

Simultaneous Removal of Oxytetracycline and Sulfamethazine Antibacterials from Animal Waste by Chemical Oxidation Processes

MERİH ÖTKER USLU AND İŞİL AKMEHMET BALCIOĞLU*

Institute of Environmental Sciences, Bogazici University, 34342 Bebek, Istanbul, Turkey

Simultaneous degradation of oxytetracycline (OTC) and sulfamethazine (SMZ) antibacterials in synthetically contaminated cow manure (20 mg of antibacterials/kg of manure) in the presence and absence of bedding was investigated by the application of ozone, Fenton, and persulfate oxidation processes. Almost the complete removal of antibacterials was attained with all oxidation processes, which were combined with a pretreatment of manure with magnesium (Mg^{2+}) salt desorption. Among the investigated oxidation processes, thermally activated persulfate oxidation with 25 mM $Na_2S_2O_8$ at 50 °C was also applied to the animal feeding operations wastewater, and the pseudo-first-order degradation rate constants of OTC and SMZ were found as 3.22 and 1.25 (1/h), respectively. Thermally activated persulfate treatment resulted in the reduction of 82% inhibition of OTC and SMZ to 7%, indicating the production of almost nontoxic degradation products in the wastewater.

KEYWORDS: Veterinary antibacterials; manure; chemical oxidation; Fenton oxidation; ozonation; persulfate oxidation

INTRODUCTION

Tetracyclines (TCs) and sulfonamides (SAs) are commonly used antibacterial agents in livestock production and aquaculture for therapeutic and prophylactic purposes. After administration, up to 90% of these compounds are excreted as a parent compound through urine and feces. Consequently, both sulfonamides and tetracyclines have been detected in manure samples at up to 91 and 46 mg/kg, respectively (1, 2). Residues of antibacterial compounds can also be found in wastewater originated from animal feeding operations (3), and subsequent use of manure or wastewater as a fertilizer results in contamination of agricultural soil with the aforementioned substances. TCs adsorb strongly to solid matrices that reduce their bioavailability, whereas SAs have low sorption tendency (4, 5). As a result of their high mobility, SAs are more frequently detected in surface water and ground-water samples compared to TCs (6).

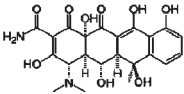
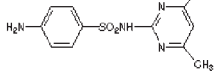
A major concern related with the presence of antibacterials in the environment is the emergence of resistance; antibacterial resistance increases the difficulty of treating infections, with a resulting effect on morbidity, mortality, and economic cost. Numerous publications have shown the occurrence of various resistant bacteria in the environment (7). Sengelov et al. (8) observed increased levels of tetracycline resistance in soil after the application of pig manure slurry containing tetracycline residues. Therefore, use of manure as a fertilizer might be a risk factor for public health because it may cause the transfer of antibacterial-resistant bacteria to humans through water or plants (7). The adverse effect of antibacterials in the environment

is not restricted only to resistance development. Methane inhibition in anaerobic swine lagoons due to the presence of SAs and TCs (10 mg/L) was reported at up to 54 and 43%, respectively (9). The inhibition of sulfonamides on soil respiration in the first two days with EC_{10} values of 7 mg/kg sulfamethaxazole and 13 mg/kg sulfamethazine was reported by Liu et al. (10). With regard to these adverse effects, it is important to remove antibacterial agents completely at their source (e.g., manure) in a short time period, before resistance to the agents can be developed in microorganisms and therefore spread into the environment. Recent studies revealed that chemical oxidation processes are able to treat water and wastewater containing antibacterials (11, 12). These processes are also considered to be efficient treatments to degrade recalcitrant compounds in solid matrices. Most commonly used chemical oxidation processes for the treatment of contaminants in soil, sewage sludge, sediments, and manure are iron-catalyzed hydrogen peroxide oxidation (Fenton or Fenton-like oxidation), ozonation, and, more recently, persulfate oxidation (13–17).

The present study is aimed at the complete and simultaneous removal of two veterinary antibacterials, oxytetracycline (OTC) and sulfamethazine (SMZ), from cow manure by means of integrated treatment processes involving magnesium salt pretreatment with subsequent ozone, Fenton, or persulfate oxidation. The effects of oxidant doses and bedding materials on the effectiveness of integrated oxidation processes were investigated. This research also aims to remove OTC and SMZ from wastewater produced by animal feeding operations via persulfate oxidation. The effect of persulfate oxidation on macro- and micronutrients and the bacterial toxicity of wastewater that can be considered as a fertilizer were also evaluated.

*Corresponding author (telephone + 90 212 359 70 36; fax + 90 212 257 50 33; e-mail balciogl@boun.edu.tr).

Table 1. Selected Characteristics of OTC and SMZ

Antibiotic	Molecular Structure	Molecular Weight (g/mole)	pK _a	K _d soil ^c (l/kg)
Oxytetracycline (OTC)		496.9	3.57;7.49;9.44 ^a	417-1026
Sulfamethazine (SMZ)		278.36	2.07;7.49 ^b	1.0-3.1

^a Reference 18. ^b Reference 19. ^c Reference 4.

MATERIALS AND METHODS

Chemicals. Oxytetracycline hydrochloride (OTC) (Sigma, C₂₂H₂₄N₂O₉·HCl, >95% purity) and sulfamethazine (SMZ) (Sigma, C₁₂H₁₄N₄O₂S, 99% purity) were chosen as model antibacterial substances, and some of their properties are given in **Table 1**. Fenton's reagent (H₂O₂ (30%) and FeSO₄·7H₂O) were purchased from Riedel de Haen and Carlo Erba. Due to its high solubility, Na₂S₂O₈ (Sigma-Aldrich) was chosen for persulfate oxidation. Mg(NO₃)₂·6H₂O (Merck) was used in the pretreatment operation for the extraction of OTC and SMZ from cow manure. Formic acid and HPLC grade acetonitrile were obtained from Sigma-Aldrich for the analysis of OTC and SMZ. According to the analytical procedure, antibacterials were extracted from the manure by citric acid monohydrate (Merck), Na₂EDTA·2H₂O (Fluka), Na₂HPO₄ (Merck), and HPLC grade methanol (Sigma-Aldrich). Trypton type 1 (HiMedia) and yeast extract (AppliChem) were used for the preparation of the growth medium of *Staphylococcus aureus* (ATCC 6538; Refik Saydam Hygiene Center) in toxicity assessment tests. All solutions were prepared with Milli-Q ultrapure deionized water (Millipore, Milford, MA), and reagents used for chemical analyses were of analytical grade.

Manure and Bedding Materials. Cow manure was obtained from an ecological farm located in western Turkey, and it was confirmed that the cow manure did not contain oxytetracycline and sulfamethazine antibacterial contamination. After drying at 70 °C for 24 h, manure was passed through a sieve (2 mm) and subsequently sterilized in an autoclave at 120 °C for 15 min to prevent any biological activity; finally, it was stored at 4 °C until used in the treatment experiments. To mimic the use of bedding material in animal husbandry, sawdust and zeolite were added to manure at a ratio of 20% (w/w). Bedding materials were also sterilized and stored in the same manner with the manure. Both manure and manure with bedding mixtures were synthetically contaminated by spiking with OTC and SMZ stock solutions. Stock antibiotic solutions were prepared in methanol at a concentration of 1 mg/mL and stored in the dark to prevent their photodegradation. The initial concentration of OTC and SMZ in manure was 20 mg/kg of dry manure. After thorough mixing with a vortex (Nüve NM 110), the solvent was allowed to evaporate overnight in the dark.

Synthetic Wastewater. The manure was mixed with deionized water (1:10 w/v) at 150 rpm (Nüve, NM 110) for 24 h to prepare synthetic animal feeding operation wastewater. It was subsequently centrifuged at 4000 rpm (Hettich Universal 16A) and then filtered through an ordinary filter paper. Similar to the manure samples, the synthetic wastewater was sterilized and contaminated with OTC and SMZ stock solutions. The initial concentration of OTC and SMZ in synthetic wastewater was 5 mg/L.

Extraction Pretreatment of Contaminated Manure. Prior to the oxidation experiments, a pretreatment operation was applied to the synthetically contaminated manure by the addition of magnesium salt solution. Five grams of manure or manure with bedding mixtures was agitated with 25 mL of 1 M Mg(NO₃)₂ solution at 150 rpm and 25 °C for 30 min in a thermostated shaker (Julabo SW 22). After the mixing period, the manure slurry, which contained 5 g of manure and 25 mL of extraction solution, was subjected to ozonation, Fenton, and persulfate oxidation processes. The pH of the manure slurry was not adjusted for pretreatment and oxidation processes, and all analyses were performed in duplicate.

Ozonation of Manure Slurry. Thirty grams of the manure or manure with bedding mixture slurry in a reactor that consisted of a 100 mL gas washing bottle was ozonated for 2 h at room temperature in semibatch mode with a continuous flow of the ozone and oxygen gas mixture (applied

ozone concentration = 15 g/m³) at a rate of 0.01 m³/h. Ozone was produced from dry, pure oxygen in a laboratory-scale corona discharge ozone generator (Fisher OZ 500). All tubing from the ozone generator to the reactor was made of silicone, and fittings were made of Teflon. Excess ozone gas passed out through the top of the reactor into an ozone gas analyzer (Fisher Ozotron 23) to determine the quantity of ozone generated and applied to the manure slurry per unit time and the partial pressure of ozone in the feed stream. The mixing of manure slurry was provided by a magnetic stirrer to ensure homogeneity and contact between the manure and oxidants.

Fenton Oxidation of Manure Slurry. Fenton's reagent was slowly added to 100 mL capacity flasks containing 30 g of manure or manure with bedding mixture slurry at a Fe(II)/H₂O₂ molar ratio of 1:10 with various H₂O₂ doses (H₂O₂ = 50–1600 mM). The Fenton oxidation process was conducted in a thermostated shaker (Julabo SW 22) at 150 rpm and 25 °C for 2 h until all added hydrogen peroxide was consumed.

Persulfate Oxidation of Manure Slurry and Synthetic Wastewater. Different concentrations of Na₂S₂O₈ solution were added to a 100 mL capacity flask containing 30 g of manure or manure with bedding mixture slurry and then agitated in a thermostated shaker at 150 rpm for 2 and 24 h treatment periods. The concentration of Na₂S₂O₈ (10–100 mM) and temperature (25–50 °C) were varied in the experiments.

Synthetically prepared contaminated wastewater in a 100 mL capacity flask was treated by the addition of Na₂S₂O₈ solution (2.5–25 mM). The persulfate oxidation of wastewater (50 mL) was performed in a thermostated shaker at 150 rpm and 50 °C. At designated time intervals, 2 mL samples were taken, filtered (0.45 μm regenerated cellulose, Sartorius), and then immediately analyzed for antibacterials and nutrients.

Antibacterial Analysis. After the application of oxidation processes, the treated manure slurry was subjected to the extraction procedure for soil samples as described by Blackwell et al. (20) with some modifications (15). The antibacterial analysis in the manure extracts and synthetic wastewater was performed with an HPLC system (Agilent Technologies 1100 series) equipped with a tertiary pump, a photodiode array, and an autosampler with an automated injection system. Quantification of antibiotics was accomplished using external standard calibration. The recoveries of OTC and SMZ in the manure extracts were determined as 89.5 ± 2.5 and 60.3 ± 3.5%, respectively. When the antibacterial substances in the manure slurry extracts could not be detected by HPLC, they were qualitatively analyzed with LC-MS/MS consisting of a Thermo Scientific TSQ Quantum mass spectrometer (Thermo Fisher Scientific, San Jose, CA) coupled with the company's Surveyor LC pump and autosampler. **Table 2** provides the operational conditions and column properties used of both systems.

Toxicity of Raw and Treated Synthetic Wastewater. Acute toxicity of contaminated and persulfate-treated contaminated wastewater was evaluated by their inhibition effect on the growth of *S. aureus*, which was chosen as the test species due to its abundance in the environment and susceptibility to tetracycline and sulfonamide group antibacterials (21, 22). To overcome the possible interference of remaining persulfate to the bacterial growth, it was decomposed by the addition of sodium thiosulfate solution prior to administration of the bacterial toxicity test.

A colony of *S. aureus*, which was plated on Luria–Bertani (LB) agar, was inoculated with 25 mL of LB medium (1% (w/v) tryptone, 0.5% (w/v) yeast extract, and 1% (w/v) NaCl) for 18 h at 37 °C and 150 rpm. Three milliliters of this culture was transferred to 150 mL of fresh LB medium and agitated at 37 °C and 150 rpm until they reached to optical density of 0.12 at 660 nm wavelength. At this period, 20 mL of the culture was added to

Table 2. Operational Conditions for HPLC and HPLC-MS/MS Analyses

	HPLC	LC-MS/MS
mobile phases	A, ACN (0.1% formic acid) B, water (0.1% formic acid)	A, ACN (0.1% formic acid) B, water (0.1% formic acid)
flow	0.3 mL/min	0.7 mL/min
gradient	0 min, 10% A 0–5 min, linear increase to 40% A 5–8 min, linear decrease to 10% A 8–11 min, 10% A	0 min, 10% A 0–1 min, linear increase to 90% A; 1–8 min, 90% A 8–8.5 min, linear decrease to 10% A 8.5–15 min, 10% A
column	Eclipse XDB C ₁₈ (5 μ m, 150 \times 4 mm) detection OTC, 360 nm detection SMZ, 268 nm	Synergy Max RP (4 μ m, 150 \times 2 mm) parent mass OTC, 461 parent mass SMZ, 279 product mass OTC, 426–444 product mass SMZ, 156–204

the flasks containing 5 mL of uncontaminated wastewater and raw and persulfate-treated contaminated wastewater. Incubation of the microorganisms exposed to wastewater samples was continued for 4.5 h. At the end of the incubation period the growth inhibition was calculated using the formula

$$\%I = (\text{OD}_c - \text{OD}_f) / (\text{OD}_c - \text{OD}_i)$$

where OD_c = optical density (OD_{660}) of wastewater at the end of incubation, OD_i = optical density (OD_{660}) of the contaminated or persulfate-treated contaminated wastewater as the culture was added to the flask, and OD_f = optical density (OD_{660}) of the mixture of the culture and contaminated or persulfate-treated contaminated wastewater at the end incubation.

Other Chemical Analyses. The organic carbon (OC) content of the manure and bedding materials was analyzed by using the Walkley–Black method with some modifications (23). For this purpose, 0.02 g of manure or bedding materials was digested with 3 mL of 1 N dichromate solution in the presence of 3 mL of concentrated sulfuric acid. Finally, the excess dichromate was titrated with 0.5 N ferrous ammonium sulfate solution to determine the organic carbon content of manure and bedding materials. The cation exchange capacity (CEC) of the manure and bedding materials was determined according to EPA method 9081. Because macro (Ca, Mg, K) and micro (Cr, Zn, Fe) minerals were detected in several types of manure samples at high concentrations (2), their analyses were conducted in a filtered (0.45 μ m regenerated cellulose, Sartorius) wastewater sample using ICP (Perkin-Elmer, Optima 2100 DV). Analyses of NO_3^- , NO_2^- , Cl^- , and SO_4^{2-} contents were performed by IC (Dionex ICS-3000) with Ionpac AS19 analytical (7.5 μ m, 250 \times 4 mm) and Ionpac AG19 guard (11 μ m, 50 \times 4 mm) columns. NH_4^+ -N and reactive PO_4^{3-} contents were determined by Nessler and ascorbic acid methods using a Hach spectrophotometer.

Persulfate analysis in the liquid phase of manure slurry and synthetic wastewater was conducted according to the iodometric method (24). Hydrogen peroxide determination in the liquid phase of manure slurry was also performed according to the iodometric method in the presence of an ammonium molybdate catalyst (25).

RESULTS AND DISCUSSION

Pretreatment of Manure. In our previous study (16), the dissolution of antibacterial substances from cow manure into the aqueous phase of the slurry by means of complexation with Mg^{2+} ions enhanced the subsequent oxidation treatment efficiency of OTC. Similarly, in the current study, prior to the oxidation, a pretreatment with magnesium salt solution was applied on manure contaminated with OTC and SMZ at two different solid to liquid ratios (manure/extraction solvent = 1:5 and 1:3). Without the addition of magnesium salt, >75% desorption of SMZ was obtained in the aqueous phase of the manure slurry (solid/liquid = 1:5), which has a pH value of 8.6. On the other hand, under the same experimental conditions, the

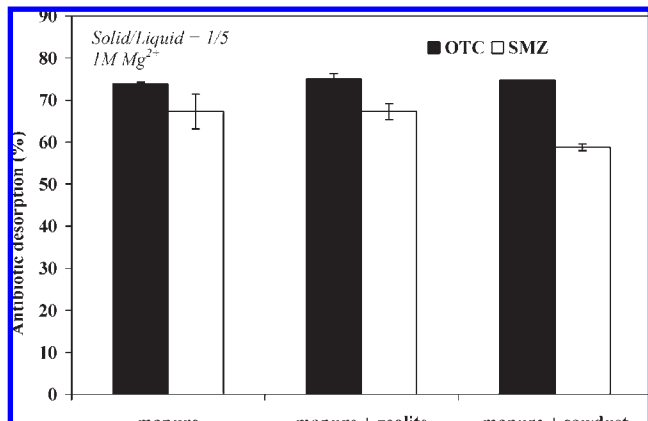
desorbable part of OTC was only 4%. As mentioned previously, TCs generally exhibit higher sorption tendencies in soils and clays compared to SAs at environmentally relevant pH values (4, 5). Furthermore, the sorption of sulfonamides has minor importance above their pK_{a2} values when anionic species of sulfonamides dominate (19). Therefore, the high desorption rate of SMZ is an expected result. The addition of 1 M Mg^{2+} salt at the same solid/liquid ratio greatly increased the extraction of OTC from the manure (73.7%) and reduced the extraction of SMZ by about 10% (data not shown). The reason for the unfavorable effect of extraction with Mg^{2+} salt solution for SMZ can be explained by the decrease of the manure slurry pH to 7.3, at which more sorption of SMZ is anticipated considering the pK_{a2} value (Table 1). Enhancement in the extraction efficiency of OTC by the addition of Mg^{2+} salt solution can be attributed to a higher ionic strength in the aqueous phase of manure slurry and metal complexation probability with OTC that lowers the sorption of the antibiotic on the manure. On the other hand, decreasing the volume of Mg^{2+} salt solution (solid/liquid = 1:3) lowered the extraction of OTC and SMZ from 73.7 and 67.2% to 67.4 and 66.3%, respectively. Considering the results obtained, subsequent experiments were performed with the manure pretreated by magnesium salt solution at a solid/liquid ratio of 1:5. Because manure has a high buffering capacity, no effort was spent to adjust the pH of the manure slurry.

Effect of Bedding Materials on the Performance of Pretreatment. Use of bedding in livestock and poultry production is a common practice to improve farm animal welfare, and the presence of bedding material in manure can affect its treatment. In this study, the effect of bedding material on the treatment of cow manure was investigated by using natural zeolite and sawdust. It was known that OC and CEC are two important parameters affecting the sorption of antibacterials on solid matrices (26, 27). Therefore, first, these parameters were analyzed in the selected bedding materials and manure (Table 3). Thereafter, both beddings were mixed with manure separately at a ratio of 20% (w/w) (28) and, finally, their effects on the extraction of antibacterials were determined (Figure 1).

Although the lower extraction of OTC would be anticipated in manure with bedding mixture due to possibly stronger sorption, there was no observable effect of bedding addition on the extraction of OTC (data not shown). The excess amount of magnesium ions in the aqueous phase of the manure slurry could sustain the dissolution of OTC. On the other hand, the addition of sawdust to the manure slightly decreased (9%) the extraction efficiency of SMZ, because sawdust has a high amount of organic matter, which has been reported to have greatly contributed

Table 3. Organic Carbon and Cation Exchange Capacities of Manure and Bedding Materials

	OC (%)	CEC (mequiv/100 g)
manure	7.3 ± 0.5	28 ± 0.8
sawdust	58.8 ± 1.25	65 ± 1.0
zeolite	0.18 ± 0.08	63 ± 1.2

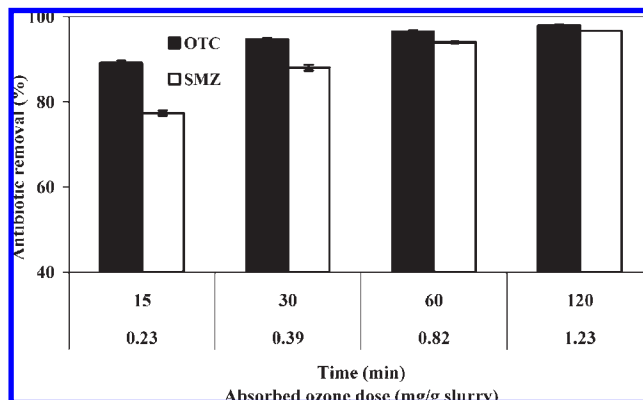
**Figure 1.** Effect of bedding materials on antibacterial extraction from manure.

toward sulfonamide sorption in solid matrices (27). Furthermore, the addition of sawdust resulted in a slight decrease in the pH value of manure slurry (from 7.3 to 6.8), which may also contribute to the sorption of SMZ on solid mixture.

Ozonation of Manure Slurry. Ozone treatment has been applied to several wastes from animal feeding operations for the purposes of disinfection (29), the removal of natural steroid hormone (30), and the reduction of malodorous substances (31), pathogens, and toxic fermentation metabolites (32). Considering the benefits of ozonation on the treatment of animal wastes, after the pretreatment, ozonation was applied to the manure for a 2 h treatment period to accomplish the simultaneous removal of OTC and SMZ (Figure 2).

As represented in Figure 2, in the absorbed ozone dose ranges of 0.23–1.23 mg of ozone/g of slurry, a slightly lower removal rate of SMZ was obtained from the manure slurry compared to that of OTC. Dodd et al. (33) also reported a lower transformation of sulfonamide antibacterials than tetracyclines in municipal wastewater by ozonation with ozone reaction rate constants of $5.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (sulfamethoxazole) and $3.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (tetracycline) at pH 7.7, respectively.

Fenton Oxidation of Manure Slurry. Depending upon the results of our previous study related with the abatement of OTC (16), Fenton oxidation was applied to the pretreated manure spiked with OTC and SMZ within a wide hydrogen peroxide dose range ($[\text{H}_2\text{O}_2]_0 = 50\text{--}1600 \text{ mM}$; $[\text{H}_2\text{O}_2]_0/[\text{Fe}^{2+}]_0 = 10$) for a 2 h treatment period. Because the solutions of hydrogen peroxide and iron were acidified before the addition to the manure to increase the stability and solubility, respectively, the initial pH of all manure slurry samples treated with the different doses of oxidant decreased to about 4.5 except for the sample subjected to the addition of 1600 mM H_2O_2 . The initial pH descended to the value of 3.6 only at the highest dose of Fenton's reagent. Further pH adjustment was not applied to manure slurry samples in the Fenton oxidation process as in the case of other used oxidation processes. At the end of the 2 h treatment period, when hydrogen peroxide added to the manure slurry was completely consumed, the pH of the manure increased

**Figure 2.** Removal of OTC and SMZ as a function of ozonation time and absorbed ozone dose.**Table 4.** Effects of Hydrogen Peroxide and Iron(II) Concentrations on Fenton Process Efficiency

H_2O_2 (mM)	Fe (mM)	removal (%)	
		OTC	SMZ
50	5	66.7 ± 0.3	75.1 ± 0.1
100	10	90.0 ± 0.5	95.5 ± 0.8
275	27.5	95.9 ± 0.3	94.8 ± 0.3
680	68	96.8 ± 0.2	96.4 ± 1.5
970	97	96.4 ± 0.4	97.4 ± 0.4
1600	160	98.3 ± 0.1	97.8 ± 0.3

from about 4.5 to 6 (data not shown). The results of oxidation with various doses of Fenton's reagent for the simultaneous degradation of OTC and SMZ are listed in Table 4.

Contrary to the results of ozonation, the removal of SMZ was higher (75.1%) than that of OTC (66.7%) at the lowest applied dose of Fenton's reagent (50 mM H_2O_2 /5 mM Fe^{2+}). This result is also not consistent with the reported reaction rate constants of the sulfamethoxazole and tetracycline antibacterials with hydroxyl radicals (33). However, the degradation reaction pathways for the antibacterials in the manure could be affected by the variation of slurry composition during the oxidation process. Moreover, hydrogen peroxide as well as hydroxyl radical could be responsible for the oxidation of target pollutants under the experimental conditions of Fenton oxidation. Therefore, the abatement rates of antibacterials can be different from those obtained by ozonation.

On the other hand, similar to the results of the ozonation process, the increasing dose of oxidant resulted in a decrease between the removal rates of the two antibacterials, and the residuals of both antibacterials remained in the slurry even at excessive dose of Fenton's reagent. As presented in Table 4, the rise of hydrogen peroxide concentration in Fenton's reagent from 50 to 275 mM significantly enhanced simultaneous OTC and SMZ removal, whereas a further increase in hydrogen peroxide concentration did not cause remarkable improvement for the abatement of antibacterial residuals. The strongly sorbed antibacterials on the manure could not consume the produced radicals because of the rapid decomposition of high amounts of hydrogen peroxide in the presence of high amounts of iron through Fenton's reaction and increase in temperature. Instead, reactive radicals may oxidize the other organic compounds of the manure released into solution. Because the longer lifetime of hydrogen peroxide in soil slurry systems was reported to enhance contaminant removal (34), it would be more efficient to catalyze hydrogen peroxide decomposition by the indigenous iron content of manure at acidic pH. This suggestion is confirmed by the

Table 5. Effect of Oxidant Concentration on Thermally Activated Persulfate Process

Na ₂ S ₂ O ₈ (mM)	temperature (°C)	removal (%)			
		OTC		SMZ	
		<i>t</i> = 2 h ^a	<i>t</i> = 24 h	<i>t</i> = 2 h	<i>t</i> = 24 h ^b
10	40	73.7 ± 1.1	nd ^c	91.2 ± 1.2	nd
10	50	83.8 ± 0.8	98.9 ± 0.1	91.3 ± 0.5	99 ± 0.9
25	40	95.4 ± 0.1	98.0 ± 0.2	92.4 ± 0.4	98 ± 0.4
25	50	nd	100	nd	100
50	40	98.3 ± 0.2	nd	93.0 ± 0.8	nd
100	40	98.7 ± 0.1	>99	95.5 ± 0.7	>99
100 + 10 Fe ²⁺	25	nd	93.2 ± 0.08	nd	>99

^a Decrease in antibacterial concentrations after a 2 h control experiment at 40 °C was negligible. ^b Decrease in concentration of SMZ after a 24 h control experiment at 40 °C was 40%, whereas that of OTC was negligible. ^c Not determined.

findings of our previous study (16) in which a lower amount of oxidant in Fenton-like treatment (434 mM H₂O₂; no addition of soluble iron) was able to degrade OTC in the manure compared to Fenton treatment at acidic pH.

At low doses of Fenton's reagent (50 mM H₂O₂/5 mM Fe²⁺), magnesium salt pretreatment enhanced the OTC removal by about 10% but did not affect overall SMZ removal because desorption of SMZ was already provided by deionized water. It was reported that high hydrogen peroxide dose (≥300 mM) can enhance the efficiency of Fenton oxidation by desorbing the pollutants from the soil surface into solution where oxidation takes place (35). By taking into account this property of hydrogen peroxide, contaminated manure was also treated with the highest dose of Fenton's reagent (1600 mM H₂O₂/160 mM Fe²⁺) without application of pretreatment. Although it eliminated the necessity of pretreatment for the more efficient OTC removal, it did not cause the complete degradation of antibacterials (data not shown). Actually, this result is not remarkably different from that obtained by the lower dose of Fenton's reagent.

Persulfate Oxidation of Manure Slurry. Besides the ozone and Fenton oxidation processes, metal ion-activated and heat-activated persulfate oxidation processes were applied to the contaminated manure slurry. It was reported that sulfate radical ($E^\circ = 2.6$), which has an oxidation potential comparable to that of the hydroxyl radical ($E^\circ = 2.7$), is more selective than the hydroxyl radical in its reaction with organic compounds, and anilinic and phenolic groups exhibit high reactivity to this radical (36). Therefore, OTC and SMZ, which have the aforementioned reactive groups in their structures, could be efficiently degraded in the manure slurry having high organic matter content by the persulfate process. Moreover, its benign end product, sulfate, is considered to be a secondary nutrient for plant growth when the treated manure is used as fertilizer.

In this part of the study, Fe(II)-activated persulfate oxidation of the pretreated manure slurry was implemented with a single dose of persulfate (100 mM) at an oxidant/catalyst ratio of 10:1. Twenty-four hour oxidation at 25 °C resulted in 93.2% of OTC and >99% of SMZ removal. Although OTC has a structure that could exhibit higher reactivity to the oxidation, SMZ abatement in manure slurry was found to be higher than that of OTC, similar to the results of Fenton oxidation. The variation of the recovery rate of antibacterials from manure slurry by the application of an oxidation process can also be the reason for this controversial oxidation yield. Similar to Fenton oxidation, persulfate oxidation resulted in a decrease in the pH value of manure slurry to about 5.2, at which the sorption of SMZ on the manure is expected. The recovery rate of sulfonamide antibacterials in soil is strongly correlated with the sorption, which is in turn increased by increasing the contact time of an antibacterial with solid matrix (37,38). Accordingly, the results of control experiments in the

absence of oxidants showed relatively high SMZ abatement (30%), whereas that of OTC was negligible within 24 h, and no significant variation in the concentration of either antibacterial was observed within a 2 h treatment period. Although the decrease of OTC and SMZ concentrations may be related to the stability of antibacterials in the manure, the contribution of this factor could not be predominant at 25 °C because the occurrence of sulfonamide antibacterials has been reported in manure and soil samples collected from various fields (see, e.g., refs 1 and 39).

Alternatively, the activation of persulfate was performed by increasing the temperature of the medium; this activation method can be more suitable for the treatment of manure. Because elevated temperatures can be obtained by the action of microorganisms during the storage of manure heaps (40), a thermally activated persulfate process at two different temperatures (40 and 50 °C) was applied to the pretreated manure at various oxidant doses (10–100 mM Na₂S₂O₈). Furthermore, the effect of the reaction period on antibacterial removal was investigated (Table 5).

The removal of OTC was improved by approximately 20% when the persulfate dose was increased from 10 to 25 mM at 40 °C, whereas >90% SMZ was removed even with the 10 mM oxidant dose within a 2 h treatment period. A further increase of the oxidant dose to 100 mM resulted in only a small enhancement in antibacterial removals within the 2 h oxidation period. As the reaction time was extended to 24 h, thermally activated persulfate oxidation caused >99% removal for both antibacterials, which could not be achieved by Fenton and ozone oxidation processes at the described experimental conditions.

Despite the high organic content of the manure, OTC and SMZ antibacterials were removed by the application of the thermally activated persulfate process. More than 90% removal of antibacterial substances with only 20% persulfate decomposition ([Na₂S₂O₈]₀ = 25 mM) in a highly complex manure matrix is evidence for the high selectivity of persulfate and sulfate radicals for the studied antibacterial substances compared to the organic matter content of manure.

The increment of the reaction temperature by 10 °C and the extension of the reaction period up to 24 h with a persulfate concentration of 10 mM increased the OTC and SMZ removals from 74 and 91% to approximately 99% (Table 5). Control experiments conducted at 50 °C without the addition of the oxidant showed 25 and 50% decreases in OTC and SMZ concentrations, respectively, at the end of 24 h. Although with increasing temperature the increased generation of sulfate radicals in the persulfate process (41) and accelerated desorption of OTC from animal manure (42) were reported, the results of the current study revealed that thermal degradation of antibacterials could contribute to their overall removal in manure slurry at

50 °C. The importance of thermal degradation of OTC in manure during storage or composting processes was also investigated by Arikan et al. (43) and Wang and Yates (42).

As the persulfate dose was increased from 10 to 25 mM at 50 °C, antibacterials in 24 h treated manure extracts were not detected by HPLC analysis. However, LC-MS/MS analysis of the same manure extracts denominates >99% removal of OTC and SMZ. The complete removal of antibacterial residuals was confirmed by LC-MS/MS analysis in manure extracts that were treated with 100 mM persulfate at 50 °C for 24 h. Consequently, the results obtained revealed that either increasing the dose of persulfate or elevating the temperature can enhance the removal rates of either antibacterials.

The effect of pretreatment on the efficiency of a thermally activated persulfate process performed with 25 mM $\text{Na}_2\text{S}_2\text{O}_8$ at 50 °C for 24 h was also investigated. As opposed to the effects on Fenton oxidation carried out with a low dose of oxidant, the contribution of Mg salt pretreatment on OTC removal from the manure slurry by persulfate oxidation was only 2%, and it had no effect on SMZ removal. This result could be explained by the thermal desorption and degradation of antibacterials at high temperatures as discussed previously. Furthermore, it was speculated that persulfate could be responsible for the desorption of organics from the soil surface similar to the effect of excess doses of hydrogen peroxide (44). Hence, this effect also decreased the importance of pretreatment of manure for the degradation of OTC and SMZ. Consequently, persulfate treatment could be proposed for the efficient treatment of contaminated manure at high temperatures because it provides nearly complete antibacterial removal even at low oxidant doses within only a 2 h treatment period. Moreover, its effect on soil organic matter was reported to be analogous to the compositional changes resulting from humification (45), which is an indicator of the maturity and stability of compost before its application to agricultural areas (46).

Effect of Bedding Materials on the Performance of Oxidation Treatment Processes. The effects of bedding material were evaluated in the manure (bedding/manure = 20% (w/w) on the treatment performance of three different oxidation processes, which were applied after the pretreatment. **Figure 3** presents overall antibacterial removal in manure, manure + zeolite, and manure + sawdust mixtures obtained by the applications of the Fenton (1600 mM H_2O_2 /160 mM Fe(II); 2 h), ozone (2 mg/min ozone dose; 2 h), and persulfate (100 mM S_2O_8 ; 40 °C; 2 h) oxidation processes.

As can be seen from **Figure 3**, >94% antibacterial removal was obtained for all matrices. However, the use of sawdust as a bedding material resulted in a minor decrease in OTC and SMZ removal rates, probably due to competition of the high organic content of sawdust for reactive radicals generated during the oxidation processes.

Persulfate Oxidation of Synthetic Animal Feeding Operation Wastewater. Because persulfate oxidation was found to be the more effective treatment process on antibacterial removal from manure, only the thermally activated persulfate oxidation process was applied to the synthetically prepared and contaminated wastewater (OTC and SMZ = 5 mg/L for each) for the simultaneous removal of OTC and SMZ. The effect of the persulfate dose on OTC and SMZ removals at 50 °C is presented in **Figure 4**, which also includes the results of control experiments carried out in the absence of oxidants.

As can be seen from **Figure 4**, degradation of SMZ and OTC antibacterials in synthetic wastewater follows pseudo-first-order kinetics and highly depends on the oxidant dose. Contrary to the results obtained in manure treated by persulfate oxidation, a

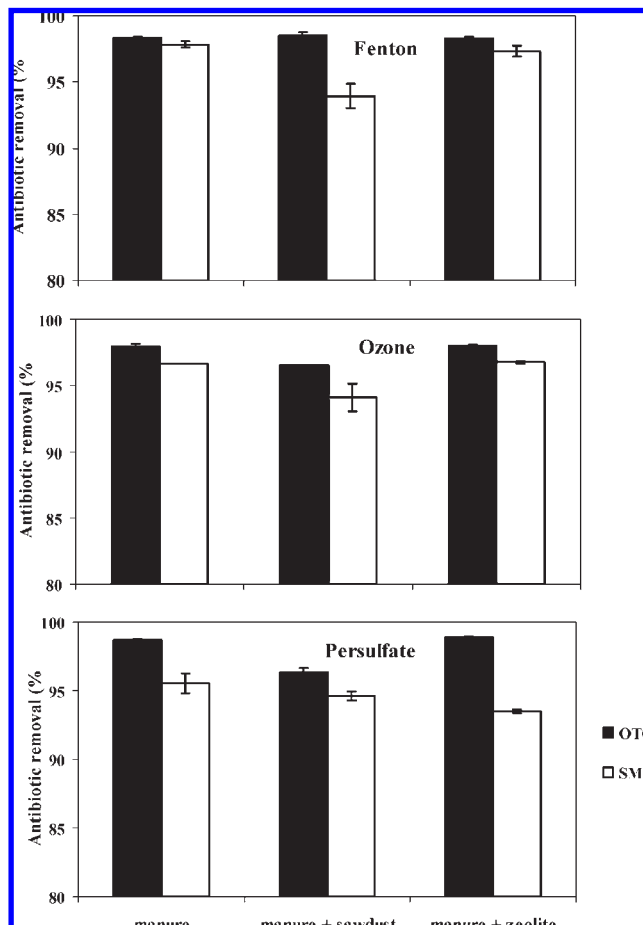


Figure 3. Effect of bedding material on the efficiencies of oxidation processes.

lower degradation rate of SMZ was obtained compared to that of OTC in synthetic wastewater. Actually, this result is consistent with the favorable oxidation tendency of the OTC structure. The main reaction pathway of sulfate radicals with organic compounds occurs by an electron transfer mechanism as well as hydrogen abstraction and addition (36, 47). Therefore, the presence of strong electron-withdrawing groups in the structure of organic compounds can reduce their degradation rate (see, e.g., ref 14). Accordingly, the $-\text{SO}_2$ group in the SMZ structure could explain the slower reaction rate of this antibacterial in this process. However, the pseudo-first-order removal rate constants of both antibacterials are still remarkably higher than the reported values of some organic compounds treated by persulfate oxidation (48).

The complete removal of antibacterials from the wastewater toxicity of reaction products is important for the evaluation of persulfate treatment efficiency. Although the reduction of sulfonamide and tetracycline group antibacterials' toxicity in aqueous solutions treated by different chemical oxidation processes has been investigated (49, 50), their toxicity in wastewater samples after the application of chemical oxidation processes has not been studied extensively.

The bacterial toxicity of raw and persulfate (25 mM) treated synthetic wastewater was comparably assessed by determining their inhibition on the growth of *S. aureus* bacteria. The growth curves of *S. aureus* in the presence of untreated and treated contaminated wastewater are presented in **Figure 5**. Experiments were also carried out in water and uncontaminated synthetic wastewater to determine the effects of manure components on the growth of bacteria.

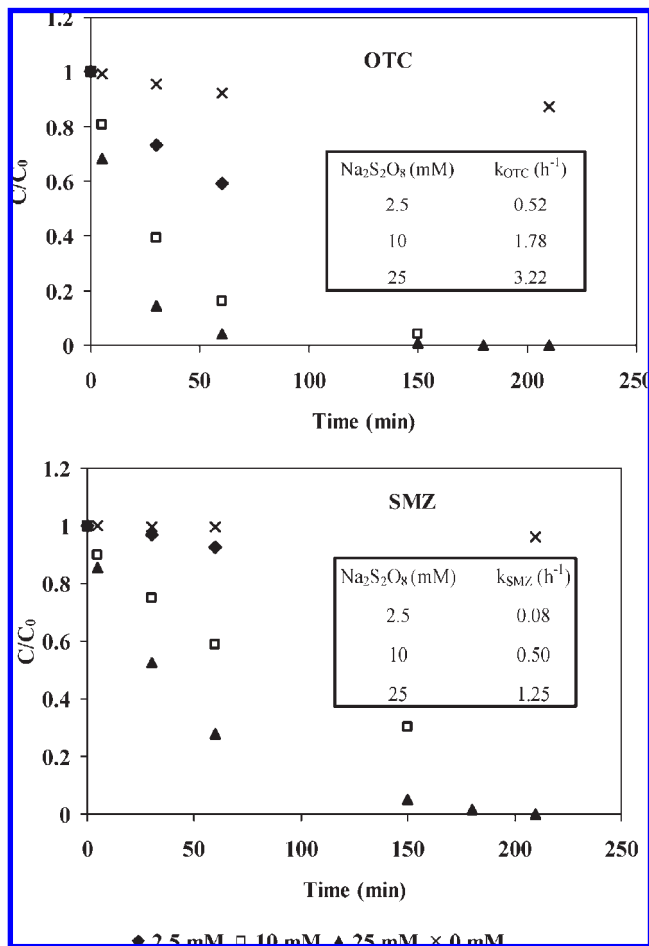


Figure 4. Effect of persulfate dose on antibacterial removal (50 °C) and pseudo-first-order removal rate constants.

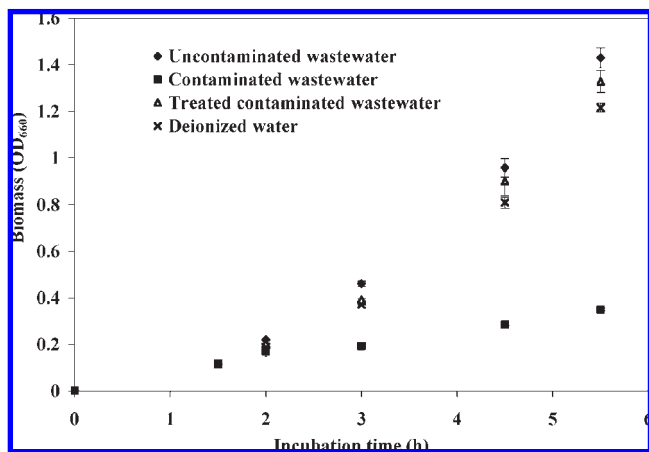


Figure 5. Growth curves of *S. aureus* in uncontaminated, contaminated, and treated contaminated wastewater.

As can be seen from **Figure 5**, *S. aureus* exhibited a higher growth rate in wastewater than that obtained in water due to the higher nutrient content of synthetic uncontaminated wastewater (**Table 6**). After 4.5 h of exposure, the inhibition of untreated contaminated wastewater on *S. aureus* growth was calculated as 82% with respect to uncontaminated wastewater. The application of the persulfate oxidation process to the wastewater with 25 mM persulfate at 50 °C for 3.5 h reduced the inhibition value to 7%.

Because the wastewater generated from animal feeding operations can be used as a valuable fertilizer (3), the variations in the

Table 6. Some Nutrient Values of Contaminated and Treated Contaminated Wastewater

parameter	concentration (mg/L)	
	contaminated wastewater	treated contaminated wastewater
TOC	110	110
NO_3^-	145	195
NO_2^-	10	7
$\text{NH}_4^+\text{-N}$	10	14
PO_4^{3-}	28	43
SO_4^{2-}	57	270
Ca	36	48
Mg	20	25
K	213	237
Fe	1.3	1.3
Zn	0.07	0.13
Cr	0.03	0.03

nutrient content of the wastewater were also monitored by the application of the persulfate oxidation process (**Table 6**).

Some nutrient values of synthetic wastewater were found to be compatible with the values reported for beef feedlot and dairy lagoon samples (3) and were not changed significantly upon the implementation of persulfate oxidation. Whereas the concentrations of heavy metals were under the maximum limits, the nitrate value of synthetic wastewater was very high relative to irrigation water standards (0–30 mg/L irrigation water) set by the Turkish Ministry of Environment and Forestry. However, treated animal feeding wastewater can be used as a fertilizer in agricultural areas as it is applied to soils at agronomic rates using risk management procedures.

Considering the effectiveness of persulfate treatment on OTC and SMZ removal from both solid and liquid animal waste, it can be suggested as an effective process for the control of antibiotic pollution in manure within a relatively short time period, which thereby prevented the development of antibiotic resistance.

SAFETY

Care must be taken during the addition of Fenton's reagent at high doses to prevent possible explosions due to sudden increase in temperature.

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