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Physical and Chemical Analysis of Composting in Cephalosporium Residue and Chicken Manure

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Abstract. In this paper, the related physical and chemical parameters in the composting were studied through the composting experiment of cephalosporin residue and chicken manure. The changes of odor and traits of composting process were analyzed. The temperature, moisture content, pH, TOC, TN and C/N were analyzed in detail. The results showed that the cephalosporins in D1 (cephalosporin residue: chicken manure = 1: 4) and D2 (cephalosporin residue: chicken manure = 2: 4) were all degraded, and the degradation rate in D3 (cephalosporin residue: chicken manure = 3:4) reached to 99.81%. As for as temperature and moisture content, four groups all meet the relevant standards, but the pH of D2 and D3 didn't meet the requirements of maturity. What's more important of the experiment is that the optimum ratio of cephalosporin residue to chicken manure was 1: 4.

1. Introduction

Antibiotics is widely produced and used in China every year, which lead to the phenomenon that a large amount of antibiotic bacteria residue can be found everywhere. But as we know, antibiotic bacteria residue contains toxic and harmful substances such as residual antibiotics, polycyclic aromatic hydrocarbons and heavy metals which is classified as hazardous waste in China. It can be seen that the reasonable disposal of antibiotic bacteria residue has become the most difficult problem to restrict the development of antibiotic pharmaceutical industry.

Antibiotic bacteria residue contains a certain amount of antibiotics, therefore it's obvious that if the antibiotic bacteria residues are not properly disposed, it will pose a potential danger to environment and human. Boleas et al [1] found that when tetracycline reached to 1mg/kg in soil, it can significantly inhibit the activity of soil dehydrogenase and phosphatase. Boonsaner et al [2] studied the effects of norfloxacin and tetracycline on soybean in saline soils, and found that they inhibited the growth of soybean and the growth rate slowed down. In addition, Boxall found that Microcystis aeruginosa and microalgae are much more susceptible to antibiotics than pseudokirchneriella subcapitata, whereas non-antibacterial algae show little difference in their sensitivity to antibiotics [3]. Antibiotic bacteria residues are rich in mycelium protein, polysaccharides and minerals, nutrient balance, Tang insist that biological pharmaceutical bacteria residues can be used as soil fertilizer to planting flower [4]. Simultaneously, she made the experiment using a bunch of red and peacock grass as the test plant, the results showed that the flower soil added bacteria residue significantly improved the growth and the quality of flowers.

At present, there are many researches of antibacterial bacteria residue composting, but few reports on the composting of cephalosporin residues. In this experiment, we selected the cephalosporin residues as the research object, which are typical antibiotic bacteria residues, and mixed with chicken manure and green waste to examine the efficacy of the composting. Research on the effects of composting could reflect reality and provide suggestions for potential engineering applications.

2. Materials and Methods

2.1 Experimental Materials

The cephalosporin residue used in this experiment was taken from a pharmaceutical factory in Hebei Province, chicken manure came from a chicken farm in Shijiazhuang, and grass clippings and green wastes were gathered from the campus of Hebei University of Science and Technology. Before the composting, grass clippings and green wastes were crushed to less than 5cm by a small grinder. The physical and chemical properties of composting materials are shown in the Table 1.

Table 1. The basic physical and chemical properties of composting materials.

Material	Moisture /%	pH	TN/%	TOC/%	C/N
Cephalosporin residue	43.6	4.89	7.15	45.56	6.37
Chicken manure	49.8	7.63	2.34	27.2	11.62
Green waste	65.8	7.2	0.89	55.6	62.4

Ni in Cephalosporin residue was not detected and other five heavy metals were all meet to NY525-2012 organic fertilizer standard of heavy metals, with the exception of Cu, Ni, Zn were not reach to heavy metals standard. Therefore, this test didn't consider the impact of heavy metals on the experimental results.The test results of heavy metal content were shown in the Table 2.

Table 2. The test results of heavy metal content.

Heavy metals	Cu	As	Hg	Cd	Cr	Ni	Zn	Pb
content	10	0.4250	0.4276	0.0200	7.112	—	99.60	3.580
standard	20	≤15	≤2	≤3	—	—	—	≤50

Note: "-" means below detection limit.

2.2 Experimental Equipment and Instrument

The composting device used in this experiment is a roller compost box. The instruments required for this experiment are electric hot water bath (DK-S26); Analytical Balances (EL204); pH meter (FE20); Whirlpool mixer (XW-80A); High-speed multi-purpose crusher(LBH-280T); Electric blast drying oven (101-0S); Multi-purpose electric furnace (DK-98-11); Ultra-pure water machine (UPH-IV-10T); CNC ultrasonic cleaner (KQ3200DA); High-speed refrigerated centrifuge (ST16R).

2.3 Experimental Methods

In this experiment, three exposed groups (D1, D2, and D3) and one control group (CK group) were designed. The material ratio of each group is shown in Table 3.

Table 3. The material ratio.

Group	Cephalosporin residue/kg	Chicken manure/kg	Green waste/kg
D1	10	40	3
D2	20	40	3
D3	30	40	3
D4	—	40	3

2.4 Assay Methods

After composting, the composting temperature and ambient temperature were measured and recorded at 9:00 and 17:00 daily. As for moisture content, we accurately weighed a certain amount of composting samples, placed in a constant temperature oven, dried at 105 ℃ for 24h to keep the weight constant, and then calculated the water content. The fresh compost samples were mixed with distilled water at a mass volume ratio of 1:5(g/mL), and continuously shaken at room temperature for 20 minutes on a shaker. After centrifugation at 3000 rpm for 10 minutes, the supernatant was taken and measured by a pH meter. The compost samples were air-dried, crushed and passed through a 100-mesh sieve. Total organic carbon (TOC) and total nitrogen (TN) were measured by TOC instrument.

As for the determination of cephalosporin residues, firstly took 10g compost samples , added 50mL of ultrapure water leaching 2h, filtered and centrifuged at 9000r / min 20min (4℃). After that took 20mL centrifuge supernatant, added 5mL zinc acetate solution and 1mL potassium ferrocyanide solution, vortexed 1min, and precipitated 20 minutes in the condition of 4℃ dark, and then centrifuged 9000r/min 20 minutes (4℃). The pellet was pipetted and the supernatant was transferred to another centrifuge tube when the tube appeared obvious protein. This operation was repeated twice, and the resulting supernatants were combined and filtered through a 0.45 μm glass fiber membrane twice. A sample of 2mL in the needle filter was aspirated into a sample using a disposable sterile syringe and analyzed by HPLC.

2.5 Statistical Analysis

All statistical analyses were expressed as mean ± standard deviation (SD) and were checked using SPSS 17.0. Significant differences were analyzed using the least significant difference (LSD) method. In this method, $P<0.05$ was considered statistically different, $0.01<P<0.05$ means statistically significantly different, and $p<0.01$ represents statistically very significantly different.

3. Results and Discussions

3.1 The Changes of Odor and Traits in the Composting Process

The composting test lasted for 35 days. The three exposed groups and CK group all experienced three stages of temperature rising, high-temperature and cooling.

During the temperature rising period (days 1 to 3d), the four groups all exuded odor, and the color deepened from light brown day by day. Meantime the color of CK group was lighter than that of 3 exposed groups. Four groups of fresh materials from the original state of loose slowly appear to lumps, and the surface become damp.

During the high-temperature stage (from the 4th to the 14th day), the four groups exuded a pungent smell of ammonia, especially the odor of D3 was heavier than others. This may be due to the nutrients of cephalosporin residue in D3 were higher than other groups. Frequent microbial activity speed up the decomposition of nitrogen-containing organic matter and release more ammonia. During this period, the color of groups were gradually deepen, and the structure became more viscous.

Composting cooling stage (days 15 to 35d), the pungent smell of ammonia gradually weakened, and the color turn into dark brown, at the same time ,the structure was more loose. After the compost matured, almost no unpleasant odor was exuded, the structure was loose and the color was black. The appearance of the four groups was almost consistent during the composting.

3.2 Changes in the Physical and Chemical Parameters

3.2.1. Temperature

During composting, the ambient temperature was relatively stable, with a small change between 32 and 37°C. During the composting period of 35d, the groups D1, D2, D3 and CK all experienced three stages of temperature rising, high-temperature and cooling, and finally reached to the ambient temperature(Fig.1). In the meantime, at the beginning of composting, the heating rates of group D1, D2 and D3 were obviously higher than those of CK group.

Comparing the heating rates of the groups D1, D2, and D3 in the early stage of composting, it was found that the heating rate of the three groups gradually decreased. The ratio of the bacteria and residue of D3 (cephalosporin residue: chicken manure = 3: 4) is higher than D1 and D2, and the heap of D3 is slower than D1and D2. D3 reached to 72.4℃, which was the peak temperature on the 6th day, it showed that with the increase of the proportion of microbial residue in the pile, the initial heating rate of composting decreased, therefore we can draw the conclusion that residual cephalosporins have a certain inhibitory effect on the pile of microorganisms. This result of temperature trends is similar to that of Zhang Hongjuan [5], who carried lincomycin bacteria residue and cow dung composting test.

According to "Hygienic requirements for harmless disposal of night soil" (GB 7959-2012) [6] in China, four groups have reached to a sound standard of hygiene. In the high-temperature period of composting, with the increase of the proportion of cephalosporin, the peak temperature of the pile body is higher, and the duration of the high temperature is longer. This shows that a large number of bacteria can effectively promote the efficiency and quality of the process. Composting for about 30d, the temperature of each pile got close to the ambient temperature, and the temperature changed gently, indicating that the four groups were well-composted.

3.2.2. Moisture Content

The moisture content of the four groups in the early composting showed a clear rise, indicating that organic matter began to decompose during this time (Fig.2). The temperature increased lowly, and the decomposition of organic matter produced more than water evaporation moisture. Meanwhile the moisture content of the groups were rising. The relative increase in water content of CK was probably due to the frequent microbial activities in chicken manure which accelerated decomposition of organic matter, resulting in producing more water. In addition, the temperature of CK pile was lower than that of the three exposed groups and evaporated very slow. Eventually, the moisture content of D1, D2, D3 and CK fell to less than 30%, which showed that four groups all meet the requirement of moisture content of organic fertilizer (≤30%) in NY525-2012.

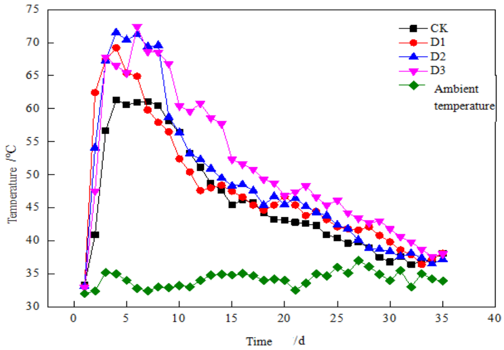

Figure 1. Temperature changes.

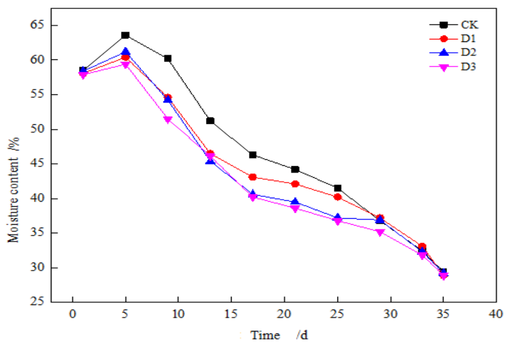

Figure 2. Moisture content change.

3.2.3. Ph

PH of four groups showed a trend of first increasing and then decreasing with composting time (Fig.3). PH of cephalosporin residue is 4.89, and this lead to pH of D1, D2, D3 were weakly acidic. As we can see from Fig.1, pH of each group increased dramatically in the temperature rise period, rose tardy in the high-temperature period and drop slowly in the cooling period. Finally, pH of D1 and CK were 8.06 and 8.04, respectively, which reached the standard of composting maturity (pH ranged from 8 to 9). PH of piles D2 and D3 were 7.48 and 7.10, respectively, which did not meet to the composting maturity standard obviously.

3.2.4. Total Organic Carbon

Throughout the composting process, TOC of each group showed a declining trend over time. During the temperature rising and high-temperature periods, the content of TOC decreased rapidly, but in the late composting stage, the temperature decreased and the activity of microorganisms gradually weakened owning to the nutrients were almost exhausted. The remaining organic substances were difficult to be reduced, and the rate of decline became smooth. Finally its content remained almost unchanged. The decrease of TOC in CK was relatively fast, which was mainly due to the highest proportion of chicken manure that contain a small amount of cellulose and were more easily degraded.

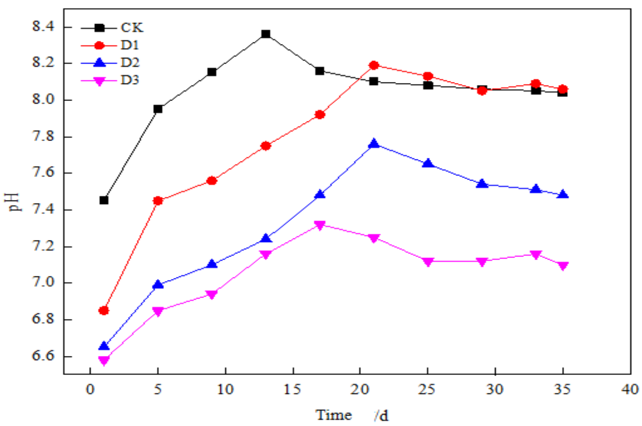

Figure 3. pH changes.

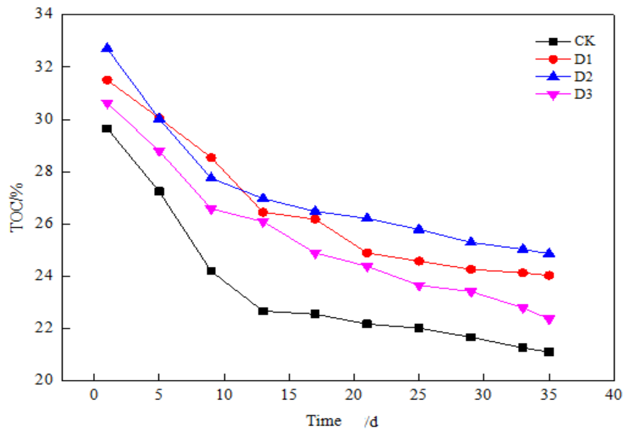

Figure 4. TOC changes.

3.2.5. Total Nitrogen

The change of total nitrogen (TN) in composting was directly related to the nitrogen which is used to provide rapid microbial growth and reproduction, and the nitrogen dissipated in the form of ammonia gas.

With the development of time, the change of TN in four groups were roughly consistent, all of them reduced untill to steady(Fig.5). The initial total nitrogen contents in the D1, D2, D3 and CK groups were 2.86%, 3.01%, 3.28% and 2.03%, respectively.

The rate of decline of D1 and CK were faster than that of D2 and D3, because the ratio of chicken manure contained in CK and D1were relatively high, and there are a large amount of microorganisms in chicken manure, resulting in the decomposition of nitrogenous organisms by microorganisms and releasing more of ammonia. It can be seen that microbial decomposition of nitrogen-containing organic matter into ammonia nitrogen is the main way of nitrogen loss in the composting process, which results in ammonia volatile.

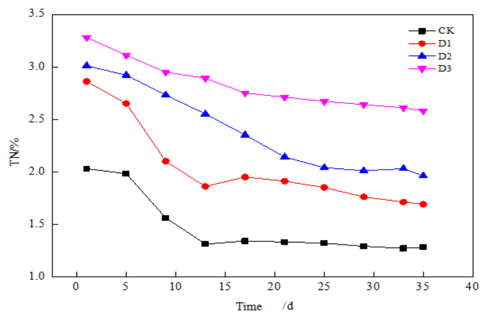

Figure 5. TN changes.

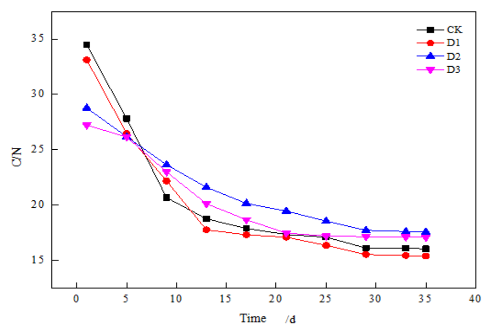

Figure 6. C/N changes.

3.2.6. C/N

With the progress of composting, the C/N of each group showed a decreasing trend, especially in the rising temperature and the high-temperature period, the C/N descending trend was most significant, and then the trend was gentle (Fig.6). The downward trend of D1 and CK were significantly faster than that of D2 and D3. At the end of compost, the C/N of D1 and CK were lower than that of D2 and D3, indicating that a large proportion of chicken manure accelerate the degradation of organic matter, which reduce the C/N of the composting material and affect the C/N of the composting product at the end of composting.

3.3 Cephalosporin Residue Before and After Composting

Residues of cephalosporins before and after composting are shown in Table 4.

Table 4. Residues of cephalosporins before and after composting.

Sample	Initial content/($mg \cdot g^{-1}$)	Final content/($mg \cdot g^{-1}$)	Degradation rate/(%)
D1	0.9837	—	100
D2	1.2783	—	100
D3	1.4969	0.0028	99.81
D4	—	—	—

As we can see from Table 4, cephalosporins in D1 and D2 piles all have been degraded after 35 days and no cephalosporin residues. Cephalosporin of D3 (the largest proportion of microbial residue) degraded 99.81%. Visibly, cephalosporin in the composting of a variety of microorganisms can be degraded under the joint action. Therefore, composting can be used as one of the treatment methods of cephalosporin bacteria residue resource.

4. Conclusion

The composting test lasted for 35d, and the appearance of four groups showed basic consistency during the composting. The four groups have all met the hygiene requirements of the "Hygienic requirements for harmless disposal of night soil" (GB7959-2012) regarding temperature. At the same time, moisture contents were in line with organic fertilizer moisture content (≤ 30%) in NY525-2012.

PH of D1 and CK were 8.06 and 8.04, respectively, which reached the composting maturity standard (pH ranged from 8 to 9). But the group D2 and D3 didn't meet the composting standard.

With the progress of composting, the C/N of each pile showed a decreasing trend. Chicken manure could help to speed up the degradation of organic matter in composting materials and reduced the C/N of composting materials. D1 reached composting maturity of the indicators. The optimum ratio of cephalosporin to chicken manure is 1: 4. At the end of composting, the cephalosporins D1 and D2 were all degraded, and the degradation rate of D3 was 99.81%.

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