Biodegradation of antibiotic residues in chicken manure by composting processes

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Abstract. Chicken manure is rich in macro and micro nutrient compared to livestock manures. Unfortunately, it may also contain higher antibiotic residues that has to be minimized before applying as a manure. The objective of this research was to eliminate antibiotic residues in chicken manure using three kinds of decomposer. The experiment was conducted in greenhouse conditions. A completely randomized design with 5 replications was applied. The treatments consisted of chicken manure (CM) with 40% water content, composting CM with local microorganisms (MOL), composting CM with Consortium of Cellulolytic Fungi (CCF), and composting CM with commercial decomposer. Parameters measured were the content of Tetracycline, C, C/N, and CO₂ evolution. The results showed that applying microbial decomposer eliminated 100% Tetracycline residue after 4 weeks composting. The C content and C/N decreased 34.8 to 41.8% and 15.46 to 20.30% after 6 weeks composting, respectively. The highest CO₂ evolution resulted from the application of CCF and commercial decomposers. Composting chicken manure by CCF or commercial decomposer prior to be used as organic fertilizer are promising to eliminate antibiotic residues.

1. Introduction

Strengthening public awareness of healthy living and environment has an impact on the increasing demand on organic agricultural products from year to year, both in Indonesia and abroad. The government strongly supports the development of organic agriculture, through the issuance of regulations on organic farming systems (Permentan No.64/2013 and SNI 6729:2016), input assistance, and the development program of 1,000 organic villages. Organic agriculture is an integrated farming system that avoids the use of artificial fertilizers, pesticides and genetically modified products, and suppresses environmental pollution (air, soil, and water). Organic agriculture absolutely requires organic fertilizer as the main nutrient source, coming from in-situ organic matter or through integration with livestock.

The positive effect of livestock manure both as organic fertilizer and soil ameliorant on horticultural crops has been no doubt. Farmers rely heavily on the use of livestock manure to support growth and yields, especially for organic farming. Its application for horticultural crops can reach 25 t ha⁻¹. Macro and micro nutrient content in chicken manure are higher than those in other manure. Unfortunately, the residue of antibiotics content in chicken manure is quite high. About 70–90% of the antibiotic in the feces and urine of animal’s discharges was in active metabolite [1]. The Tetracycline elimination occurs in the kidneys through the glomerular filtration mechanism and is excreted through urine.
Several studies have confirmed that antibiotics used in animal production are present in fresh manure, manure storage tanks, soil, surface and underground water [1,2]. Antibiotics content in chicken manure is quite high and may reach 200 ppm [3]. Chen et al. [4] found tetracycline, oxytetracycline, chlortetracycline, and doxycycline (0.1–205 ppm) in manure-amended soils. Most of the antibiotic residues in manure form complexes with soluble organics and remain stable during manure storage [1]. The research conducted by the Bogor Veterinary Research Institute (Balitvet) showed that 71.43% of feed mills in Bogor, Cianjur, Tangerang, Bekasi and Sukabumi districts provided additional tetracycline and sulfonamide antibiotics in chicken feed products [5]. The use of antibiotics for livestock health purposes cannot be avoided, but sometimes breeders also use them as growth promoters in excessive dosage. Survey conducted by Civas in 2016 in Central Java showed that 37.5% breeders still used tetracycline not only as an anti-bacterial but also for growth promoters.

Application of chicken manure on agricultural land spreads antibiotics to the environment in large scale, although antibiotics are recalcitrant to be degraded. When chicken manure is applied to agricultural land, the fraction of its antibiotic becomes mobile with the water flow in the soil and contaminates the environment including surface and groundwater [1]. This phenomenon will affect 1) the presence of antibiotics detected in food products from animals and plants [3, 6], 2) the development of resistant antibiotic bacteria [7], and 3) contamination of the agricultural environment due to manure land application [1, 8]. Contamination of antibiotics in soil influences the microbial community or diversity composition which may result in alteration of ecological function [7].

Although most antibiotics remain stable during manure storage, their degradation to various extents depend on 1) the concentration and class of antibiotic, 2) bioreactor operating conditions, 3) type of feedstock and 4) inoculum sources. Generally, antibiotics are degraded during composting > anaerobic digestion > manure storage > soil [1]. Several studies using white rot fungus as a source of inoculants to degrade antibiotics have been reported by [9–12], but the uses of *Aspergillus niger* and *Trichoderma* sp have not been widely reported. For this reason, technology is needed to eliminate antibiotic residues in chicken manure using kinds of decomposer, so the application will be environmentally friendly. The purpose of this research was to eliminate Tetracycline residue in chicken manure by applying decomposer.

### 2. Materials and methods

#### 2.1. Cellulolytic fungi sources and their viability on agar media contained 250 ppm tetracycline

The experiment was conducted at Biology Laboratory of ISRI. Five cellulolytic fungus strains of ISRI collection were used in this experiment namely 2 strains of *Trichoderma viride* (Triv 13.4 and Triv 13.2), 1 strain of *T. koningii* (Kun4), 1 strain of *Aspergillus niger* (Asn1) and 1 strain of *Lentinus* sp (L7). All strains were grown in 2 kinds of liquid media, 1% malt extract (ME) and 1% malt extract + 250 ppm Tetracycline (ME+Tc). Shaking incubation was conducted in 120 rpm for 15 days at room temperature (29 °C to 31 °C). Viability cell was counted by Total Plate Count on PDA media at 3, 6, 9, 12 dan 15 days after inoculation. The Consortium of Cellulolytic Fungi (CCF) was created by combining the best growing isolates on Tetracycline contained media. Subsequently, the liquid CCF culture was used in experiment 2.2.

#### 2.2. Green house experiment: composting of chicken manure containing tetracycline residues

The experiment was conducted in Greenhouse conditions using 25 pots, each filled with 20 kg chicken manure taken from Jetak village, Semarang Regency. A completely randomized design with 5 replications was applied. Water content of chicken manure (CM) was adjusted to 40%, except control. The treatments consisted of composting of CM with existing water content (as control), without microbial decomposer, with local microorganisms (MOL), with CCF, and with commercial decomposer. Concentrations of MOL and CCF each applied were 5% (v/w), and the concentration of commercial decomposer applied was 1 kg t⁻¹ manure. All the pots were covered with black plastic and incubated for 30 days. Parameters measured were the contents of Tetracycline, CO₂ evolution rate,
Ultrasound-Assisted Extraction was used to extract Tetracycline from the manures [13], [14] and measured by applying HPLC (Shimadzu). CO₂ evolution rate was measured by alkali absorption methods [15]. The method for assaying the contents of organic C (dry combustion), total N, and C/N follows [16].

3. Results and discussion

The growth curve of fungi and rates of each fungus on 1% ME and 1% ME + 250 ppm Tetracycline media are presented in Figure 1. Malt extract of 1% was used only as a starter for the energy source of the fungus at the beginning of growth. It was expected that the fungus could use the C source by breaking the C tetracycline chain. In general, both fungal growth patterns were firstly shown by the Triv13.2 and L7 strains, and secondly by the Triv13.4, Asn1, and Kun4 strains. In 1% ME medium, the Triv13.2 and L7 strains showed the same growth pattern in both media, but the total fungal population was higher in 1% ME + 250 ppm Tetracycline media. Whereas the fungal growth curves produced by the Triv13.4, Asn1, and Kun4 strains showed that the number of fungal population decreased drastically on days 12-15 on ME media, but the population was still in a stationary phase until days 12 up to 15 in 250 ppm Tetracycline contained media.

![Figure 1](image-url)
The population of Triv13.4 and L7 strains still reached 1x10^6 cfu ml^-1, while Triv13.2, Asn1, and Kun4 strains had decreased to 1x10^4 - 1x10^5 cfu ml^-1. Based on the number of fungal population on ME + TC media, the CFC formula contained Triv13.4 and L1 strains.

Dynamics of temperature changes that occur during the composting process are presented in Figure 2. The highest temperature achieved during the CM composting was 55°C coming from microbial treatments (D and E). Applications of microbial decomposer treatments such as C, D, and E showed a higher average temperature compared to those without microbial decomposer treatments (Figure 3). The temperature increase was due to microbial activity. Composting process is divided into 3 phases, namely mesophilic phase 1, thermophilic phase, and mesophilic phase 2. In the thermophilic phase, the recalcitrant materials like cellulose and lignin will be broken down and temperature will reach > 45°C. Antibiotics are relatively recalcitrant to degradation.

Table 1 shows the content of Tetracycline during the composting and the percentage of its decreasing contents. The content of existing Tetracycline on CM taken from Semarang district was 1.16 ppm. After 15 days composting, Tetracycline content on CM decreased about 54.35 - 72.10%. The highest reduction of Tetracycline was obtained from the composting using a commercial decomposer and followed by using MOL. However, the assays performed on the 30th day were still found Tetracycline contained in the control and MOL treatments, namely 0.488 and 0.473 ppm, respectively. Tetracycline was not detected in other treatments on the 30th day.

Figure 2. Dynamic of temperature changes during composting the CM. A = Control, B = composting CM without adding microbial decomposer, C = composting CM with local microorganisms (MOL), D = composting CM with CCF, E = composting CM with commercial decomposer.

Figure 3. The average temperature achieved during composting the CM. A = Control, B = composting CM without adding microbial decomposer, C = composting CM with local microorganisms (MOL), D = composting CM with CCF, E = composting CM with commercial decomposer.
**Table 1.** Tetracycline content (ppm) and decreasing percentage (%) in CM during the composting.

| Composting treatment* | composting period (days) | ppm       | %        | ppm       | %        |
|-----------------------|--------------------------|-----------|----------|-----------|----------|
|                       | 15                       | 30        |
| A                     | 0.475 ± 0.05             | 59.17 ± 0.43 | 0.488 ± 0.027 | 57.94 ± 2.90 |
| B                     | 0.529 ± 0.018            | 54.36 ± 1.59 | nd       | 100.00 ± 0.00 |
| C                     | 0.405 ± 0.032            | 65.09 ± 2.96 | 0.473 ± 0.001 | 59.18 ± 0.12 |
| D                     | 0.491 ± 0.023            | 57.71 ± 1.94 | nd       | 100.00 ± 0.00 |
| E                     | 0.324 ± 0.039            | 72.10 ± 3.83 | nd       | 100.00 ± 0.00 |

*) A = Control, B = composting of CM without adding microbial decomposer, C = composting of CM with local microorganisms (MOL), D = composting of CM with CCF, E = composting of CM with commercial decomposer.

Tetracycline which was still detected on day 30 caused by the compost temperature was not high enough to damage its structure or because the number of microbes degrading Tetracycline did not reach the quorum. The CO₂ evolution during the composting is presented in Table 2. The highest average of CO₂ evolution was found in the composting CM using CCF. CO₂ was produced as a result in the decomposition of organic compounds due to microbial activity. There was a significant difference between control and other treatments. In treatment B (without addition of microbes), the CM water content was adjusted to approximately 40% being enough to activate indigenous microbes in CM. Composting treatment by adding microbial decomposer showed a higher CO₂ evolution than without microbial decomposer.

**Table 2.** CO₂ evolution (mg C-CO₂/kg CM) during composting the chicken manure.

| Composting Treatment¹ | Composting period (days) |            |            |            |            |            |            |
|-----------------------|--------------------------|------------|------------|------------|------------|------------|------------|
|                       |                          | 7          | 15         | 21         | 30         | Average    |
| A                     | 30.19 a                  | 67.75 a    | 58.32 a    | 45.36 a    | 50.41 a    |
| B                     | 998.18 b                 | 962.83 b   | 675.41 b   | 891.22 b   | 881.91 b   |
| C                     | 1065.77 b                | 853.87 b   | 848.33 b   | 1022.16 b  | 947.53 b   |
| D                     | 1173.94 b                | 1092.17 b  | 769.87 b   | 1058.16 b  | 1023.54 b  |
| E                     | 1029.77 b                | 1083.89 b  | 821.81 b   | 1019.23 b  | 988.22 b   |

¹ A = Control, B = composting CM without adding microbial decomposer, C = composting CM with local microorganisms (MOL), D = composting CM with CCF, E = composting CM with commercial decomposer.

**Table 3.** The organic C, total N and C/N of chicken manure during the composting.

| Composting Treatment* | Composting period (days) | Organic C (%) | Total N (%) | C/N |
|-----------------------|--------------------------|---------------|-------------|-----|
|                       | 15 30 average            | 15 30 average | 15 30 average |
| A                     | 37.72 c 29.54 c 33.63 c | 3.09 2.74 2.92 | 12 11 11.5   |
| B                     | 34.92 b 27.71 c 31.32 b | 3.06 2.91 2.99 | 12 10 11.0   |
| C                     | 34.58 b 25.28 b 29.93 b | 2.99 2.58 2.79 | 12 10 11.0   |
| D                     | 27.56 a 23.42 b 25.49 ab| 2.90 2.36 2.63 | 9 10 9.5     |
| E                     | 27.07 a 16.87 a 21.97 a | 2.75 2.04 2.40 | 10 8 9.0     |

*) A = Control, B = composting CM without adding microbial decomposer, C = composting CM with local microorganisms (MOL), D = composting CM with CCF, E = composting CM with commercial decomposer.
During the decomposition, the C content of organic compounds will decrease because some C is released in the form of CO₂, while the N content is relatively stable resulting in a decrease in the C/N value.

Table 3 presents the determination of the organic containing C, N, and C/N during composting measured on the 15th and 30th days. The treatment using commercial decomposer was very effective to decompose compound C in CM, in where on day 30 the organic C content decreased drastically up to 16.87% with a C/N value of 9.0.

4. Conclusions
The viability of *Trichoderma viride* strains Triv13.4 and *Lentinus* sp strain L7 growth in 1% Malt Extract + 250 ppm Tetracycline medium during 15th day of incubation was 1.52x10⁶ and 1.33x10⁶ cfu ml⁻¹, respectively. Composting chicken manure with 40% moisture content inoculated with commercial decomposer and CCF (containing *Trichoderma viride* strain Triv13.4 and *Lentinus* sp strain L7), reduced the Tetracycline content in chicken manure by 72.10% and 57.71% on day 15th of composting, respectively. Chicken manure must be composted before land application to eliminate antibiotics residue. Composting using a decomposer is highly recommended because it can increasing the temperature during composting.

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