Can Composting Solve Specified Risk Material Issues?

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What Are Specified Risk Materials?

Prion diseases, i.e., transmissible spongiform encephalopathies (TSE), are a group of fatal neurodegenerative diseases including scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob disease (CJD) in humans.

As of February 2014, 19 cases of BSE have been confirmed in Canada and 4 cases in the U.S. The occurrence of BSE in North America has resulted in over an $11 billion loss to the cattle industry (Le Roy et al., 2006; Coffey et al., 2005). Due to the identification of 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 materials (SRM; e.g., spinal cord, skull, brain, vertebral column, eyes, tonsils, trigeminal and dorsal root and distal ileum) into the food chain. If these tissues are not removed from the carcass, then the entire carcass is designated as SRM. The U.S. government subsequently passed a similar regulation in 2008. It is estimated that there are 250,000 tonnes of SRM generated in Canada annually with 74,000 tonnes of this originating in the province of Alberta (Gilroyed et al., 2010; AARI, 2005). The cervid industry in Canada is much smaller, but still with 145,000 animals and an overall mortality rate of 5.7% (Canadian Cervid Alliance, 2009; Haigh et al., 2005), it generates significant quantities of SRM. Substantial quantities of SRM are also generated as a result of road kills in the United States. For example, in New York, over 25,000 road-killed deer and other animals need to be disposed of annually by the State Department of Transportation (Bonhotal et al., 2007). Proper disposal of these carcasses and associated SRM is important for North America to maintain its TSE controlled status within the World Organisation for Animal Health and control the transmission of TSE diseases among ungulates.

Currently, the majority of SRM are rendered, dehydrated, and disposed of in landfills in Canada and the U.S. Even though this approach is preferable to natural decomposition, it is still environmentally questionable as prions can likely remain stable in landfills for decades. Moreover, the large geographical distribution and transportation distances required to gather livestock and road kills, makes rendering or incineration often impractical as a disposal method for SRM. Therefore, alternative more economical and adoptable methods of SRM disposal are needed to dispose of the SRM generated from sheep, cattle, and cervids, meat processing plants and road kills. Use of on-farm composting for SRM disposal is of interest as it is a relatively simple procedure and environmentally sound. Moreover, composting has the advantage of being comparatively low cost and generating a final product that can be used as a fertilizer and valuable soil amendment.

Previous Investigations on SRM Composting

Composting of SRM and animal carcasses has been investigated using windrows, static piles, and bins or vessels for poultry, swine, sheep, deer, and cattle. Our previous studies using a ratio of 5 parts feedlot manure to 1 part cattle mortalities in a windrow composting experiment demonstrated that <1% of residual bone from cattle remained in the cured compost (Stanford et al., 2009). Sheep carcasses including keratinized wool were

completely degraded in composting bins in which temperatures of over 131°F (55°C) were maintained for a period of 41 days (Stanford et al., 2000). Moreover, more than 90% dry matter of cattle brain tissues and 80% dry matter of cattle hoof decomposed after 7 and 56 days, respectively, in biosecure compost piles (Xu et al., 2009a). In the same compost piles, a 99% reduction in genomic DNA of composted cattle tissue was observed after 147 days of composting, suggesting almost complete decomposition of soft carcass tissues (Xu et al., 2009b). Therefore, composting, when done with consideration for design, layout, monitoring, maintenance, and environmental impacts, has potential as an efficient and safe method of disposing of SRM.

Physicochemical Parameters Required for SRM Composting

A number of factors can affect the degradation of organic matter in compost. However, temperature, moisture, oxygen concentration, porosity, pH, and carbon to nitrogen (C:N) ratio are among most influential parameters to consider when composting SRM. In practice, thermophilic composting (≥131°F or 55°C) is recommended for disposal of SRM. Our previous research (Xu et al., 2009a) showed that the majority of SRM (>90%) was degraded within the first 7 days of thermophilic composting. However, excessively high temperatures, i.e., >158°F or 70°C, are difficult to achieve and can actually reduce the diversity of bacteria that participate in the composting process (Tkachuk et al., 2014), a factor that could limit the diversity of substrates degraded during the latter stages of composting

Compost moistures ranging from 60 to 80% are desirable for SRM composting (Ahn et al., 2008). However, directly applying water to SRM compost is not recommended due to the risk of generating leachate that could transport infectious pathogens into the surrounding environment. A more proper approach to optimizing moisture during composting is by measuring water activity (aw), which is the amount of free water available to microbial populations. Water activities ≥0.9 for bacteria and ≥0.7 for fungi can ensure sufficient water is available to sustain microbial activity during composting (Reuter et al., 2010). Adequate oxygen concentration also promotes the activity of aerobic microorganisms and should at least be ≥5% with levels ≥10% being even more desirable for SRM composting. In practice, SRM compost can be transferred from a primary compost pile to a secondary compost pile or bin to ensure adequate aeration for SRM decomposition (Keener et al., 2000).

In addition, the size of compost substrate ranging from 3 to 50 mm (<2 inches) with C:N ratios of 20:1 to 35:1 is recommended for efficient SRM composting (Rynk, 1992; Carr et al., 1998). In this manner, whole carcasses can be composted within this optimized matrix, eliminating the need to split up carcasses, a practice that can also increase the likelihood of disseminating infective agents. As SRM possess levels of nitrogen and moisture outside the optimal ranges, bulking agents such as wood shavings, wood chips, sawdust, post peelings, and crop residues can be added to increase compost porosity and carbon content. Mixing carbonaceous materials with SRM at a ratio of 1:1 (v v-1) can create a suitable matrix for SRM windrow or pile composting (Kalbasi et al., 2005).

Compost pH can affect the growth of microorganisms and the inactivation of pathogens within SRM compost. Typical pH in compost is a range of 6.5 to 10 (Langston et al., 2002; Xu et al., 2009a). However, controlling pH during SRM composting process is not recommended. More parameters or factors, such as weight of SRM or carcasses and season of the year can also affect the decomposition of SRM in compost.

Necessity for Studies on Fate of Prions during Composting

Composting is particular attractive as the temperatures during the process (≥131°F or 55°C) are sufficient to inactive a wide variety of bacterial pathogens, including Salmonella, VTEC *Escherichia coli*, Campylobacter, Clostridium and Listeria, the cysts/oocysts of the zoonotic parasites, Giardia and Cryptosporidium, and viruses responsible for foot and mouth disease, avian influenza, and Newcastle disease. However, safe disposal of SRM or carcasses possibly infected with TSE in a manner that inactivates infectious prions has

proven challenging as prions resist most conventional means of disinfection. Several microbial proteinases exhibit the ability to degrade infectious prions (Table 1) and many of these proteolytic enzymes are produced by microorganisms frequently found in compost. Furthermore, the high temperatures 122 to 140°F (50 to 60°C) and pH (>8) achieved during composting promotes the denaturing of proteins and their susceptibility to enzymatic degradation. Additionally, the duration that prions could be exposed to enzymatic activity could be considerable (i.e., weeks to months) further promoting protein degradation. To date, a single study has shown that scrapie prions were degraded to below detectable levels by Western blot analysis after 108 to 148 days of composting (Huang et al., 2007). Therefore, to adopt this practice as a method for SRM disposal, confirming the inactivation of infectious prions during composting has become increasingly important.

Table 1. Enzymes capable of degrading infectious prions.

Source enzymes	Source microbes	Reaction conditions	References
MSK 103 serine protease	*Bacillus licheniformis*	50°C, pH 9	Yoshioka et al., 2007
Proteinase K	*Tritirachium album*	50°C, pH 7-8	Langeveld et al., 2003
PWD-l keritanse	*Bacillus licheniformis*	50°C, pH 7-8	Langeveld et al., 2003
Keratinolytic protease	*Nocardiopsis* sp.	50°C, pH 11	Mitsuiki et al., 2006
Keratinolytic protease	*Thermoanaerobacter* subsp.	60°C, pH 7	Tsiroulmikov et al., 2004
MC2 alkaline protease	*Bacillus lentus*	60°C, pH 8-12	Dickinson et al., 2009
Properase, protease M, Purafect Ox, Purafect	*Bacillus* sp.	60°C, pH 12	McLeod et al., 2004
Protease E, Protease F	*Thermus* sp.	80°C, pH 7	McLeod et al., 2004
Alkaline serine proteinase	*Streptomyces* sp.	60°C, pH 10	Hui et al., 2004

Composting Systems for Use

Laboratory-scale composters

Passively aerated laboratory-scale composters (Figures 1a and 1b) can be used for studies on the degradation of scrapie, CWD, and BSE prions under Level 3 biocontainment conditions. Our early work (Xu et al., 2013) demonstrated that mixing of feathers with cattle manure enhanced SRM degradation, likely by enriching for proteolytic bacteria capable of degrading recalcitrant protein within the compost matrix. Therefore, feedlot cattle manure can be used as the matrix for composting and feathers can be added to promote the degradation of recalcitrant proteins such as keratin and prions.

Our recent data (Xu, 2012) showed a ≥90% degradation of scrapie, BSE, and CWD prions after 28 days of composting using this laboratory-scale model and that this degradation was enhanced if chicken feathers were added to the composting matrix. These results support the contention that composting can degrade infections prions.

Field-scale composters

Due to the limited biomass, temperatures in laboratory-scale composter only stay elevated for a few days as compared to weeks or months in field-scale compost piles. It is likely that field-scale composting may result in even greater degradation of prions than lab-scale composting. To investigate this possibility, we used a biosecure field-scale composting structure as shown in Figure 2a to investigate the degradation of scrapie prions during composting.

Figure 1A.

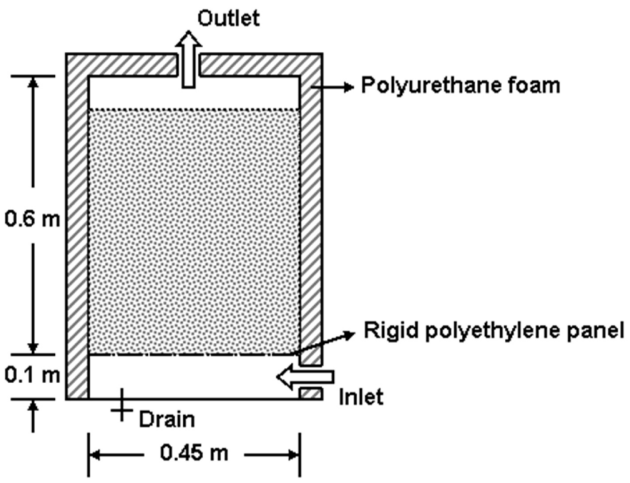

Figure 1B.

Figure 1. (a) and (b): 110 L passively aerated laboratory-scale composters used for studies of infectious prions under containment conditions. Schematics source: Xu et al., (2010a) (reprinted with permission); (c) and (d): procedures for sampling infectious prions during laboratory-scale composting process.

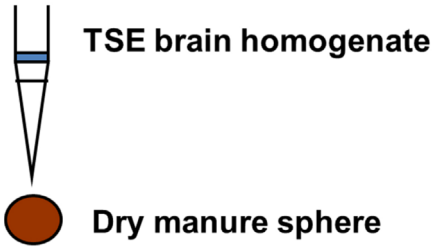

Figure 1C.

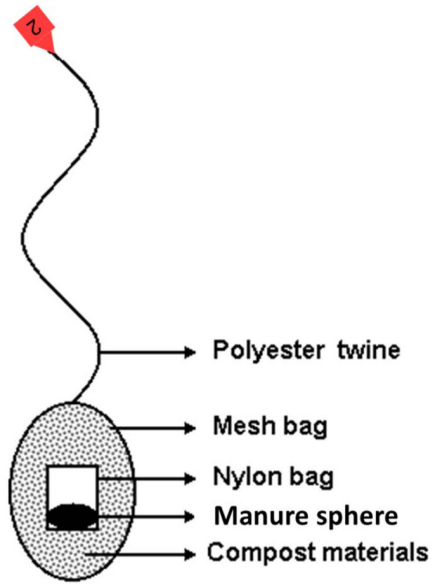

Figure 1D.

Compost matrices were contained in a bunker composter constructed of large straw bales with a final dimension of 25 m × 5 m with a height of 2.4 m (roughly 27 yd × 5.4 yd and 7.8 ft tall). Each bunker contained approximately 85,000 kg (90 tons) of compost and 16 mature cattle carcasses. Previous research demonstrated that stainless steel surfaces readily bind prions, a property that has resulted in the transmission of CJD among people as the result of contaminated surgical instruments (Zobeley et al., 1999; Flechsig et al., 2001). In our study, scrapie prions were bound to stainless steel beads and then placed within the compost structures at known locations in structures that allowed for their retrieval (Figure 2b; Reuter et al., 2008). Composted beads were collected subsequently and then inoculated directly into Syrian hamsters and hamsters were then monitored for developing TSE disease. Results indicated that infectivity of the prions was reduced by at least 100,000 times during composting.

Use of Final SRM Compost as a Fertilizer

In general, the final compost product is free of pathogens and plant seeds and sufficiently nutrient stable for land application. In the U.S., some states allow SRM composting. However, they do not have specific regulations about land application of the final product (Morse, 2009). Application of final SRM compost product onto land owned by the composting site could be allowed as long as it does represent a public health hazard (Bass et al., 2012). Currently, the use of final SRM compost as a fertilizer is restricted in Canada. The Canadian government does not recommend the direct spread of SRM compost onto pasture land or land that is used for crop production. However, final SRM compost could be used for land reclamation, if the land is not

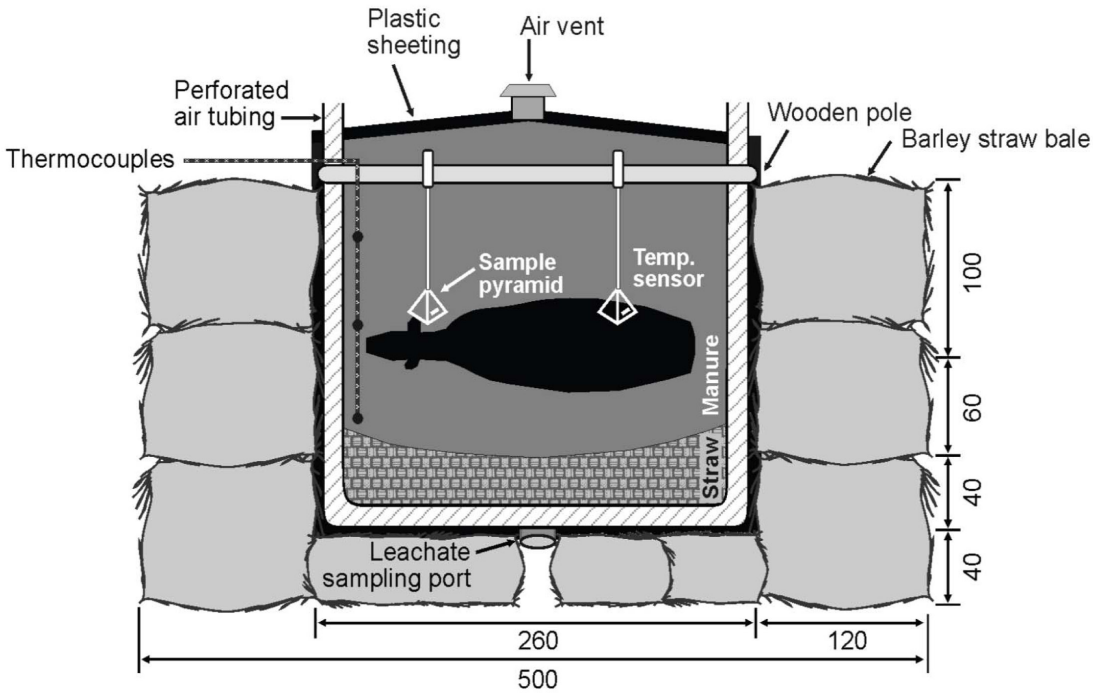

Figure 2A.

Figure 2. (a): a schematic of a biosecure field-scale composting structure designed for composting of cattle carcasses and scrapie prions. Units are in cm. Source: Xu et al., (2010b) (reprinted with permission). (b): a photo of Baker retrieval pyramid used for sampling scrapie prions during field-scale composting process.

Figure 2B.

grazed for at least 5 years (Hawkins, 2010). Moreover, removal of SRM compost from the farm of origin is allowable with a permit issued by CFIA but the direct sale of final SRM compost is prohibited (CFIA, 2011).

Summary and Future Research

Disposing of SRM through composting is not a common practice in Asia, Africa, or South America, probably due to the continued use of these tissues as human food or animal feed. However, this option is being re-investigated by intensive farming communities in North America and Europe. Therefore, inactivation of infectious prions in compost needs to be further investigated. Our laboratory-scale experiment suggests a greater than 90% reduction of TSE prions after composting for 14 to 28 days. Bioassays at field scale should be further employed to determine if composting can effectively inactivate these infectious proteins in bodily fluids or excrement. Moreover, the dilution of any remaining infectious particles upon the completion of

composting and their widespread dispersion during land application should further reduce the likelihood of hosts coming in contact with an infectious dose.

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