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Decline in extractable kitasamycin during the composting of kitasamycin manufacturing waste with dairy manure and sawdust

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ABSTRACT

The aim of this study was to propose a feasible treatment of kitasamycin manufacturing waste by examining extractable kitasamycin and evaluating its compost maturity during the composting of waste with different ratios of dairy manure and sawdust over a 40-day period (volume/volume/volume; M1, 0/80/20; M2, 10/70/20; and M3, 30/50/20). During composting, the concentration of extractable kitasamycin in kitasamycin-contaminated composts declined rapidly, and was undetectable in M2 within 15 days. M2 also achieved the highest fertility compost, which was characterised by the following final parameters: electrical conductivity, 2.34 dS cm^{-1}; pH, 8.15; total C/N, 22.2; water-soluble NH_4^+, P, and K, 0.37, 3.43, and 1.05 g kg^{-1}, respectively; and plant germination index values, 92%. Furthermore, DGGE analysis showed a dramatic increase in the diversity of bacterial species during composting. In contrast, a high concentration (121 mg kg^{-1}) of extractable kitasamycin still remained in the M3 compost, which exerted an inhibitory effect on the composting, resulting in reduced bacterial diversity, high values of electrical conductivity and water-soluble NH_4^+, a low C/N ratio, and a low plant germination index value. Furthermore, 3.86 log (CFU g^{-1}) kitasamycin-resistant bacteria were still present on day 40, indicating the biological degradation contributed to the decline of extractable kitasamycin.

1. Introduction

The treatment of antibiotic manufacturing waste, which is a residue of the organic medium used for microbial fermentation during antibiotic production, has become a difficult problem in China. Due to the high levels of antibiotic residuals, this biowaste has been utilised as an additive to animal feed to promote growth and to prevent pathogenic bacterial infection. However, incomplete assimilation in animals has resulted in 70–90% of the antibiotic being excreted in manure (Phillips et al., 2004; Kumar et al., 2005); the manure is not intensively treated and is often applied directly to agricultural fields, which contributes to the increase in antibiotics and antibiotic-resistance genes in the environment. The spread of antibiotic residues in the environment poses a potential risk to aquatic and terrestrial organisms (Hamscher et al., 2000). In addition to reducing the diversity of micro-organisms in the environment (Zielezny et al., 2006), this phenomenon can also enhance human antimicrobial resistance due to the consumption of crops grown in the antibiotic-contaminated soils (Keen and Montforts, 2011). Thus, appropriate techniques are needed introduced to reduce the antibiotic level of manufacturing waste prior to agricultural utilisation.

Composting is an effective methodology for the decomposing of organic wastes via the biological degradation of organic constituents under controlled conditions, and has been successfully applied for the treatment of persistent organic pollutants (Laine and Jorgensen, 1997; Zeng et al., 2011). Recently, additional information regarding antibiotic degradation during manure composting has become available. A study on chlortetracycline in aged and spiked poultry manure demonstrated that more than 90% of the initial level of chlortetracycline was depleted under aerobic composting (Bao et al., 2009). Both full-scale and lab-scale investigations have indicated that decreases in tetracycline and sulphonamide concentrations are highly dependent on the presence of sawdust during composting (Kim et al., 2012). Dolliver et al. (2008) suggested that stockpiling may be a practical and economical option for livestock producers to reduce antibiotic levels in manure.

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As a by-product of microbial fermentation, antibiotic manufacturing waste cannot be composted alone due to its high nitrogen content (Table 1), which could enhance the loss of N via volatilisation and phototoxic molecules may simultaneously build up due to insufficient biodegradation of the organic matter (Bernal et al., 2009). Normally, high-nitrogen wastes are mixed with adjusted by the carbon-rich materials prior to composting to obtain an initial C/N ratio of between 25 and 35 (Bernal et al., 2009); however, these values are not absolute. An economic analysis showed that composting with a low initial C/N ratio (20) is beneficial to efficiently dispose of a high amount of N-rich waste (swine manure) (Zhu, 2007). Furthermore, to achieve high grade composting, other factors that affect the composting process, such as pH, electrical conductivity (EC), and the available nutrients, should also be well balanced. However, the formulation of composting mix between the antibiotic manufacturing waste and other organic materials is currently unavailable.

Another important issue is the effect and fate of the antibiotics present in the manufacturing waste throughout the composting process. Previous studies have shown that antibiotic residues in manures did not have a significant effect on the composting process because the concentrations of extractable antibiotics decreased rapidly in during the first 3–10 days of composting (Arikan et al., 2007; Dolliver et al., 2008; Bao et al., 2009; Kim et al., 2012). However, antibiotic manufacturing wastes contain much higher extractable antibiotic (>10 fold) than those reported in previous manure studies (Table 1; Dolliver et al., 2008; Bao et al., 2009; Arikan et al., 2006, 2007; 2009; Kim et al., 2012). At present, the effect of high concentrations of extractable antibiotics in antibiotic waste on microbial activities during composting is poorly understood. A comparison of the bacteria composition between the antibiotic-free and antibiotic-contaminated composts during the process of composting may contribute to a better understanding of the antibiotic degradation process. This process is associated with the succession of microbial communities during composting. Advanced molecular biological techniques, such as polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE), have proven to be useful for the detection of this succession (Cahyani et al., 2003; Nakasaki et al., 2009).

Kitasamycin is one of the antibiotics that is highly active against a wide range of Gram-positive bacteria and has been widely used in the rearing of food-producing animals to prevent and treat diseases (Jordan and Knight, 1984). It has been estimated that 6300 tons of kitasamycin waste are annually released from antibiotic manufacturers in China. This is of considerable concern because persistent antibiotic residues may result in the development and spread of antibiotic-resistant bacteria, which could undermine life-saving antibiotic therapies (Keen and Montforts, 2011; Zhu et al., 2013). In the present study, the kitasamycin manufacturing waste was mixed with dairy manure and sawdust at different ratios prior to composting. The objectives of this study were (1) to investigate the fate of kitasamycin during composting and to determine whether biological degradation contributes to the decline in extractable kitasamycin and (2) to evaluate the compost maturity and define the appropriate ratio of kitasamycin manufacturing waste to the dairy manure and sawdust in order to provide a feasible protocol for the utilisation of antibiotic manufacturing waste after composting.

Table 1
Selected characteristics of the three composting materials.

Characteristics	Dairy manure	Sawdust	Kitasamycin waste
pH	8.61	7.95	4.43
EC (dS cm^{-1})	0.63	0.86	3.98
Total N (%)	1.35	0.43	6.19
Total C (%)	43.3	55.2	38.8
Total P (g kg^{-1})	10.3	0.64	6.32
Total K (g kg^{-1})	32.4	44.1	2.62
Bulk density (kg m^{-3})	794	540	276
Moisture content (%)	73%	13.4%	18%
Extractable kitasamycin (mg g^{-1})	0	0	11.8
Total Cu (mg kg^{-1})	425	8.23	7.32
Total Zn (mg kg^{-1})	936	7.63	760

2. Materials and methods

2.1. Experimental set-up and sample collection

The sawdust and dairy manure were obtained from a livestock farm and sawmill in Lin-an City, China, respectively. The size of the sawdust ranged from 6 mm to 8 mm. The dairy manure was sourced from a non-antibiotic-dependent dairy farming and was scraped from concrete pens and stored in a covered area prior to use. The kitasamycin manufacturing waste was collected from the residual organic medium obtained after bacterial fermentation, solid–liquid separation and drying in Laiyi Bio-Technology Co, Xinchang City, China. Selected characteristics of the three materials used are presented in Table 1.

Composting experiments were performed in March of 2011, in a compost site with rectangle-shaped bays (50 m × 5 m × 1.2 m, length × width × height) in Lin-an Jinda Biological Science and Technology Co. Ltd, China (Fig. 1S). This composting mode has been generally applied in China. Similar experimental design can be found in a previous work (Liu et al., 2011). The kitasamycin manufacturing waste was mixed with dairy manure and sawdust were mixed at different ratios (by volume): M, 10/80/20; M2, 10/70/20; and M3, 30/50/20 (Figs. 1S and 2S). Water was added to each mixture, and the mixtures were mixed with a front-end loader to achieve moisture content of approximately 65% and were then subdivided the mixtures into different parts of the bays. The total volume of each mixture was 25 m^3 (5 m × 5 m × 1 m, length × width × height), and the total weights of M1, M2, and M3 were estimated to be 16.0 t, 16.2 t, and 16.6 t, respectively. The piles were aerated using a turning machine twice a week at 4:00 pm. The ambient temperature and mixture temperature in the centre of the composting material were recorded using a temperature meter every day at 9:00 am. The composting was performed for 40 days, and samples were taken at 0, 5, 10, 15, 20, 25, 30, 35, and 40 days at 9:00 am. Three replicates were collected from each mixture. To ensure representative sampling, three longitudinal sections (from 30, 50 and 70 cm from the base of mixture, respectively) were randomly dug, and the samples drawn from different sections of the same pile were mixed. A total of approximately 2 kg of samples were obtained using this sampling method. The collected samples were divided into two equal parts: one part was preserved at 4 °C for microbiological analysis, and the other part was air-dried, ground and sieved for analysis after air-drying.

2.2. Chemical analyses

The pH and electrical conductivity (EC) measurements were performed on aqueous suspensions of the fresh compost samples (1:10, W/V, compost/water ratio) using a pH electrode (MP511, Shanghai, China) and a conductivity indicator (DDSJ-308A, Shanghai, China), respectively. The compost samples were analysed for organic carbon through oxidation with potassium dichromate and for the total N using the Kjeldahl method. The results are expressed per dry weight of material.

To analyse the water-soluble fractions of the composting material, 15 g of fresh sample was extracted with 150 mL of distilled water (1:10 w/v ratio) by shaking for 24 h on a horizontal shaker at