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ABSTRACT

Oxytetracycline (OTC) and tetracycline (TC) are the most common TC antibiotics used in human and veterinary medicine. Residual amounts of these antibiotics in manure pose a potential threat to public and ecological health as a result of the potential for them to be released to the environment following land application of manure from animals treated with antibiotics. We investigated the degradation of OTC and TC during anaerobic composting. We tested the effects of temperature and antibiotic concentration on degradation rates in a control and in manure spiked with TCs. We examined changes in pH, biological degradation material (BDM), and moisture corresponding with antibiotic degradation of TCs in the swine manure. Results showed that the OTC and TC concentrations decreased by between 68.54% and 95.50% in all nine treatments following 14 days of anaerobic composting, and the highest removal efficiencies were observed at an incubation temperature of 55 °C and initial concentrations of 10.36 $\mu\text{g}\cdot\text{g}^{-1}$, and 5.96 $\mu\text{g}\cdot\text{g}^{-1}$ of OTC and TC, respectively, which were degraded by 95.50%, and 90.06%. During composting at 55 °C and at added concentrations of 5 $\mu\text{g}\cdot\text{g}^{-1}$, OTC decreased rapidly, and the time required for 50% and 90% degradation was 4.1 and 9.8 days, respectively; for TC, these values were 4.4 and 14.0 days, respectively. Removal efficiencies for all TCs correlated well with moisture content of the manure. These results show that composting may be a practical and useful means to reduce concentrations of OTC and TC in swine manure prior to its land application.

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1. Introduction

Since the antibiotic penicillin was discovered in 1928, thousands of antibiotics have been discovered and synthesized [1]. Antibiotics continue to be widely used at therapeutic levels to treat infectious disease and as a feed supplement to prevent disease in livestock, and at sub-therapeutic levels to promote the growth and development of domestic animals [2]. However, antibiotics, which are mainly given to animals *via* oral or intramuscular injection, are absorbed poorly in the gut, and as much as 70%–90% of antibiotics may be excreted from animals; this figure varies with the molecular structure and dosage level of the antibiotic, and age of the animal [3].

Tetracyclines (TCs), such as oxytetracycline (OTC) and tetracycline (TC), are the most commonly-used antibiotics in animal husbandry [4]. In China, about 465 million tonnes of swine manure are generated annually, OTC and TC were the most prevalent contaminants detected in swine manure samples; their maximum concentrations were

354 and 139 $\text{mg}\cdot\text{kg}^{-1}$, respectively [5,6]. More frequent usage of TCs will result in higher concentrations in animal manure. Several studies have reported that approximately 72% of TCs added to animal feeds are rapidly excreted *via* urine and feces as the parent compound, and thus enter the environment through the application of manure to farmland as organic fertilizer. Additionally, individual animals continued to excrete TCs over a long period of time [1,3]. Thus, the disposal of manure containing OTC and TC is a possible means of reducing the amount of these antibiotics that are ultimately released into the environment.

In many parts of the world, including China, land application is the most common method for recycling manure and is therefore the most common means by which antibiotic residues are released to the environment following the storage of manure in lagoons or pits by run off or leaching [7,8]. Although livestock manure can supply various nutrients for crops and can improve soil quality, they may contain pathogens and unstable components, such as organic nitrogen and soluble phosphorus, as well as various antibiotics, which pose a hazard to human health and ecosystems once they enter the food chain [9]. Previous studies have showed antibiotics' residues in animal manures can do harm to organisms and also cause the occurrence of resistant genes in the environment through land application [10,11]. Cheesanford *et al.* [12] detected that the persistence of TC residues in the environment may contribute to bacterial resistance to TCs. This increasing resistance

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may be a concern for both human and animal health if TC antibiotics are present in food crops. In addition, Halling-Sørensen [13] found that TC antibiotics are toxic to the microalgae and bacteria. Therefore, it is important to development appropriate regulations with respect to its application on the farmland as fertilizer.

Composting is a practical and economical method for the disposal of various types of animal manure. Through composting, pathogens are destroyed under controlled conditions and the organic materials of swine manure are biologically decomposed by groups of microorganisms, producing stable compounds [14,15]. Previous studies of Selvam *et al.* [16], and Ho *et al.* [17] showed that the degradation of tetracyclines in manure occurs mainly in the first 2 weeks by composting. In Arian's research [18], the concentration of OTC during the manure composting declined rapidly and achieved a 95% reduction within the first six days. Hu *et al.* [10] presented that more than 93% of TC declined during a 45-day composting. Dolliver *et al.* [19] found that more than 99% removal of chlortetracycline was achieved in less than 10 days. However, there is very limited scientific information on the degradation of TCs as most researchers usually focus on the degradation of sulfonamides during manure composting, and in particular, information on antibiotic degradation during anaerobic composting is lacking. The objectives of this study are to determine how OTC and TC are degraded during anaerobic composting of swine manure, and to investigate the effects of temperature, antibiotic concentration, and other factors (pH, biological degradation material (BDM), and moisture content) on TC degradation.

2. Experimental Work

2.1. Chemicals and standards

We analyzed two target parent TCs in this study: OTC and TC, which we purchased from Sigma Co. (St. Louis, MO, USA). Their molecular structures are shown in Fig. 1. We dissolved the stock solutions of the TC standards in methanol and stored them at 4 °C. We purchased HPLC-grade acetonitrile and methanol from Merck (Darmstadt, Germany). All the chemicals used, including oxalic acid dihydrate, citric acid monohydrate, and ethylene diaminetetraacetic acid disodium salt (Na_2EDTA) were of analytical pure grade.

2.2. Raw materials

We collected swine manure from an animal farm located in Yuhang, a suburb of Hangzhou, in the province of Zhejiang, China. We transported the samples immediately to the lab and analyzed them for the target antibiotics. We then stored the samples at 4 °C in preparation for analysis of other parameters (total N, total P, pH, biological degradation material (BDM), and moisture content). The characteristics of the swine manure are shown in Table 1.

2.3. Equipment

We performed the manure composting experiment using nine identical lab vessels (25 cm in height, 15 cm in diameter) for anaerobic composting. The lab vessels containing the livestock manure were

Table 1

Physical and chemical properties of raw swine manure used in this study

Material	Characteristics				
	ZN/ $\text{g} \cdot \text{kg}^{-1}$	TP/ $\text{g} \cdot \text{kg}^{-1}$	pH	BDM/%	Moisture/%
Swine manure	29.63	7.04	5.11	51.60	70.36

Note: all concentrations are reported on a dry mass (DW) basis.

ZN, the concentration of total nitrogen; TP, the concentration of total phosphorus; BDM, biological degradation material.

placed in a water bath which was used to maintain the compost at a set temperature of 25 °C (room temperature), 35 °C (mesophilic temperature), or 55 °C (thermophilic temperature), and we sealed them with rubber stoppers. We connected a ventilation tube through the upper part of each container, which we connected to the buffer bottle (a wide mouth bottle), from which it vented to the outdoors. We inserted a thermometer into each bottle through the cap. The temperature was changing during the composting, which was an important indicator of the maturity of compost. In addition, the temperature evolution contributed to understanding the composting process and the comparison of different treatment methods. We installed a sampling port at the bottom of each container. We used water baths to maintain the compost at set temperatures of 25 °C, 35 °C, and 55 °C.

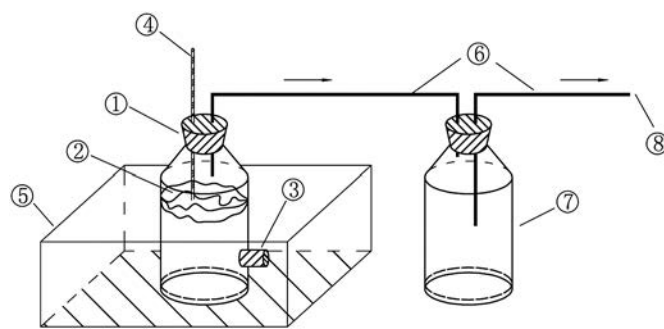


Fig. 2. The anaerobic compost experimental setup. ① sample inlet; ② swine manure; ③ sampling port; ④ thermometer; ⑤ water bath; ⑥ air guide tube; ⑦ buffer bottle; ⑧ external environment.

The experimental setup is shown in Fig. 2.

2.4. Composting experimental design

The initial concentrations of TCs in swine manure were equivalent to the concentrations in the control. We added OTC and TC to the nine treatments (T1–T9); each treatment vessel contained 4 kg of swine manure (Table 2). A stock solution of the TC standard ($5000 \mu\text{g} \cdot \text{mL}^{-1}$) was prepared and diluted with an appropriate volume of sterile water. The diluted TC solution was added and then mixed with the swine manure at two target concentration levels ($5 \mu\text{g} \cdot \text{g}^{-1}$ or $10 \mu\text{g} \cdot \text{g}^{-1}$, dry mass (DW)). All samples were thoroughly mixed manually for 15 min in a plastic basin and maintained at 25 °C, 35 °C, or 55 °C for 14 days. These added concentrations are consistent with the concentrations

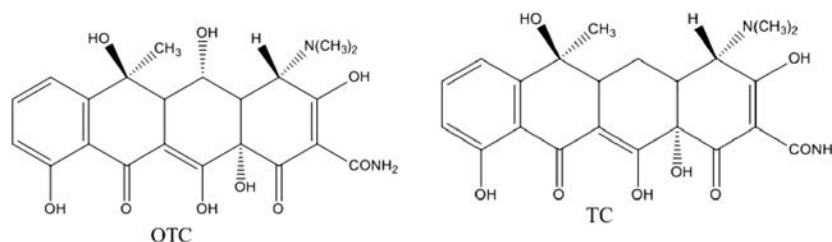


Fig. 1. Molecular structures of the tetracycline antibiotics: oxytetracycline (OTC) and tetracycline (TC).

Table 2
Experimental conditions of the nine treatments

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9
Added TC concentration/ $\mu\text{g} \cdot \text{g}^{-1}$	0	5	10	0	5	10	0	5	10
Temperature/ $^{\circ}\text{C}$	25	25	25	35	35	35	55	55	55

assessed in our previous studies, through which we determined the concentrations of TC antibiotics in swine manure in the province of Zhengjiang. The swine manure with no TCs was the control. In all samples, three composting samples were taken from each treatment, each composite sample was collected by mixing three subsamples respectively taken from the top, middle and bottom layers of each pile at days 0, 2, 4, 6, 8, 10, 12, and 14 and composite samples were analyzed for TC concentrations.

2.5. Extraction

The TCs in the swine manure or compost samples were extracted according to the modified method of Selvam *et al.* [20]. Briefly, We extracted approximately 5 g of each sample (fresh wet mass) with 20 ml of prepared McIlvaine– Na_2EDTA buffer, which we shook on a reciprocal shaker at $200 \text{ r} \cdot \text{min}^{-1}$ for 10 min, then placed the solution into an ultrasonic bath at 300 mA for 10 min to equilibrate the phases at room temperature. We centrifuged the samples for 10 min at $8000 \text{ r} \cdot \text{min}^{-1}$ and collected the supernatant. We then repeated the same extraction twice, each time using an additional 20 ml of EDTA solution. We then combined all the supernatant and transferred it to a new centrifuge tube, and centrifuged the extract at $8500 \text{ r} \cdot \text{min}^{-1}$ for 20 min. We filtered the supernatant solution through a syringe filter with a pore size of $0.45 \mu\text{m}$. We then performed solid-phase extraction using Waters Oasis HLB cartridges (150 mg, 6 ml) (Waters Corp., Milford, MA, USA) conditioned with 6 ml of methanol followed by 6 ml of ultra-pure water. Following the conditioning step, we added the compost sample extract to the SPE cartridge in 8-ml increments until all the liquid percolated through the adsorbent at a flow rate of approximately $1.0 \text{ ml} \cdot \text{min}^{-1}$. We then rinsed the cartridges sequentially with 6 ml of ultra-pure water. Finally, we eluted the cartridges with 3 ml of methanol, collected the eluate and concentrated it to dryness under a stream of nitrogen, then reconstituted it with 1 ml of methanol containing the ultra-pure water (3:2 v/v). We then filtered the solution through a $0.45\text{-}\mu\text{m}$ nylon membrane, and transferred it to 2-ml amber auto-sampler vials. To determine the extraction efficiencies, we calculated the swine manure concentrations as the mean of triplicate samples in each lab vessel every time.

2.6. Analysis

We analyzed antibiotics by HPLC (Waters e2695) as described by Sun *et al.* [21] with minor modifications. The instrument was equipped with a C18 column (i.d. $4.6 \text{ mm} \times 150 \text{ mm}$, $5 \mu\text{m}$) and we used an isocratic run. We maintained the column at 25°C . For TCs, the mobile phase was a mixture of oxalic acid–acetonitrile (85:15 v/v) in an isocratic system at a flow rate of $0.6 \text{ ml} \cdot \text{min}^{-1}$ and the detection wavelength was 355 nm. We let the column stabilize for 30 min at this setting prior to the next analysis.

There were pronounced differences between recoveries of the two TCs. We observed recoveries of 61.4% and 84.2% for OTC and TC, respectively. We corrected the concentrations obtained in all the following experiments using these recovery values. On predetermined days, we analyzed the concentrations of the two TCs in the composting samples (DW).

We measured temperature daily by inserting a digital thermometer into the containers at a depth of 20 cm. We determined the moisture content gravimetrically by drying the samples in an oven at 105°C for

24 h, or until no change in dry mass was observed [22]. We determined total phosphorus content by block digestion and flow injection analysis. We determined total N concentrations of samples using the catalytic tube combustion method and a Vario Max CNS Macro Elemental Analyzer with a TCD detector. We determined pH in the extraction solution of compost products at a compost/water ratio of 1:10 [23]. We analyzed BDM of the municipal solid waste using the potassium dichromate method [24].

3. Results and Discussion

3.1. The evolution of composting pH, BDM, and moisture of manure

Previous studies have investigated the evolution of different physico-chemical properties during manure composting, such as pH, moisture, temperature, and heavy metal and nutrient content [20]. In this study, we investigated pH, BDM, and moisture during composting (Fig. 3).

The evolution of pH throughout the composting period in all treatments is shown in Fig. 3(a). At 55°C , the initial pH values were 5.11, 5.12, and 5.13 in the T7, T8, and T9 treatments, respectively. During the initial stage of composting, microbial activity and the degradation of organic acid resulted in the production of ammonia [25] that increased the pH rapidly to maximum values of 5.93, 5.28, and 5.44 on day 8, while the pH declined gradually to reach values of 5.17, 5.18, and 5.13 at the end of composting (Fig. 3(a)). Moreover, ammonia reacted with H_2O to form NH_4^+ and free OH^+ causing the increase of pH [26]. The decline in pH could be attributed to the volatilization or microbial assimilation of ammoniacal nitrogen. The removal of $\text{NH}_4\text{-N}$ resulted in a slight decrease in pH following composting in a study by Hua *et al.* [27]. The study also demonstrated that the release of carbon dioxide may lead to acidification of the mixture and cause a decrease in pH. Previous studies have reported that when the pH value was 5–6, the predominant reaction was reversible epimerization of TCs to 4-epi-tetracycline [28]. Kühne *et al.* also indicated that 4-epi-tetracycline can be formed from stable TC in water and liquid swine manure [1,29]. In this degradation study, we did not determine whether 4-epi-tetracycline was formed. Additionally, the pH in the T7 treatment varied widely during the composting process, which is likely the result of the high temperature. The pH varied to a similar extent in the T1, T2, T3, T4, T5, and T6 treatments at a lower intensity, which was probably due to a lower level of microbial activity. This trend in pH is typical and similar to that observed in previous reports on livestock manure composting [30].

Of the parameters evaluated in this study, the BDM best reflects the degree of biodegradation of the manure [31]. As shown in Fig. 3(b), the BDM of the composting mass in all the treatments, decreased rapidly from 46.33%–52.66% to 18.09%–31.92% on day 14 (Fig. 3(b)), possibly as a result of the degradation of biodegradable organic material in the swine manure as a consequence of microbial utilization, which could lead to the decomposition of organic matter into stable humic components and inorganic compounds [27]. Furthermore, we observed no significant difference in BDM among different treatments, and all declining trends were similar over time.

The moisture content is shown in Fig. 3(c). The moisture contents of swine manure compost in the T3, T6, and T9 treatments were all 71.98% at time zero and declined to 59.49%, 57.52%, and 53.12%, respectively, after 14 days (Fig. 3(c)). The decrease could be attributed to the volatilization of moisture from the swine manure. Furthermore, it demonstrates that the moisture content of swine manure at 55°C decreased more rapidly than at 25°C and 35°C and that the moisture content of swine manure was higher at 25°C than at 35°C . The differences may be attributable to differences in moisture volatilization rates, temperature, and evaporation rates. Additionally, some moisture was lost as a result of the sampling procedure.

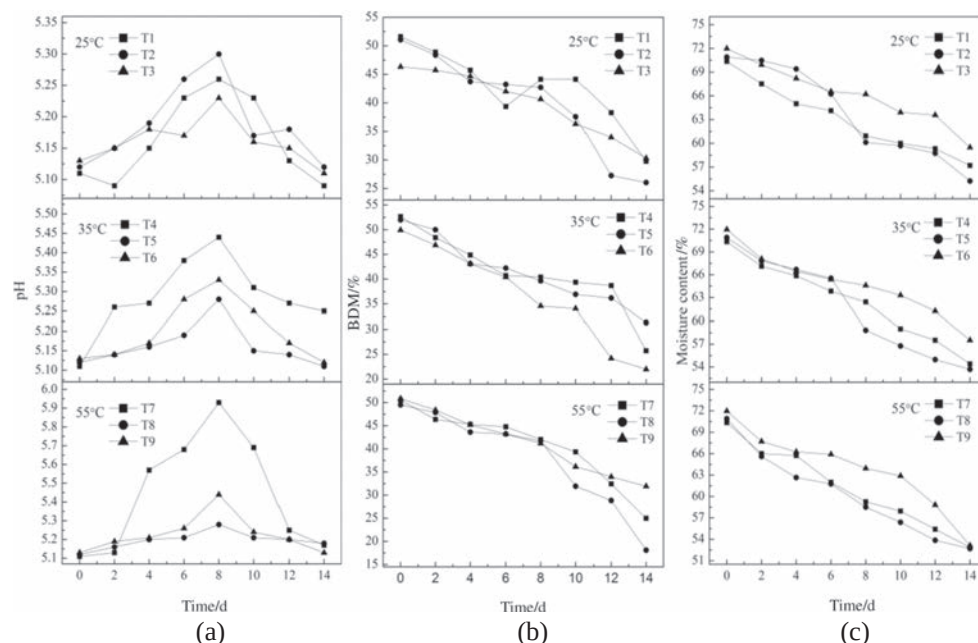


Fig. 3. Variations in pH, BDM, and moisture of manure over time during the swine manure composting process in different treatments. (T1): swine manure, $0 \mu\text{g}\cdot\text{g}^{-1}$ TC, 25°C ; (T2): swine manure, $5 \mu\text{g}\cdot\text{g}^{-1}$ TC, 25°C ; (T3): swine manure, $10 \mu\text{g}\cdot\text{g}^{-1}$ TC, 25°C ; (T4): swine manure, $0 \mu\text{g}\cdot\text{g}^{-1}$ TC, 35°C ; (T5): swine manure, $5 \mu\text{g}\cdot\text{g}^{-1}$ TC, 35°C ; (T6): swine manure, $10 \mu\text{g}\cdot\text{g}^{-1}$ TC, 35°C ; (T7): swine manure, $0 \mu\text{g}\cdot\text{g}^{-1}$ TC, 55°C ; (T8): swine manure, $5 \mu\text{g}\cdot\text{g}^{-1}$ TC, 55°C ; (T9): swine manure, $10 \mu\text{g}\cdot\text{g}^{-1}$ TC, 55°C .

3.2. Effect of temperature on the degradation of OTC and TC

TCs are considered to be unstable because of their unique chemical structure, and may undergo abiotic degradation depending on the surrounding conditions, such as pH, temperature, and light conditions [32]. Our results showed that all composting treatment conditions could definitely result in a decrease in TC content (Table 3). In this study, the declining trend in the nine treatments was similar, irrespective of differences in compost temperatures and TC concentrations.

Fig. 4 illustrates the behavior of OTC and TC during the anaerobic swine manure composting process. The initial concentrations of OTC and TC in the swine manure were $5.36 \mu\text{g}\cdot\text{g}^{-1}$ and $0.96 \mu\text{g}\cdot\text{g}^{-1}$, respectively. In the spiked swine manure, the initial OTC concentrations were $10.36 \mu\text{g}\cdot\text{g}^{-1}$ and $15.36 \mu\text{g}\cdot\text{g}^{-1}$, and initial TC concentrations were $5.96 \mu\text{g}\cdot\text{g}^{-1}$ and $10.96 \mu\text{g}\cdot\text{g}^{-1}$, respectively.

We investigated the removal of the TCs at different phases of the swine manure composting process (Fig. 4). Briefly, at 25°C , 35°C , and 55°C and a spiking level of $5 \mu\text{g}\cdot\text{g}^{-1}$, the total removal efficiencies of OTC were 81.94%, 87.49%, and 95.50%, respectively (Fig. 4(a)); and of TC were 80.78%, 86.57%, and 90.06%, respectively (Fig. 4(b)). The results above indicate that OTC and TC were removed more efficiently at 55°C than at 25°C and 35°C , and that the degradation of the TCs was higher at 35°C than at 25°C . The role of temperature on the loss of TCs has been revealed in a few earlier studies. Kühne *et al.* [29] reported a half-life of 9 days for TC in non-aerated swine manure at 25°C . Shi *et al.* [33] presented that OTC decreased by 100% in less than 20 days of anaerobic composting at 35°C . Stone *et al.* [34] found that temperature affected chlortetracycline treatment in swine manure: 57% degradation was observed at $10\text{--}20^\circ\text{C}$, but 74%–92% occurred at 35°C . According to Arikian, the reduction of extractable TC residues increased

with incubation time and temperature [35]. Therefore, a thermophilic temperature ($>40^\circ\text{C}$) [19], which we achieved in the T7, T8, and T9 treatments could significantly accelerate the degradation of TCs during composting. Shen *et al.* found that the degradation rates of residual TCs in swine manure increased with temperature, with the maximum occurring at 55°C [36]. The swine manure may contain various potentially pathogenic organisms, and the high temperatures achieved through metabolic heat generated by microbial activity through the composting process could have a detrimental effect on these harmful microbes [14]. In addition to thermophilic temperatures, intense biological activity could probably contribute to the rapid degradation of TCs during the entire composting process. In general, TCs are apt to be absorbed by organic matter because of their molecular structure and physico-chemical properties; and organic matter potentially generates more binding sites for antibiotics as the temperature rises during composting, and consequently are transformed into non-extractable forms [37]. Meanwhile, the TC removal rates were lower at 25°C than at 35°C in this study; this may be the result of the higher microbial activity at 35°C than at 25°C . Microbial activity played an important role during composting, not only through the effects of increased temperature, but also through their role in promoting the degradation of TCs [38].

3.3. Effect of concentration on the degradation of OTC and TC

We added TCs at different concentrations ($5 \mu\text{g}\cdot\text{g}^{-1}$ and $10 \mu\text{g}\cdot\text{g}^{-1}$) to the swine manure to investigate the differences in TC depletion between the control and the spiked manure compost samples. As shown in Fig. 4, the trends of TC concentrations in compost samples spiked with TCs + were similar to those of the control, in which TC concentrations decreased with composting time.

Table 3

Percentage of reduction in the concentrations of OTC and TC in the different treatments after 14 days of composting

Composting treatment		T1	T2	T3	T4	T5	T6	T7	T8	T9
Percentage of reduction/%	OTC	69.63	81.94	76.52	78.93	87.49	83.85	83.48	95.50	89.49
	TC	68.54	80.78	75.81	75.95	86.57	80.75	82.30	90.06	86.28

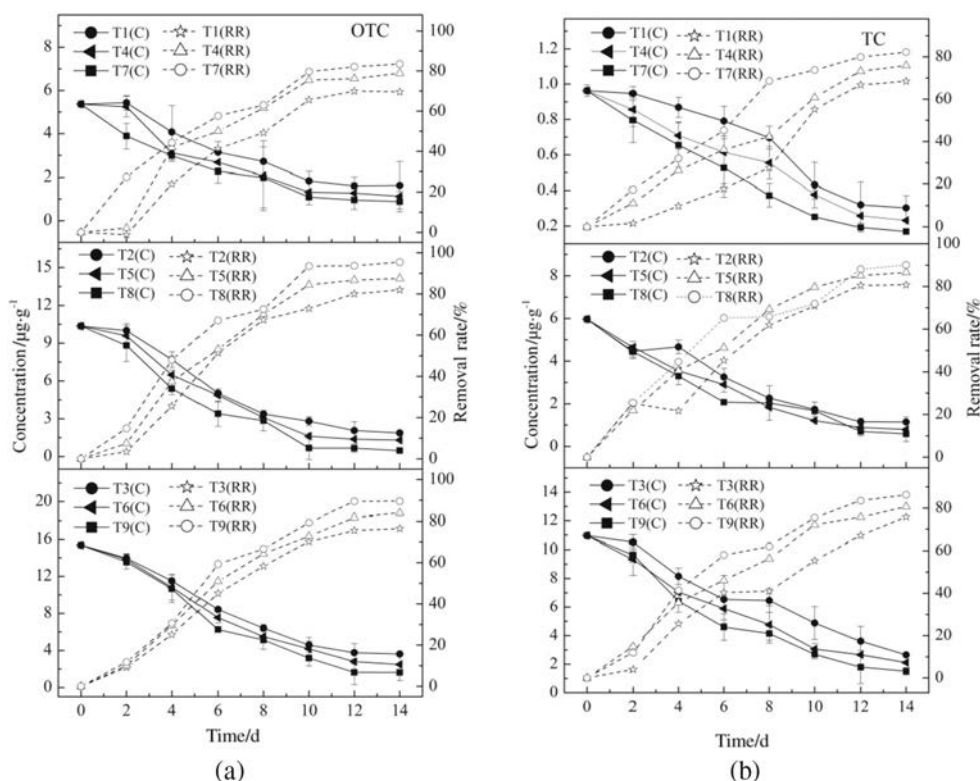


Fig. 4. Variations in the concentrations and removal efficiencies of OTC and TC during the swine manure composting process. (T1): swine manure, 0 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 25 °C; (T2): swine manure, 5 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 25 °C; (T3): swine manure, 10 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 25 °C; (T4): swine manure, 0 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 35 °C; (T5): swine manure, 5 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 35 °C; (T6): swine manure, 10 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 35 °C; (T7): swine manure, 0 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 55 °C; (T8): swine manure, 5 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 55 °C; (T9): swine manure, 10 $\mu\text{g}\cdot\text{g}^{-1}$ TC, 55 °C. C: concentration ($\mu\text{g}\cdot\text{g}^{-1}$); RR: removal rate (%).

We also investigated the removal of two TCs at different phases of composting. The results show that the removal of two TCs was higher in the spiked manure than in the control at all phases of the composting process. Removal of the two TCs in the control was lower than at added concentrations of 5 $\mu\text{g}\cdot\text{g}^{-1}$ and 10 $\mu\text{g}\cdot\text{g}^{-1}$. For example, at 55 °C and at added concentrations of 0, 5, and 10 $\mu\text{g}\cdot\text{g}^{-1}$, the total removal efficiencies of OTC were 83.48%, 95.50%, and 89.49%, respectively (Table 3). A similar degradation trend occurred in the presence of TC. The difference between the removal efficiencies of the two TCs between the control and the treatments could be the result of sorption. According to the Thiele-Bruhn theory, at 55 °C and at added concentrations of 10, 100, and 1000 $\mu\text{g}\cdot\text{g}^{-1}$, OTC removal efficiencies in a 14-day soil incubation study were 90%, 70%, and 60%, respectively, which indicates that the degradation of OTC is likely due to non-linear adsorption [37]. In the control, the TCs first passed through the gut of the animals, and were finally excreted via urine and feces that end up in manure. In the spiked manure, the two TCs were strongly sorbed to the original manure with increasing contact time between them and the manure, which decreases the speed and the extent of the release of the TCs from manure. However, the two TCs may have been degraded before being sorbed by the spiked manure. Thus, removal of the two TCs in the 0–8 day composting phase was lower in the control than in the spiked manure as shown in Fig. 4. For instance, at 55 °C and added concentrations of 0, 5, and 10 $\mu\text{g}\cdot\text{g}^{-1}$, OTC removal efficiencies in the 0–8 day phase were 63.02%, 72.55%, and 66.64%, respectively. Removal of both TCs from the control was lower than from the treatments, which may be the result of strong sorption of manure to the TCs in the control. This finding is consistent with those of Chadwick *et al.* [39] who reported that TCs absorbed or complexed strongly to manure solids, a tendency that is largely related to the coexistent divalent cations in manures [35]. In addition, Loke *et al.* [40] found that OTC readily binds to proteins, particles and organic matter in manure. Moreover, Kühne *et al.* [29]

found inactivation of TCs by binding to macromolecules or chelates was also possible. Although we had not examined adsorption of TCs, we cannot rule out the possibility that disappearance of antibiotics was partly due to their adsorption onto solid particles.

In comparison, removal of the two TCs at a spike level of 5 $\mu\text{g}\cdot\text{g}^{-1}$ in this study was higher than at 10 $\mu\text{g}\cdot\text{g}^{-1}$; this may be because the activity of most microbes may be inhibited at 10 $\mu\text{g}\cdot\text{g}^{-1}$. In general, the microbial community is simpler at higher concentrations of TC antibiotics and a high concentration of antibiotics will inhibit the microbial community. Previously, it was reported that although initial extractable antibiotic concentrations in field soils are generally low, concentrations immediately following manure application are high enough to impact soil microorganism activity [37,42]. Furthermore, the increase could be attributed to bioaccumulation in the effective concentration, which should also be taken into account [41].

During antibiotic composting, OTC was degraded more rapidly than TC in all nine treatments. For example, the total removal efficiency of the OTC at 55 °C (added concentration of 5 $\mu\text{g}\cdot\text{g}^{-1}$) achieved in the T8 treatment, the concentration of OTC decreased 95.50%, from 10.362 to 0.466 $\mu\text{g}\cdot\text{g}^{-1}$ during 14 days of composting, while TC decreased 90.06% from 5.963 to 0.593 $\mu\text{g}\cdot\text{g}^{-1}$ from day 0 to day 14. Several studies have investigated the relationship between removal characteristics of antibiotics and the molecular structure. It was reported that the major transformation products of OTC, which have high molecular weight and high structural complexity were more resistant to biotransformation than TC, which have low molecular weight [42]. Also, OTC is susceptible to photolysis and hydrolysis [43]. Furthermore, similar results of other studies have demonstrated that OTC was the most rapidly degraded of the TCs. The fate of OTC during composting of swine manure was investigated in a study by Wu *et al.* in which removal of OTC from the composting mass reached 92% within 1 week [38].

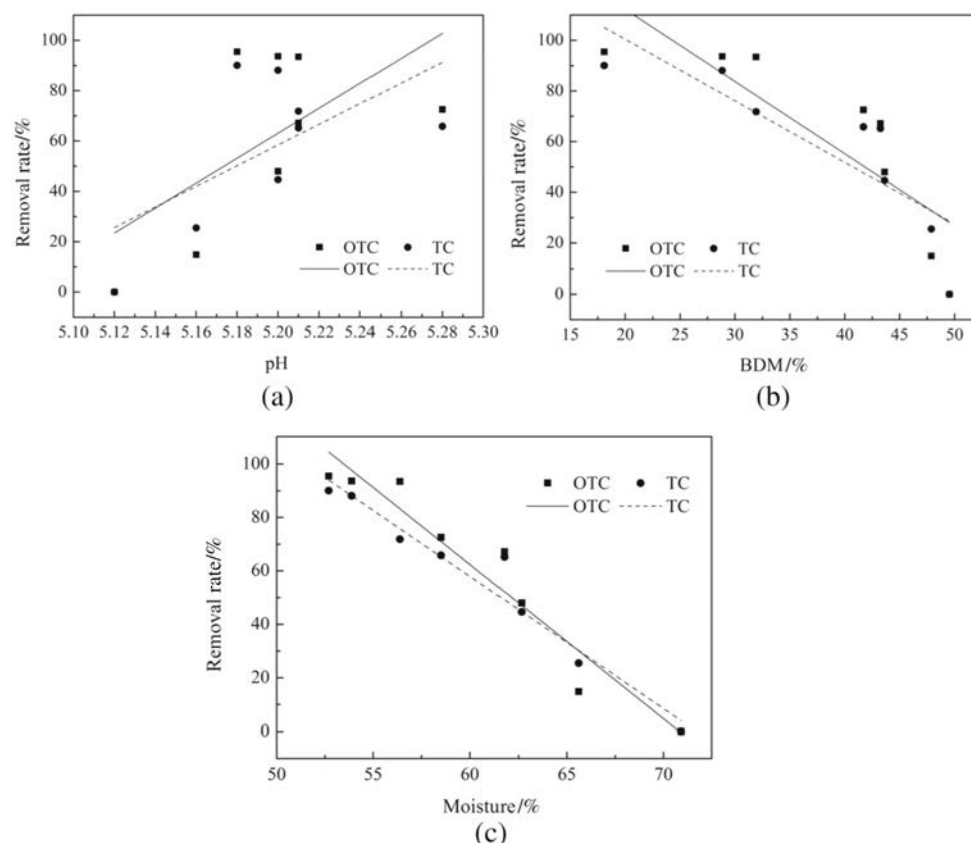


Fig. 5. Removal efficiencies of both TCs ($5 \mu\text{g}\cdot\text{g}^{-1}$) as a function of compost pH, BDM, and moisture, at 55°C .

3.4. Correlations between removal efficiency of the two TCs and compost pH, BDM, and moisture

The first-order kinetic model has been used in most studies to describe the degradation behavior of antibiotics during composting [14,17,19]. We therefore modeled the depletion of the TCs using a first-order equation to observe the influence of different important experimental factors (pH, BDM, and moisture) on the removal of TCs during manure composting. Fig. 5 shows the single factor analysis of the linear regression, which we conducted to compare the removal efficiency of the two TCs ($5 \mu\text{g}\cdot\text{g}^{-1}$, added at 55°C) with compost pH, BDM, and moisture.

We observed good correlations with moisture and removal efficiency of TCs for composting manure: the correlation coefficients (R^2) between moisture and removal efficiency were 0.930, and 0.952 for the OTC and TC, respectively, which demonstrates that moisture strongly influences TC removal. However, the correlations between pH and removal efficiency for both TCs was very low: the correlation coefficients (R^2) between pH and removal efficiency were 0.382 and 0.366 for OTC and TC, respectively. The correlation coefficients (R^2) for BDM and removal efficiency were 0.703 and 0.711 for OTC and TC, respectively (Table 4). These results indicate that the moisture content of swine manure may be the key factor influencing the biodegradation of both TCs. Within the specific limits of the previous study, the decline in moisture

content has been shown to reduce the bacteria numbers in manure, and consequently, the reduction in bacteria numbers may lead to a decrease in the biological degradation of antibiotics. Additionally, the results show that the correlation coefficients (R^2) of TC and other parameters, except pH, are higher compared with those of OTC, which could be a result of differences in their molecular structure.

4. Conclusions

Composting effectively promoted the depletion of both TCs in the control and the spiked manure in the nine treatments. Both TCs degraded most rapidly in swine manure at a concentration of $5 \mu\text{g}\cdot\text{g}^{-1}$ and a temperature of 55°C , while they were most slowly degraded in the swine manure of the control at 25°C . The removal efficiency varied between 70% and 95%, with a maximum of 95.50% for OTC after 14 days of composting in response to an added concentration of $5 \mu\text{g}\cdot\text{g}^{-1}$ and a temperature of 55°C . Moisture was the key factor influencing biodegradation of the two TCs, and OTC was degraded more rapidly than TC. These results suggest that there were important interactions between the initial concentration of antibiotic and temperature, which accelerate antibiotic degradation rates during composting. Composting is considered to be a practical and useful option for reducing TCs in swine manure prior to its application to fields.

Table 4

Regression equations of the removal efficiencies of the two TCs and pH, BDM, and moisture during manure composting

	pH		BDM/%		Moisture/%	
	Fitting equation	R^2	Fitting equation	R^2	Fitting equation	R^2
OTC	$y = 4.9542x - 25.131$	0.382	$y = -2.8518x + 1.693$	0.703	$y = -5.7619x + 4.081$	0.930
TC	$y = 4.1042x - 20.757$	0.366	$y = -2.4310x + 1.490$	0.711	$y = -4.9375x + 3.541$	0.952

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