Ecotoxicology and Environmental Safety 140 (2017) 1–6

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting

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ARTICLE INFO

Keywords:
Antibiotic resistance gene
Bamboo charcoal
Chicken manure
Composting

ABSTRACT

Composting is widely used for animal waste disposal, and bamboo charcoal (BC) can be used for nitrogen conservation during composting. However, the effects of BC on antibiotic resistance genes (ARGs) during chicken manure composting are still unclear. This study investigated the effects on ARGs of adding different proportions of BC (0%, 5%, 10%, and 20% w/w) to chicken manure compost. After 26 days, the relative abundances (RAs) of most ARGs (*tetC*, *tetG*, *tetW*, *tetX*, *sul2*, *drfA1*, *drfA7*, *ermB*, *ermF*, *ermQ*, and *ermX*) and *intI1* declined by 21.6–99.5%, whereas *sul1* increased by 7.5–17.7 times. The average RAs reductions with 0%, 5%, 10%, and 20% BC were 0.85, 1.05, 1.08, and 1.15 logs, respectively. The most important environmental factor for the ARG profiles was temperature according to redundancy analysis. Furthermore, BC significantly decreased the bio-Cu and bio-Zn levels, thereby reducing the co-selection pressure from heavy metals. Different proportions of BC had no significant effects on the removal of *tetG*, *tetW*, *tetX*, *sul2*, *drfA1*, and *ermB*. Supplementation with 10% BC was more effective at removing *tetC* and *drfA7* compared with the other treatments. The results suggested that 10% BC supplementation is appropriate for reducing ARGs in chicken manure compost.

1. Introduction

The increasing emergence and spread of antibiotic resistance genes (ARGs) have become major global public health issues in recent years (WHO, 2014; Wright, 2010). Antibiotics are used widely by the livestock industry, so animal manure is considered to be an important reservoir of antibiotics and ARGs (Heuer et al., 2011; Zhu et al., 2013). Farmers often use composted livestock manure to improve the quality of the soil, and thus compost derived from livestock manure could allow large amounts of ARGs to enter farmland (Zhu et al., 2013). The emergence of ARGs has also been detected in both soils and vegetables (Rahube et al., 2014). Furthermore, they could pose a threat to human health due to horizontal gene transfer (HGT) via mobile genetic elements (Su et al., 2015; Fang et al., 2015). Thus, the removal of ARGs from animal manure before their application to soil is becoming an important environmental issue.

Composting is a widely accepted method for transforming organic waste into a stable product, which is nontoxic and nonpathogenic, and it can be applied as an appropriate fertilizer and soil conditioner to support plant growth (USEPA, 2002; Huang et al., 2006). Several studies have investigated the evolution of ARGs during composting, but variable results were obtained by different researchers. Zhang et al. (2016a) found that after sludge composting, some ARGs (*blaCTX-M*, *blaTEM*, *ermB*, *ereA*, and *tetW*) were reduced by 0.3–2 logs, whereas others (*ermF*, *sulI*, *sulII*, *tetG*, *tetX*, *mefA*, and *aac(6′)-Ib-cr*) increased by 0.3–1.3 logs. Selvam et al. (2012) found that after 56 days of composting, tetracycline, sulfonamide, and fluoroquinolone resistance genes were undetectable among the selected ARGs tested in the compost product, but not *parC*. Cui et al. (2016) showed that after 42 days, the average ARG removal rate was 0.86 log units in a controlled composting experiment. Therefore, composting is a potentially useful method for the management of manure to decrease the abundance of ARGs.

Nitrogen loss is a significant problem during livestock manure composting, thereby reducing the quality of the compost product and causing environmental problems (Chan et al., 2016). Biochar is a potential amendment for the conservation of nitrogen in composting due to its unique properties, such as its chemical composition, high surface area, microporosity, and sorption capacity (Sánchez-García et al., 2015). Biochar can also increase the temperature (Malińska et al., 2014), thereby prolonging the thermophilic phase (Liu et al., 2014) to accelerate the degradation of organic matter during compost-

Abbreviations: ARG, antibiotic resistance gene; BC, bamboo charcoal; bio-Cu, bio-available Cu; bio-Zn, bio-available Zn; HGT, horizontal gene transfer; qPCR, quantitative PCR; RAs, relative abundances

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http://dx.doi.org/10.1016/j.ecoenv.2017.01.007
Received 20 August 2016; Received in revised form 3 January 2017; Accepted 3 January 2017
Available online 27 February 2017

ing (Sánchez-García et al., 2015). Jindo et al. (2012) showed that biochar induces specific changes in the microbial community structure of agricultural waste and livestock manure during composting. Moreover, typical heavy metals such as Cu and Zn play important roles in determining the abundances of certain ARGs (Ji et al., 2012). Cu and Zn are considered typical heavy metals because they are used widely in the livestock industry, thereby leading to high concentrations of Cu and Zn in animal manure (Zhang et al., 2012). Bamboo charcoal (BC) can decrease the mobility of heavy metals (Chen et al., 2010), so biochar might be beneficial for reducing the mobility of heavy metals and it may help to remove ARGs in an effective manner. Various types of biochar also have different effects on ARGs during the composting process (Cui et al., 2016), but using the same type of biochar in variable proportions may have diverse effects on ARGs. Furthermore, China is one of the biggest producers of bamboo, so BC is an important part of biochar in China (Zhang et al., 2001). Thus, identifying an appropriate proportion of BC to control ARGs has practical significance.

In the present study, four different proportions of BC (0, 5%, 10%, and 20%) were added to chicken manure and the changes in three types of ARGs (tetracycline resistance genes, *tet*: *tetC*, *tetG*, *tetW*, and *tetX*; sulfonamide resistance genes, *sul*: *sul1*, *sul2*, *dfrA1*, and *dfrA7*; and macrolide resistance genes, *erm*: *ermB*, *ermF*, *ermQ*, and *ermX*) and an integron-integrase gene (*intI1*) were evaluated. The aims of this study were: (1) to investigate the effects of adding different proportions of BC on ARGs during chicken manure composting, (2) to determine the relationship between environmental factors and ARGs during composting, and (3) to identify the most appropriate proportion of BC supplementation for controlling ARGs.

2. Materials and methods

2.1. Composting materials

The composting materials comprised a mixture of chicken manure, wheat stalk (2–3 cm), and BC. Wheat stalk was obtained from a straw processing factory (Shaanxi, China). Chicken manure was collected from a chicken farm (Shaanxi, China). BC was made from bamboo at a pyrolysis temperature 600 °C and it provided by Shanghai Hainuo Charcoal Co. Ltd (Shanghai, China). The physicochemical properties of the materials are shown in Table S1.

2.2. Experimental design

The composting experiments were performed in the composting area at Northwest A & F University, PR China. Twelve identical laboratory-scale foam containers were used, which contained 3 kg of a chicken manure: wheat stalk mixture (=1:1, dry weight) and the C/N ratio was ca 25. A control experiment was conducted without the addition of BC. Biochar was added to the chicken manure and wheat stalk mixture at rates of 5%, 10%, and 20% on a dry weight basis. The four composting experiments were designated as: CK (chicken manure + wheat stalk), BC5 (chicken manure + wheat stalk + 5% BC), BC10 (chicken manure + wheat stalk + 10% BC), and BC20 (chicken manure + wheat stalk + 20% BC). Each treatment had three replicates. The moisture content of each mixture was adjusted to around 60% with water. All of the composting treatments were conducted for 26 days in 36-L rectangular form containers (length × width × height × thickness = 28 × 26 × 50 × 3.5 cm). The side of each container was punched with two holes (2 × 2 cm), and thus oxygen was available via natural ventilation. The pile of compost was turned over on days 0, 2, 4, 7, 14, and 26, and water was added as necessary. The temperature was measured at 5:00 pm on every day.

2.3. Sample collection

Samples were collected on days 0, 2, 4, 7, 14, and 26 to cover the three composting stages: the thermophilic stage on days 2 and 4, the mesophilic stage on days 7 and 14, and the maturation stage on day 26. The compost material was mixed homogeneously before sampling. Each sample was split into two parts, where one was stored at 4 °C for chemical analysis, and the other was stored at –80 °C for DNA extraction and further analysis after freeze-drying.

2.4. Physicochemical analysis of the compost samples

The moisture content was measured by drying in an oven at 105 °C for 12 h, or until no change in the dry weight was observed. The pH was determined in a 1:9 compost: water (w/v) suspension using a pH meter (Rex, China) after shaking for 30 min with an end-over-end shaker. The K_2CrO_4 oxidation method (Nelson and Sommers, 1982) and Kjeldahl method (Bremner and Mulvaney, 1982) were used to determine the total organic carbon and total nitrogen contents, respectively. NH_4^+-N and NO_3^--N were extracted with 2 M KCl and assayed using a segmented flow analyzer (Skalar, Netherland). Bio-available heavy metals (bio-Cu and bio-Zn) were extracted with diethylenetriamine-pentaacetic acid (DPTA) at a solid: liquid ratio of 1:5 (w/v) and analyzed using a flame atomic absorption spectrometer (Hitachi, Japan). DTPA-extractable heavy metals were defined as bio-available heavymetals (Roosa et al., 2014).

2.5. DNA extraction and quantitative PCR (qPCR)

DNA was extracted from freeze-dried samples (0.1 g) using a FastDNA Spin Kit for Soil (MP Biomedical, USA), according to the manufacturer's instructions. During DNA extraction, 5.5 M guanidinium isothiocyanate (Amesco) was added to remove humic acid. Four tetracycline resistance genes (*tet*: t*etC*, *tetG*, *tetW*, and *tetX*), four sulfonamide resistance genes (*sul*: *sul1*, *sul2*, *dfrA1*, and *dfrA7*), four macrolide resistance genes (*erm*: *ermB*, *ermF*, *ermQ*, and *ermX)*, and an integron gene (*intI1*) were analyzed by PCR and agarose electrophoresis. The detected ARGs and 16S rRNA gene were analyzed further by qPCR. The qPCR reaction mixture comprised 1 μL of DNA template, 0.25 μL of each 20 pM primer (ShengGong, China), 10 μL of SuperReal PreMix Plus (TianGen, China), and 8.5 μL of RNase-free water. The qPCR conditions comprised an initial hold for 15 min at 95 °C, followed by 40 cycles for 10 s at 95 °C, 20 s at the annealing temperatures shown in Table S2, and then 32 s at 72 °C. To eliminate the effects of inhibitory compounds, the DNA template was a tenfold dilution of extracted DNA. qPCR was performed using Bio-Rad IQ5 (Bio-Rad, USA). The primers and qPCR conditions are described in Table S2. For the standard curves, $R^2 > 0.99$ and the amplification efficiency ranged between 90% and 110%, and they were used to calculate the copy numbers of ARGs. The relative abundances (RAs) of the ARGs were calculated as: ARG copies/16S rRNA copies.

2.6. Statistical analysis

The abundance of *intI1* and the standard errors of ARGs were analyzed using Microsoft Excel 2013. Changes in the RAs of the ARGs were tested with SigmaPlot 12.5. Pearson's correlation coefficients (r) and *P*-values between the dependent variables (ARGs) and independent variables (*intI1* and bio-available heavy metals) were obtained using SPSS 19.0. Redundancy analyses (RDA) of the correlations between ARGs and environment factors were performed using Canoco 4.5 for Windows.

3. Results and discussion

3.1. Physicochemical changes during composting

Temperature is one of the key indicators of the progress of composting (Hassen et al., 2001). As shown in Fig. 1, the temperatures