nonsterile manure at different moisture contents. Points are experimental data and lines are model-fitted results using the adjusted first-order model.

to 0.281 ± 0.015 and 0.332 ± 0.008 d^{-1} and the *a* value increases from 0.079 ± 0.017 to 0.114 ± 0.012 and 0.098 ± 0.007, respectively. The half-life of sulfadimethoxine degradation was correspondingly reduced from 4.49 to 2.90 and 2.34 d, respectively.

As $k'' = k\lambda_o$, the increase of k'' with the increasing moisture may be mainly attributed to the increase of λ_o. With increased water contained in the manure, there would be more sulfadimethoxine dissolved in the aqueous phase and more available to degradation. Keeping manure at high moistures during its storage may effectively accelerate sulfadimethoxine degradation in manure.

Sulfonamides have been considered to have little sorption affinity in soil and thus may possess a high mobility in the soil (Tolls, 2001). This has been further confirmed in the field experiments. Within 24 h of application of spiked liquid manure, sulfachloropyridazine, a sulfonamide antibiotic, was found to have leached to 20-cm depth (Kay et al., 2004). Sulfachloropyridazine was also identified to have a strong potential for runoff. With only 3 to 4 mm rainfall in 1 h, sulfachloropyridazine was detected at 703.2 μg l^{-1} in the runoff which is about 10 times that of oxytetracycline with a similar spiked initial concentration in the applied liquid manure (Kay et al., 2005). Thus, application of sulfonamide antibiotic-contaminated manure into the agricultural land may result in serious contamination of groundwater and surface water. Treatment of contaminated manure before application to enhance the degradation of sulfonamides appears to be a good strategy to reduce their contamination in the agricultural environment.

Temperature Dependency

When the incubation temperature was increased from 25 to 40°C, degradation of sulfadimethoxine in manure increased (in Fig. 6). Degradation kinetics at the two temperatures obeyed the availability-adjusted first-order model. The values of regression correlation coefficient, *r*, were both above 0.99 (data not shown). When the temperature increased from 25 to 40°C, the degradation rate constant, k'', increased from 0.332 ± 0.008 to 0.777 ± 0.073 d^{-1} and the availability coefficient, *a*, increased from 0.098 ± 0.007 to 0.311 ± 0.040. The half-life was reduced from 2.34 d at 25°C to 1.04 d at 40°C.

The increase of rate constant, k'', with the increasing temperature may be attributed to the increase of both *k* and λ_o. Based on the Arrhenius equation (Cotton et al., 1995), an increase of temperature accelerates a reaction by increasing the rate constant. Both chemical and biological degradation process of sulfadimethoxine in manure could be accelerated with the increasing temperature, thus increasing *k*. With the increase of temperature, sulfadimethoxine availability in manure for degradation could also be increased since sorption of organic contaminants in various media is generally reduced with the increase of temperature (Fruhstorfer et al., 1993). It is very likely that λ_o also increased with the increasing temperature.

Furthermore, with increasing temperature the rate of sulfadimethoxine desorption would also increase and would further increase sulfadimethoxine degradation compared to lower temperatures.

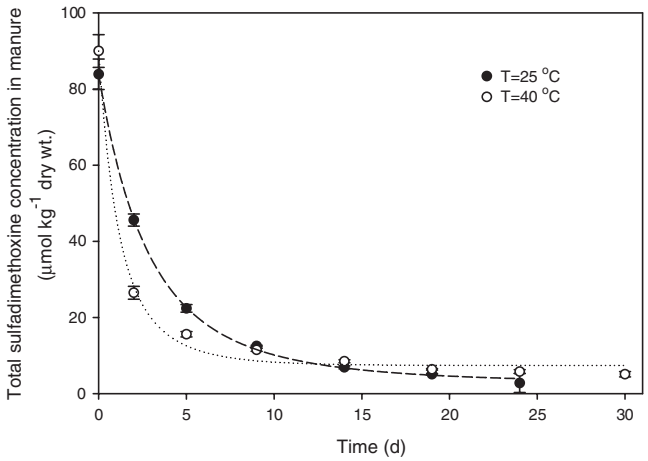

Fig. 6. The degradation of sulfadimethoxine in nonsterile manure at 25 and 40°C. Points are experimental data and lines are model-fitted results using the adjusted first-order model.

Therefore, keeping contaminated manure at moderately high temperatures may effectively enhance the degradation of sulfadimethoxine in manure. In cold places or during low temperature seasons, manure from sulfadimethoxine-treated animals may have to be stored for a longer time compared to that at warm conditions. Temperature effect might have to be considered when estimating the required storage period for contaminated manure to degrade sulfadimethoxine.

CONCLUSIONS

The degradation kinetics of sulfadimethoxine in both sterile and nonsterile manure obeyed the adjusted first-order model. The degradation rate constant in nonsterile manure was significantly higher than that in sterile manure, indicating that the microorganisms were a major contributor to the degradation of this antibiotic in manure. With an increase in the initial concentration of sulfadimethoxine in manure, the bioactivity of the degrading microorganisms may be gradually inhibited and the degradation rate constant could be subsequently reduced. Raising manure moisture or storage temperature may significantly increase sulfadimethoxine availability for degradation and effectively enhance sulfadimethoxine degradation in manure.

ACKNOWLEDGMENTS

The authors highly appreciate the assistance from Qiaoping Zhang, USDA Salinity Laboratory, in performing the experiments of this study. Help from Wen Ly, University of California, Riverside, is also highly valued.

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