Effects of Elevated Tetracycline Concentrations on Aerobic Composting of Human Feces: Composting Behavior and Microbial Community Succession

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Abstract The effects of antibiotics on aerobic composting are investigated by dosing of tetracycline (TC) in fresh human feces with sawdust as biomass carrier. Variability in process parameters such as temperature, pH, water-soluble carbon, germination index (GI) and dehydrogenase activity (DHA) are evaluated at TC dosages of 0, 100, 250 and 500 mg/kg in a 21-day composting. Moreover, microbial community succession is examined by high-throughput 16S rRNA gene sequencing. Findings indicate significant impacts to the process parameters with the increase of TC concentration such as inhibition of temperature increases during aerobic composting, lowering of pH, increasing of water-soluble carbon residue, a decrease of GI, and hindering of DHA. Furthermore, elevated TC concentrations significantly alter the microbial community succession and reduce the community diversity and abundance. Therefore, interference in microbial community structures and a hindrance to biological activity are believed to be the main adverse effects of TC on the composting process and maturity of the composting products.

Keywords Tetracycline · Aerobic composting · Human feces · Microbial community · Compost maturity

Introduction

In rural areas with water shortages, application of the current sanitation technology for human feces disposal based on its dilution with large volumes of centrally provided potable water is not feasible [1]. Consequently, the traditional dry pail latrines remain the foremost approach to the handling and storage of feces. This method, however, has a number of negative consequences for public health as well as the environment such as easy to contaminate groundwater and cause of gastrointestinal infections [2].

Since toilet flushing is impracticable in water scarce regions and dry pail latrines elicit negative effects, composting toilets, which center on aerobic composting, are thought to be the best option for human feces disposal in a sanitary manner [3]. Thus, the commercial implementation of aerobic composting to dispose of excreta is burgeoning. Composting toilets are currently available in rural regions, natural parks, and other places that experience water shortages as minimal infrastructure is needed and it can be inexpensive to implement [4, 5].

Antibiotics are broadly utilized to prevent and treat human diseases [6]. However, these medicines are not well absorbed by humans. Circa 30–90% of antibiotic dosages (unaltered and their metabolites) are released, in biologically active forms, in human excreta due to partial absorption [6, 7]. The antibiotics most frequently used by humans include tetracyclines, quinolones and sulfonamides at dosages of up to 2 g/day and with excretion ratios of up to 90% [8]. Thus, considering the maximum concentrations of these antibiotics in feces mixed into a 5 kg-scale

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household composting device [9], the original antibiotics concentration could increase significantly by up to 500 mg/kg. The effectiveness of aerobic composting for the removal of antibiotics has been demonstrated [10, 11]. However, the potential effects of these antibiotics on the composting process have not been taken into account. The types and concentrations of residual antibiotics fluctuate widely in manure. This fluctuation could cause changes to the microbial community through composting, thus, impacting the composting process and the condition of the compost product [12]. Previous studies demonstrate the effects of oxytetracycline, sulfamethazine and sulfamethoxazole on the composting process of feces [13, 14]. However, studies on tetracycline (TC) [15], the most commonly used antibiotic by humans is lacking. Understanding of the effects of TC on compost is necessary to improve the process operation, but, until now, no such studies can be found. In addition, previous reports on this subject are based on antibiotics concentrations of less than 200 mg/kg. Thus, the effects of higher antibiotics concentrations on the small-scale household composting process should be further understood.

The primary objective of this article is to establish the impacts of TC on the composting process and maturity during aerobic composting of human feces in household sanitation use. In addition, the changes in bacterial community structure are analyzed using high-throughput 16S rRNA gene sequencing.

Materials and Methods

Composting Materials

TC hydrochloride (> 97.5% purity) was acquired from Sigma-Aldrich (Germany). Human feces and sawdust were the composting materials. Fresh feces were obtained from a single university dormitory housing four healthy young males of about 24 years old. The feces were collected in plastic vessel [16] and homogenized, which were bundled in batches for storage at -20°C . The solid matrix was sawdust, gathered from a regional timber processing plant, which was screened to a particle size of 1–2 mm. The sawdust was kept in a cool and dry area. The high porosity of sawdust provided sufficient void space for water retention and drainage, and air ventilation, thus providing a proper aerobic environment for microbial growth in the composts [17]. The characteristics of the feces and sawdust are shown in Table 1.

Experimental Design

The composting experiment was performed using laboratory-scale composting reactors that were built with plastic containers (25 L), air pumps, and flow meters (Fig. 1). Air was pumped into the air chamber at 1.5 L/min, a flow rate that was optimized through several experiments. Plastic covers with small holes to stop the loss of moisture were put on the reactors. We combined 0.3 kg of human feces with 0.75 kg of sawdust particles at a dry weight feces:sawdust ratio of 1:2.5 to achieve a C/N ratio of 24:1 [18]. During the composting process, the moisture content in the containers was maintained at 60% utilizing deionized water. TC was therapeutically administered at a daily dose of 1–2 g, and 30–90% of it was excreted in urine and feces [6]. It is noteworthy that China has high rates of antibiotic abuse, and therefore, more antibiotics may be ingested in the human body [19]. Based on the above reports, the blank matrix was spiked with TC at four different concentrations: 0 (control test, CK), 100 (TC100), 250 (TC250) and 500 (TC500) mg/kg dry weight. Each composting treatment was repeated in triplicate. The mixture in the reactor was stirred twice per day during the 21 days of composting. The composting process was carried out at the mean ambient temperature of 28°C .

Sample Collection

Samples were obtained daily from the mixtures of feces and sawdust after being well homogenized. These samples were divided into equal portions: one portion was kept at 4°C for further chemical evaluation and enzyme activity assays, and the other portion was kept at -80°C for molecular analyses. Compost samples for microbiological analysis comprised of triplicate composite samples at three main stages, i.e., temperature-rise stage (day 2), temperature-fall stage (day 6) and mature stage (day 21), respectively, and was mixed thoroughly. The composts at three stages in CK, TC100, TC250 and TC500 were labeled as CK-1, CK-2, CK-3; TC100-1, TC100-2, TC100-3; TC250-1, TC250-2, TC250-3; and, TC500-1, TC500-2, TC500-3, respectively.

Physicochemical Analysis and Enzyme Activity Assaying

The temperatures of the composting mixtures were recorded twice a day. The moisture content was determined by drying at 105°C for 24 h. The pH of the samples was tested in distilled water (solid to distilled water ratio of 1:10, w/v) with a pH meter. Water-soluble carbon (WSC) fraction of compost samples was determined by extracting with water (compost: water ratio of 1:10, w/v) followed by

Table 1 Physicochemical characteristics of human feces and sawdust

Item	Moisture content (%)	Water soluble carbon (g/kg)	Total organic carbon (g/kg)	Total nitrogen (g/kg)
Human feces	82.29	441.33	507.64	57.09
Sawdust	12.11	29.39	379.12	1.50

Fig. 1 Schematic diagram of composting reactor

shaking for 1 h on a horizontal shaker. The WSC contents were extracted and measured according to the method described by Chantigny et al. [20]. The value of germination index (GI) was tested following the method described by Gu et al. [21].

Dehydrogenase activity (DHA) was measured by the reduction method of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) [22]. Briefly, a 5 g sample was mixed with 2.0 mL of 1% TTC solution (dissolve in 0.5 M Tris–HCl buffer, pH 7.6) and 2.0 mL of deionized water and incubated for 6 h at 37 °C in the dark. The sample was then blended with 100 mL of methanol to extract TPF, shaken for 1 h at 150 rpm (20 °C), and filtered to measure the absorbance of color in the extract at 485 nm. A control without the addition of TTC was included for each sample. Values of DHA are expressed as $\text{mg} \cdot \text{TPF} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$.

Microbiological Analysis

DNA Extraction

Mixed composting samples (0.2 g) were collected in 2 mL sterilized tubes for total DNA extraction with E.Z.N.A.TM Mag-Bind Soil DNA Kit (OMEGA, USA) according to the manufacturer's instructions. The concentration of the DNA was measured using a Qubit 2.0 (Life, USA) to ensure that adequate amounts of high-quality genomic DNA had been extracted [23]. The extracted DNA was stored at –20 °C for subsequent use.

PCR Amplification

The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by polymerase chain reaction (PCR). Two universal bacterial 16S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer 341F (5'-CCTACGGGNGGCWGCAG-3') and amplicon PCR reverse primer 805R (5'-GACTACHVGGGTATCTAATCC-3') [24]. The reaction was set up as follows: microbial DNA (10 ng/μL) 2 μL; amplicon PCR forward primer (10 μM) 1 μL; amplicon PCR reverse primer (10 μM) 1 μL; 2 × KAPA HiFi Hot Start Ready Mix 15 μL (total 30 μL) [23]. The mixtures were denatured using the following program: 1 cycle at 95 °C for 3 min, first 5 cycles at 95 °C for 30 s, annealing at 45 °C for 30 s, elongation at 72 °C for 30 s, then 20 cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s and a final extension at 72 °C for 5 min [23]. The PCR products were checked using electrophoresis in 1% (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide and visualized under UV light.

Sequencing

Sangon BioTech (Shanghai) conducted the 16S rRNA high-throughput sequencing utilizing the Illumina MiSeq system (Illumina, USA). After sequencing, the raw data were processed, and the method described according to Wu et al. [25].

Sequence Accession Numbers

The sequence information was deposited in the NCBI's SRA database, and the accession number is SRP103632.

Data Analysis

The experiments were conducted on triplicate samples, and the mean values with standard error are shown. One-way analysis of variance (ANOVA) and multiple comparisons test were utilized to examine the statistical differences among the control and treatment groups. The statistical analyses were conducted using PASW Statistics 18.0. Heatmap analysis was carried out using gplots (version

2.17.0) Package in R software (version 3.2). Redundancy analysis (RDA) was performed using Vegan Package (version 2.0-10).

Results and Discussion

Effects of TC on Compost Physicochemical Characteristics

In household composting, it is challenging to sustain thermophilic temperatures throughout the composting process since it is on a small-scale and there is rapid heat loss [9]. Variations in the pile temperature throughout the

composting process are illustrated in Fig. 2a. Based on these alterations in the pile temperature, the composting process can be split into three stages: the temperature-rise stage, the temperature-fall stage, and the mature stage. For the treatments CK, TC100, TC250, and TC500, the temperatures peaked on the third day at 55.6, 50.5, 48.2, and 46.1 °C, respectively. In the temperature-rise and temperature-fall stages, the temperature of the CK treatment was greater than those of TC100, TC250, and TC500, suggesting that elevated TC concentrations had inhibitory impacts on the metabolic capacity of the microbial community [26]. Nonetheless, all composts achieved a relatively steady temperature near the ambient temperature. In the mature stage, the temperature was relatively lower in CK and TC100 compared to TC250 and TC500. This difference may be due to the generation of resistant populations in the composting environment with the composting process [27], which increased the metabolic activity of microorganisms in the composting [10]. The heat generated by different metabolic activities led to inconsistent temperature changes in all treatments. Comparably, Sun et al. [26] documented an obvious decrease in temperature in contrast to the control as a result of the addition of tetracycline antibiotics. These outcomes show that elevated TC concentrations in compost may inhibit the increase of temperature in the temperature-rise stage and, thus, delay the composting maturity.

The differences in pH for all composts are shown in Fig. 2b. The trends in the pH differences for the four treatments were virtually similar throughout the composting time. In the first stage of all treatments, whereby temperatures escalated as a result of microbial metabolism, a rapid decrease of pH was observed in the initial 2 or 3 days, which is a potential sign of the formation of fatty acids due to organic decomposition [17]. Subsequently, the pH rose rapidly from about 5.2 to 8.1, which was linked to the degradation of organic acids or the liberation of ammonia compounds [28]. Minute decreases in the pH values were recorded in TC250 and TC500, in contrast to CK and TC100 at the mature stage, which demonstrated the inhibition of ammonia liberation by antibiotics [29].

Trends in the WSC contents throughout the composting process were nearly identical to those of temperature (Fig. 2c). In every one of the treatments, the WSC contents rose quickly upon initial composting and reached a peak on day 1 or 2, which then decreased gradually to the lowest level. The peak WSC contents in the CK and TC100 treatments were notably greater than those of TC250 and TC500. Nevertheless, as the composting process proceeded, the WSC contents in TC250 and TC500 steadily increased to exceed those of CK and TC100 in the mature stage. Upon completing the composting, the CK treatment was discovered to have the lowest WSC (14.39 g/kg),

Fig. 2 Variations in temperature (a), pH (b) and WSC (c) throughout the composting process. AT represents the ambient temperature. CK, TC100, TC250, and TC500 denote the tetracycline doses of 0, 100, 250, and 500 mg/kg DW in the compost, respectively

succeeded by TC100 (15.83 g/kg), TC250 (20.46 g/kg), and TC500 (25.07 g/kg), respectively. The WSC values for CK and TC100 were below the threshold value of 17 g/kg that was established to describe a mature compost by Bernai et al. [30]. These findings further show that the addition of antibiotics at high concentrations impeded the maturity of the compost.

Toxicity and Maturity Analyses

GI is a sensitive parameter utilized to examine the compost maturity and phytotoxicity of the compost. The GI values for every one of the treatments showed rapid declines in the first stage of composting, which then increased steadily until the composts achieved maturity (Fig. 3). According to Zucconi et al. [31], a GI value higher than 80% is thought to be safe for applications on land and suggests the maturity of compost. The CK treatment was the first to achieve this standard, with the highest GI value of 145.8%, succeeded by TC100 (116.2%) and TC250 (95.5%). The TC500 treatment (80.2%) barely satisfied the required standard.

Maturity is the degree or level of completeness of composting and suggests better qualities that come from ‘aging’ or ‘curing’ of a product [3]. Immature and not well-stabilized composts may present several issues throughout storage, marketing, and utilization. Criteria and parameters have been suggested for analyzing compost maturity [32]. However, due to the complexity of composting materials and conditions, widely accepted standard parameters and methods for compost maturity evaluation have not been established. Maturity is not identified by one sole property, so it is best evaluated by measuring two or more parameters of the compost. Maturity parameters are established on various properties: physical, chemical, and biological, such as microbial activity, temperature, pH, WSC, and GI of the compost, all of which are broadly acknowledged as common indicators [32]. Overall, findings from this study

Fig. 3 Changes in germination index during human feces aerobic composting

indicate that at elevated levels of up to 500 mg/kg, TC hinders the aerobic composting process for feces disposal and the maturity of the compost produced.

Dehydrogenase Activity

DHA has been described as a measure of microbial activity in composts. It is one of the main components of the respiratory chain and offers data on the compost maturity [33]. The highest DHA ($\text{mg TPF g}^{-1}\text{h}^{-1}$) was obtained in TC100 (14.04), followed by CK (12.96), TC250 (11.67) and TC500 (8.69) (Fig. 4). The rapid increase in DHA at the first stage of composting was induced by oxidation of labile organic compounds catalyzed by the enzyme [33]. Similar findings were documented by other researchers [34]. In the first stage of composting, DHA in TC100 was greater than that of CK, which suggested that relatively low concentrations of TC may encourage the activity of dehydrogenase in compost, potentially because of the hormesis of antibiotics [35]. DHA in TC500 was significantly lower than that in the other treatments, which demonstrates an evident inhibition of DHA at high concentrations of TC. The inhibitory effects of elevated concentrations of TC on DHA, consequently, led to an inhibition of the breakdown of organic matter (Fig. 2c). In the mature stage of the composting, however, DHA was lowered, which indicates that the majority of the organic matter had been degraded by microorganisms and changed to a stable material, and thus, the respiratory process slowed down [36]. Moreover, the increase in pH (Fig. 2b) also inhibited enzymatic activity during composting [36].

Influence of TC on Bacterial Community Structure

Sequencing Results and Bacterial Diversity Indices

Overall, 356,794 effective sequences were obtained from the 13 samples. The least number of effective sequences

Fig. 4 Changes in activities of dehydrogenase during human feces aerobic composting

from every sample was 22,249 (Table 2). Through clustering, 7813 operational taxonomic units (OTUs) were identified at the cutoff of 3%. The OTU coverage was enough for community diversity, with a coverage estimate of greater than 0.987 for every library. The α -diversity indices, including the Shannon and Simpson diversity indices [37], were determined to denote the community diversity (Table 2). The α -diversity index can be utilized to examine microbial abundance and community evenness from compost. A higher Shannon index or a lower Simpson index value indicates a higher number of microorganism species in more equal abundances. The values of these two diversity indices in TC100 were relatively high, which shows that the microbial community diversity increased with decreasing concentrations of TC [38]. In contrast, the Shannon and Simpson indices for TC500 were lower than those for the other treatments throughout the mature stage of composting. Furthermore, the diversity of the microbial community was slightly lowered with increasing concentrations of TC. This effect could be attributed to the sensitivity of microorganisms to increased levels of antibiotics, thus lowering the number and activity of microorganisms [26].

Changes in the Bacterial Community

The makeup of the bacterial communities showed temporal differences throughout the composting process by contrasting the composition and structure of bacterial 16S rRNA genes acquired through high-throughput sequencing. The makeup of the bacterial community was altered significantly at the phylum level, as illustrated in Fig. 5. It is well established that the composting process impacts the bacterial population [39], but exposure to increased levels of TC can produce a significant shift in the composting microbial community. The bacterial community structures

in TC500 and TC250 varied more than that in TC100, in contrast to CK, at the temperature-fall and mature stages of composting. This finding shows that TC has more resolute impacts on the bacterial community at higher concentrations [40].

Proteobacteria, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were the four predominant phyla in each of the samples, and they are typically identified in the manure compost [41]. In raw composting mixtures, *Firmicutes* (87.3%) were the most plentiful bacteria, succeeded by *Proteobacteria* (7.3%), *Actinobacteria* (1.9%), and *Bacteroidetes* (0.6%). As composting progressed, *Proteobacteria* increased significantly. *Firmicutes* and *Proteobacteria* became the most dominant phyla at the temperature-rise stage, accounting for 88.0–94.4% of the total bacterial gene sequences. Compared with the CK treatment, the proportion of *Proteobacteria* decreased as the TC concentration increased, probably due to the lower thermotolerance of *Proteobacteria* than *Firmicutes* [42]. The greatest number of bacteria at the temperature-fall and mature stages belonged to the *Proteobacteria* phylum followed by the *Bacteroidetes* phylum. The subsequent dominance of *Proteobacteria* and *Bacteroidetes* can probably be ascribed to the relatively lower temperatures of household composting [42]. *Actinobacteria*, established as a marker for compost maturity [41], showed a rising trend from 0.3% (temperature-rise stage) to 5.9% (temperature-fall stage) in the CK treatment. Nevertheless, the addition of TC may have a greater impact on these bacteria. Overall, all composting samples from the TC treatments showed decreased levels of *Actinobacteria* than the CK treatment. Thus, TC addition effected changes to the bacterial abundance. These findings contradict prior reports that detailed *Actinobacteria* as usually plentiful in compost, especially during the temperature-fall and mature stages [39], but they corroborate the findings reported by de Gannes et al. [43].

Table 2 Diversity (Shannon and Simpson) and sequencing depth (coverage) indexes for bacterial community of each sample

Sample	Seq. num.	OTU num.	Shannon	Simpson	Coverage
Raw mixture	26723	580	2.82	0.154	0.990
CK-1	26844	406	2.40	0.249	0.992
CK-2	29729	902	3.71	0.043	0.988
CK-3	23812	692	3.91	0.058	0.987
TC100-1	25218	528	2.93	0.272	0.990
TC100-2	28726	590	3.65	0.054	0.991
TC100-3	30605	748	4.05	0.061	0.989
TC250-1	32198	701	2.69	0.169	0.992
TC250-2	32345	599	3.46	0.047	0.990
TC250-3	22249	493	3.67	0.049	0.989
TC500-1	30061	570	2.11	0.163	0.990
TC500-2	25950	441	2.97	0.035	0.992
TC500-3	22334	563	3.12	0.040	0.988

Fig. 5 Bacterial community evolution during the aerobic composting of human feces at phylum level (1, 2 and 3 represent the temperature-rise, temperature-fall and mature stages of compost on day 2, 6 and 21, respectively)

The bacterial community profiles were isolated by composting time, which was also reinforced by clustering analysis of the main genera (Fig. 6). The common fecal

pathogens *Escherichia/Shigella* were the predominant species identified from the initial mixture of the CK treatment, which accounted for 47.15% of the total

Fig. 6 Heatmap of species relative abundance at the genus level in compost samples (1, 2 and 3 represent the temperature-rise, temperature-fall and mature stages of compost on day 2, 6 and 21, respectively)

bacteria. For the composts spiked with TC, the proportion of *Escherichia/Shigella* decreased significantly with increasing TC concentration. Furthermore, the proportion of *Bacillus* increased to become the predominant genus at the temperature-rise stage. The genus *Bacillus* is a widespread genus of *Firmicutes* in feces composting [39] and accounted for 46.41% of the total bacteria in TC500. The genus *Bacillus* are well known degraders of organic residue, show cellulolytic activity [44], have been found during various stages of composting and are known to produce proteases, chitinases and cellulases [45]. The dominant species at the temperature-fall stage were mainly composed of *Sphingobacterium*, *Moheibacter*, *Myroides* and *Pusillimonas*. *Sphingobacterium* was the predominant genus belonging to *Sphingobacteriales* at the last two stages of composting, especially for the control group. *Moheibacter* and *Myroides* were the dominant genera belonging to *Flavobacteriales* at the temperature-fall stage of TC100 and TC500. *Bacterioidetes* are considered effective degraders of macromolecules [43]. It has been documented that the genus *Flavobacterium* in the *Bacterioidetes* phylum can degrade hydrocarbons [46]. Therefore, *Bacterioidetes* bacteria may have added to the elimination of organics in this evaluation. The genus *Pusillimonas*, part of the family *Alcaligenaceae*, can use organic acid to generate alkali [47]. Thus, it may add greatly to the pH increase and the maintenance of the alkaline environment in the compost. The species of dominant genera increased among samples at the mature stage, indicating that species diversity was enriched at the later stage [42] when mesophilic microorganisms recolonized the compost. The community structures and dominant bacteria in TC-spiked composts at the mature stage had limited similarity in contrast to those in the CK treatment.

Impact Factors on Bacterial Community Structure

The dominant species of microorganisms have been determined to be mostly dependent on the composting process parameters [39]. Consequently, the relationships between physicochemical properties in the composting process and bacterial community variance were evaluated by RDA (Fig. 7). Findings suggest rapid alterations in bacterial communities in the temperature-rise stage but only minimal changes in the next two stages. Overall, bacterial communities tended to stabilize after the temperature-fall stage, which confirmed the results of the cluster analysis. Seven parameters, specifically, temperature, pH, WSC, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DHA, and GI, were chosen from the group of physicochemical parameters measured in this evaluation according to their variance inflation factors. Of the selected parameters, temperature, $\text{NH}_4^+\text{-N}$, and GI were positively correlated with the first

Fig. 7 Redundancy analysis of the relationships between bacterial community (OTU abundance at 3% cutoff level) and the physicochemical characteristics of composting samples. Significant composting parameters are indicated by blue lines. Samples are represented by circle (CK), square (TC100), diamond (TC250), triangle (TC500) and numbers 1, 2, 3 refer to the temperature-rise, temperature-fall and mature stages of compost on day 2, 6 and 21, respectively

RDA axis ($p < 0.05$), and pH and DHA were positively correlated with the second RDA axis ($p < 0.05$). The parameters as mentioned above were thought to impact the community structure the most. Similar findings have been documented in numerous prior evaluations [41, 48]. However, $\text{NO}_3^-\text{-N}$ and WSC had relatively minimal impacts on the different bacterial community compositions compared to the other physicochemical parameters in our study. In fact, nitrogen and carbon are required nutrient elements for bacterial growth [3]. Further, WSC is an actively cycled carbon pool, which is the immediate precursor of bacteria growth and activity, thereby fueling the decomposition processes [37]. Availability of WSC can impact large environmental processes since WSC can accelerate the transport of nitrogen, phosphorus, and sulfur, as well as the energy supply, to bacteria [28, 32].

Conclusions

Human feces often contain considerably high concentrations of antibiotics as pharmaceutical residue. Taking tetracycline as an example, it is demonstrated that elevated levels of up to 500 mg/kg hinder the aerobic composting process for feces disposal and manure reuse. In addition to process parameters such as temperature, pH and WSC, the most significant adverse effect is that on the maturity of the compost produced, shown by the lowered GI. The decreased DHA at elevated concentrations of tetracycline

indicate reduced biological activity, while the significant alteration of microbial community succession explained the main reason for these adverse effects.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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