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Microbial communities and greenhouse gas emissions associated with the biodegradation of specified risk material in compost

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ABSTRACT

Provided that infectious prions (PrP^{Sc}) are inactivated, composting of specified risk material (SRM) may be a viable alternative to rendering and landfilling. In this study, bacterial and fungal communities as well as greenhouse gas emissions associated with the degradation of SRM were examined in laboratory composters over two 14 day composting cycles. Chicken feathers were mixed into compost to enrich for microbial communities involved in the degradation of keratin and other recalcitrant proteins such as prions. Feathers altered the composition of bacterial and fungal communities primarily during the first cycle. The bacterial genera *Saccharomonospora*, *Thermobifida*, *Thermoactinomycetaceae*, *Thiohalospira*, *Pseudomonas*, *Actinomadura*, and *Enterobacter*, and the fungal genera *Dothideomycetes*, *Cladosporium*, *Chaetomium*, and *Trichaptum* were identified as candidates involved in SRM degradation. Feathers increased ($P < 0.05$) headspace concentrations of CH_4 primarily during the early stages of the first cycle and N_2O during the second. Although inclusion of feathers in compost increases greenhouse gas emissions, it may promote the establishment of microbial communities that are more adept at degrading SRM and recalcitrant proteins such as keratin and PrP^{Sc}.

1. Introduction

In response to the identification of bovine spongiform encephalopathy (BSE) in Canada in 2003, the Canadian Food Inspection Agency (CFIA) imposed an enhanced feed ban in July of 2007 to prevent the introduction of specified risk material (SRM) into the food chain. Specified risk material was designated to include the skull, brain, trigeminal ganglia, eyes, palatine tonsils, spinal cord and the dorsal root ganglia from cattle aged 30 months or older, as well as the distal ileum from cattle of all ages. These are tissues known to be at risk of accumulating infectious prion proteins (PrP^{Sc}). Currently, the majority of SRM in Canada is rendered and then disposed of in landfills. However, alternative on-farm disposal methods for SRM which are environmentally acceptable and economically feasible are desired. Composting may be an option as it has been shown to inactivate pathogens while producing a valuable fertilizer for agricultural crops (Hao et al., 2009).

The microbial consortia in compost could potentially carry out the biodegradation of SRM infected with PrP^{Sc}, due to the wide range of proteolytic enzymes it produces. Previous research has revealed bacterial species that produce proteases that are capable of degrading recalcitrant PrP^{Sc} (Hui et al., 2004; McLeod et al., 2004), some of which reside in compost (Ryckeboer et al., 2003). We have characterized actinobacterial communities that may degrade SRM in compost (Xu et al., 2011), and previously isolated a novel actinobacterium with the ability to degrade recalcitrant proteins in compost (Puhl et al., 2009). However, the nature of the various microbial communities associated with the degradation of SRM remains undefined.

Despite the obvious advantages of using composting for SRM disposal, addition of carcasses to manure during composting has been shown to increase greenhouse gas emissions (Xu et al., 2007). Hao et al. (2009) observed that methane (CH_4) emissions during co-composting of feedlot manure with cattle mortalities were higher than with slaughterhouse SRM wastes. During composting, methanogens are involved in the production and methanotrophs in the oxidation of CH_4. Heterotrophic methanogens produce CH_4 utilizing acetate, formate or methanol as a carbon source, whereas autotrophic methanogens reduce CO_2 to CH_4 (Ferry, 1993). Methanotrophs utilize methane monooxygenase to catalyze the oxidation of CH_4 to methanol, which is further oxidized to formaldehyde (Xin et al., 2004). However, the relative

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abundance of methanogens and methanotrophs during the composting of SRM has not been investigated.

Keratinases that have the capacity to degrade keratin in feathers may also exhibit activity against PrP^{Sc}, due to similarities in the structure of these proteins (Suzuki et al., 2006). Recent studies indicated that enrichment of a composting matrix with feathers not only produced an effective non-specific proteolytic activity early in the composting process, but also promoted the growth of keratinolytic fungi that degraded feathers in the later stages of composting (Korniłowicz-Kowalska and Bohacz, 2010; Bohacz and Korniłowicz-Kowalska, 2009). Specified risk material contains a large fraction of labile protein (8% fresh weight basis; McIlwain and Bachelard, 1985), so inclusion of feathers in compost may promote enzyme activity (i.e., non-specific proteolytic and keratinolytic) that could improve the degradation of SRM as well as PrP^{Sc}.

Our research group has reported that mixing of feathers with cattle manure in a laboratory-scale composter increased total N content though enhanced SRM degradation, but did not alter the physicochemical properties of compost (Xu et al., 2013). Hence, we hypothesized that inclusion of feathers may alter the composition of the microbial community in a manner that enhances SRM degradation in compost. The objectives in this study were to examine the composition of bacterial and fungal communities degrading SRM in compost with and without feathers. Furthermore, emissions of greenhouse gases (i.e., CH_4 and N_2O) and the related abundance of methanogens and methanotrophs were also investigated.

2. Materials and methods

2.1. Composting setup and sampling procedure

Passively aerated laboratory-scale composters were used as previously described (Xu et al., 2010). Triplicate composters were filled with matrices of either control compost (35 ± 0.1 kg fresh feedlot beef manure, 74.8% moisture; 3.5 ± 0.1 kg white spruce (*Picea glauca*) wood shavings) or feather compost (34.2 ± 0.1 kg beef manure; 3.5 ± 0.1 kg wood shavings; 0.8 ± 0.1 kg chicken (*Gallus gallus*) feathers). Compost substrates were prepared by mixing the initial materials in a mortar mixer (12S; Crown construction equipment, Winnipeg, Canada), resulting in 66.7% moisture in the control compost (C/N ratio = 22.2) and 65% moisture in feather compost (C/N ratio = 17.5).

Fresh bovine brain tissues (SRM) from mortalities under 30 months of age were obtained from a nearby slaughterhouse. Bovine brain tissues (50 ± 0.1 g; wet basis) were weighed and sealed in 140 × 90 mm nylon bags (53 μm pore size; ANKOM Technology, Macedon, USA). The nylon bags were then placed within larger polyester mesh bags (200 × 200 mm; 5 mm pore size) with 400 g of freshly mixed compost substrate. Polyester twine was attached to each mesh bag to enable recovery of the material during composting. As each compost vessel was filled, four mesh bags were placed at 0.3 m below the top of each composter. One bag per treatment was randomly collected after 7 and 14 days. After collecting the bags on day 14, composters were emptied and the contents were mixed using a specific shovel for each treatment. During the mixing process, water was added to the material to return it to the moisture level measured prior to initiation of composting. The moistened mixture was returned to the original composter for a second heating cycle. As the composters were refilled, the remaining two mesh bags containing a mixture of SRM and composted manure from the first heating cycle were returned and placed in each composter in the same manner as for the first cycle. In the second cycle, one of the remaining two mesh bags was collected after 21 and 28 days, respectively.

Triplicate samples of the initial mixed matrix and fresh SRM were collected at day 0. Decomposed SRM and compost samples were collected from each mesh bag after sampling on days 7, 14, 21, and 28. All samples were freeze-dried after collection and used for subsequent DNA extraction.

2.2. Gas collection and analysis

Changes in CH_4 and N_2O concentrations (ppm) in the composter headspace were used for the qualitative estimation of CH_4 and N_2O emissions from passively aerated composters. For gas collection, a flexible polyvinyl chloride tube (3.2 mm diameter; Nalgene, New York, USA) was inserted into each composter headspace through a hole drilled in the composter sidewall. One end of the tube was placed above the center of the compost matrix within the composter, while the other end, protruding out of the composter was sealed using an air-tight tube fitting (Swagelok, Medicine Hat, Canada). Gas samples (11 ml) were extracted from the tube fitting using a polypropylene syringe and then injected into 5.9-ml, pre-evacuated, septum-stoppered vials (Exetainer; Labco Limited, Buckinghamshire, UK). Gas samples were collected from each composter three times daily at 4 h intervals and analyzed for CH_4 and N_2O concentrations using a gas chromatograph (Varian 450; Varian Instruments, Walnut Creek, USA) equipped with flame ionization and electron capture detectors. Oxygen concentration (ppm) in the compost was measured twice daily using an oxygen monitor (Model OT-21; Demista Instruments, Arlington Heights, USA) at the same depth as the mesh bags were implanted.

2.3. DNA extraction

Prior to DNA extraction, freeze-dried SRM (n = 27) and compost (n = 30) samples collected at days 0, 7, 14, 21, and 28 were ground using a Ball Mill (MM200; Retsch GmbH, Haan, Germany). Subsequently, DNA was extracted from 100 mg of each sample by QIAamp DNA stool mini kit (Qiagen, Toronton, Canada). Extracted DNA was quantified using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, USA) and then stored at −20 °C prior to further PCR–DGGE and qPCR analyses.

2.4. PCR–DGGE and sequencing analysis

To decrease the variability within a given treatment, an equal amount of DNA extracted from replicate samples at each sampling date was pooled (Xu et al., 2011), resulting in a total of nine SRM and 10 compost DNA samples used for the subsequent PCR–DGGE analysis. All PCR amplifications contained 1 × HotStarTaq *Plus* DNA Master Mix (Qiagen), 0.2 μM of each primer in a final volume of 50 μl. Amplification was conducted using a thermal cycler (Mastercycler ep*gradient*; Eppendorf, Hamburg, Germany). Partial fragments of the bacterial 16S rRNA gene were amplified using primers F984GC and R1378 (Heuer et al., 1997; Table 1). Each reaction contained 40 ng of template DNA and the cycling conditions consisted of 95 °C for 5 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 62 °C, 2 min at 72 °C, and final extension for 10 min at 72 °C. For fungi, partial fragments of the fungal 18S rRNA gene were amplified using nested PCR. In the first-round PCR, template DNA (100 ng) was amplified by primers EF4 and Fung5 (Marshall et al., 2003 and Table 1) with conditions of 95 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C, 1 min at 72 °C, and final extension for 5 min at 72 °C. In the nested step, amplification was conducted with primers EF4 and NS2GC (Marshall et al., 2003 and Table 1) in the same manner as described above in the first-round PCR. The PCR products were visualized on a 1.0% (w/v) agarose gel before DGGE analysis.

Bacterial and fungal PCR products were loaded onto 6% and 7.5% polyacrylamide gels in 1 × TAE buffer at 60 °C using a DCode™ Universal Mutation System (Bio-Rad, Hercules, USA). For bacteria,