TECHNICAL NOTE

# A simple method for temporal collection of tissue and microbial samples from static composting systems

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Reuter, T., Xu, W., Alexander, T.W., Baker, B.C., Larney, F.J., Stanford, K. and McAllister, T.A. 2008. **A simple method for temporal collection of tissue and microbial samples from static composting systems**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **50***:* 6.17 - 6.20. Composting is being considered seriously as a means of disposal of livestock moralities in Canada, in part as a result of the enhanced feed ban implemented by the Canadian Food Inspection Agency in 2008. Information on the fate of pathogens and animal tissues during composting is essential to assess its suitability as a means of carcass disposal. This report describes a simple system for the implantation and temporal retrieval of microbial and tissue samples from field-scale static composting systems. A metal device, termed the Baker Retrieval Pyramid (BRP), with an internal volume of 3.6 L was constructed to enable the implantation and removal of samples, with minimal disturbance, from depths of 0.8 and 1.6 m within composting piles. Polyester bags with a pore size of 50 µm were placed in the BRP and used to introduce known amounts of *Escherichia* O157:H7, Newcastle disease virus, brain tissue, and keratin into the composting pile. Thermocouples were attached to the BRPs to enable temperature to be monitored directly at the site of implantation. The BRPs allowed for temporal removal of both microbial and tissue samples during a static composting period of 148 days. Microbial samples were shown to be non-viable within a period of three weeks and over 80% (dry matter basis) of brain tissue and keratin were decomposed after 148 days of composting. Whole carcasses were decomposed to a similar extent, suggesting that the microbial ecology within the BRP was similar to that of the outer composting matrix. It was concluded that the BRP system could be used for temporal examination of the decomposition of a variety of substrates and the fate of microorganisms, without altering the efficacy of static composting as a means of carcass disposal. **Keywords**: cattle mortality disposal, compost process, decomposition, monitoring, sampling device.

Le compostage est considéré comme une méthode prometteuse pour disposer des animaux morts dans les élevages au Canada; ceci est dû en partie à une règlementation plus stricte adoptée en 2008 par l'Agence canadienne d'inspection des aliments sur l'utilisation de produits animaux dans l'alimentation animale. Des informations sur la dégradation ou non des pathogènes et tissus animaux lors du compostage sont essentielles pour déterminer si le compostage peut constituer une méthode appropriée pour la disposition des carcasses animales. Cet article décrit un système simple pour l'implantation et le prélèvement périodique d'échantillons microbiens de même que de tissus provenant de systèmes de compostage statique à échelle réelle. Un appareil de métal possédant un volume interne d'environ 3.6 L, appelé la pyramide de prélèvements Baker (PPB), a ainsi été construit pour permettre le prélèvement d'échantillons non remaniés à des profondeurs de 0,8 et 1,6 m dans les tas de compost tout en causant un mélange minimal. Des sacs de polyester (taille de pores de 50 µm) ont été placés dans le PPB et ont été utilisés pour introduire des quantités connues de *Escherichia* O157:H7, de virus de la maladie de Newcastle, de tissus cérébraux et de kératine dans le tas de compost. Des thermocouples ont été fixés aux PPBs pour permettre l'enregistrement de la température directement au site d'implantation. Les PPBs ont permis le prélèvement périodique d'échantillons microbiens et de tissus durant la période de compostage statique de 148 jours. Les échantillons microbiens se sont avérés être non viables à l'intérieur d'une période de trois semaines et plus de 80% des tissus cérébraux (sur une base sèche) et de la kératine étaient décomposés après 148 jours de compostage. Les carcasses entières étaient décomposées de la même manière suggérant que l'écologie microbienne à l'intérieur du PPB était similaire à celle présente dans la matrice externe de compostage. Il a été conclu que le système de PPB pourrait être utilisé périodiquement pour examiner la décomposition d'une variété de substrats et la dégradation des microorganismes sans altérer l'efficacité du compostage statique comme moyen d'éliminer des carcasses animales. **Mots clés**: élimination des carcasses bovines, processus de compostage, décomposition, suivi du processus, appareil d'échantillonnage.

## INTRODUCTION

In Canada, recently passed legislation will expand restrictions on feeding ruminant by-products, including specified risk materials (e.g., brain, central nervous system tissues), to livestock. As such, the need to find alternative means of disposal of cattle mortalities and their by-products is increasing. Composting has been proposed as a viable means by which to dispose of livestock mortalities (Kalbasi et al. 2005). However, concerns remain over the biosecurity of mortality compost, particularly with regard to the extent of carcass degradation and the stability and potential survival during composting of pathogens such as *Escherichia coli* O157:H7, *Mycobacterium avium* and the prion protein responsible for bovine spongiform

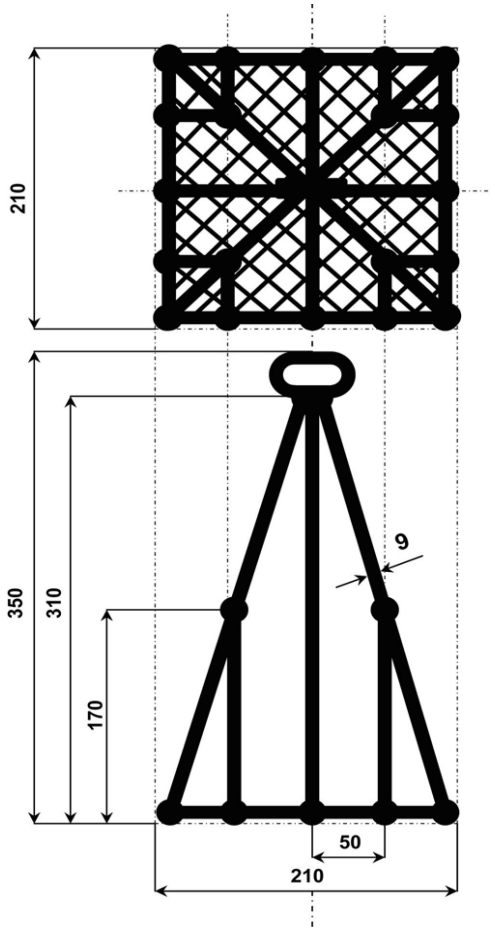


**Fig. 1. Engineering drawing for the construction of the Baker Retrieval Pyramid for the implementation and temporal removal of samples from field scale static composting piles (dimensions in millimeters).**

encephalopathy (Kalbasi et al. 2006). Determining the fate of tissues and potential pathogens during mortality composting is therefore essential for mortality compost to be acceptable as a soil amendment.

Static composting systems have great potential as an economical means of disposing of livestock mortalities (Fonstad et al. 2003; Glanville et al. 2006). These systems avoid the generation of aerosols during the composting process and thereby minimize the risk of dispersion of potentially infectious agents into the surrounding environment (Taha et al. 2006). However, collecting representative samples from the interior of static composting piles is difficult, because removal of manure to access the material to be sampled can affect resident microbial populations and alter the dynamics of the composting process (Curtis et al. 2005; Kalbasi et al. 2006). In order to evaluate the composting process and the degradation of potential risk material at different times and/or locations, a sampling device is required. The present paper describes a simple system developed for implanting and collecting, at desired intervals, microbial and substrate samples from the interior of static composting piles. The system was employed in a large field-scale composting system for disposal of cattle mortalities (Xu et al. 2008).

## MATERIALS and METHODS

### Pyramid construction

The system was designed around the construction of pyramidal steel cages, dubbed Baker Retrieval Pyramids (BRP), manufactured by seam welding in the design depicted in Fig. 1. The pyramidal shape was chosen to minimize tensile forces arising from resistance during extraction of the BRP from the compost pile. The base of the BRP was a 210 mm × 210 mm piece of 4-mm expanded metal mesh with 540-mm$^2$ trapezoidal spacing. Steel rods (228 mm length; 9 mm diameter) were affixed at each corner and positioned to converge at a point 310 mm above the base. A steel annulus was welded to the convergence point to enable attachment of retrieval chains to the top of the BRP. The BRP had an internal volume of 3.6 L, within which samples of interest could be placed. Each BRP was coated with inert Plastisol (Plastico Industries, Cambridge, ON) to minimize interaction between the sampling device and the composting environment (Fig. 2A).

### Pyramid application

Samples of brain tissue and keratin (5 g dry weight) were placed in 50 × 100 mm bags made of monofilament nitrogen-free polyester with a mean pore size of 50 µm (ANKOM rumen *in situ* bags, Ankom Technology, Macedon, NY) using an ANKOM heat sealer (model 1915). Microbial samples [*E. coli* O157:H7, $10^8$ CFU/g; Newcastle Disease virus, $\approx 10^6$ $ELD_{50}$/g (50% embryo lethal dose)] inoculated into manure (10 g) were placed in separate nylon bags. All bags were heat-sealed and one of each bag type was then placed within each BRP onto a 60-mm layer of loosely packed fresh manure that had been loaded manually into the base of the BRP (Fig. 2B). Additional fresh manure from the same source as that used to establish the static compost pile, was passed between the steel rods to surround the bags and secure them in position within the BRP (Fig. 2C). Thermocouples were affixed to each BRP and entwined with the retrieval chains for guidance out of the compost pile and connected to an externally located data logger. Thermocouples were extended into the interior of the pyramid so temperature was measured in the centre of the inner chamber.

### Pyramid placement and retrieval

A total of 48 BRPs were constructed and attached to zinc-plated steel chain (4.7 mm link i.d.; maximum tensile loading capacity 3.56 kN; Fig. 2) and placed in two replicate static composting piles (24 BRP/pile) each with a final mass of approximately 10 Mg and containing 16 feedlot cattle mortalities. Final dimensions of the structures were 25 m length × 5 m width × 2.4 m height. Large barley straw bales (2.6 × 1.2 ×0.8 m) were used to construct 1.2 m thick walls in a rectangular shape, and small straw bales (1 × 0.45 × 0.4 m) were employed to form a 0.4-m high floor. The BRPs were implanted in proximity above the carcasses in the pile at depths of 0.8 and 1.6 m below the initial upper surface. Free ends of the chains were tethered to cross-beams to maintain the chains in a vertical position until construction of the static compost piles was complete (Fig. 2D). Descriptive tags were affixed to the chains to facilitate identification of the BRP for retrieval purposes. Duplicate BRPs were removed from each depth within each of the composters on days 0, 7, 14, 28, 56, 112, and 147. Temperatures in BRPs positioned at 0.8 and 1.6 m were monitored by use of type T

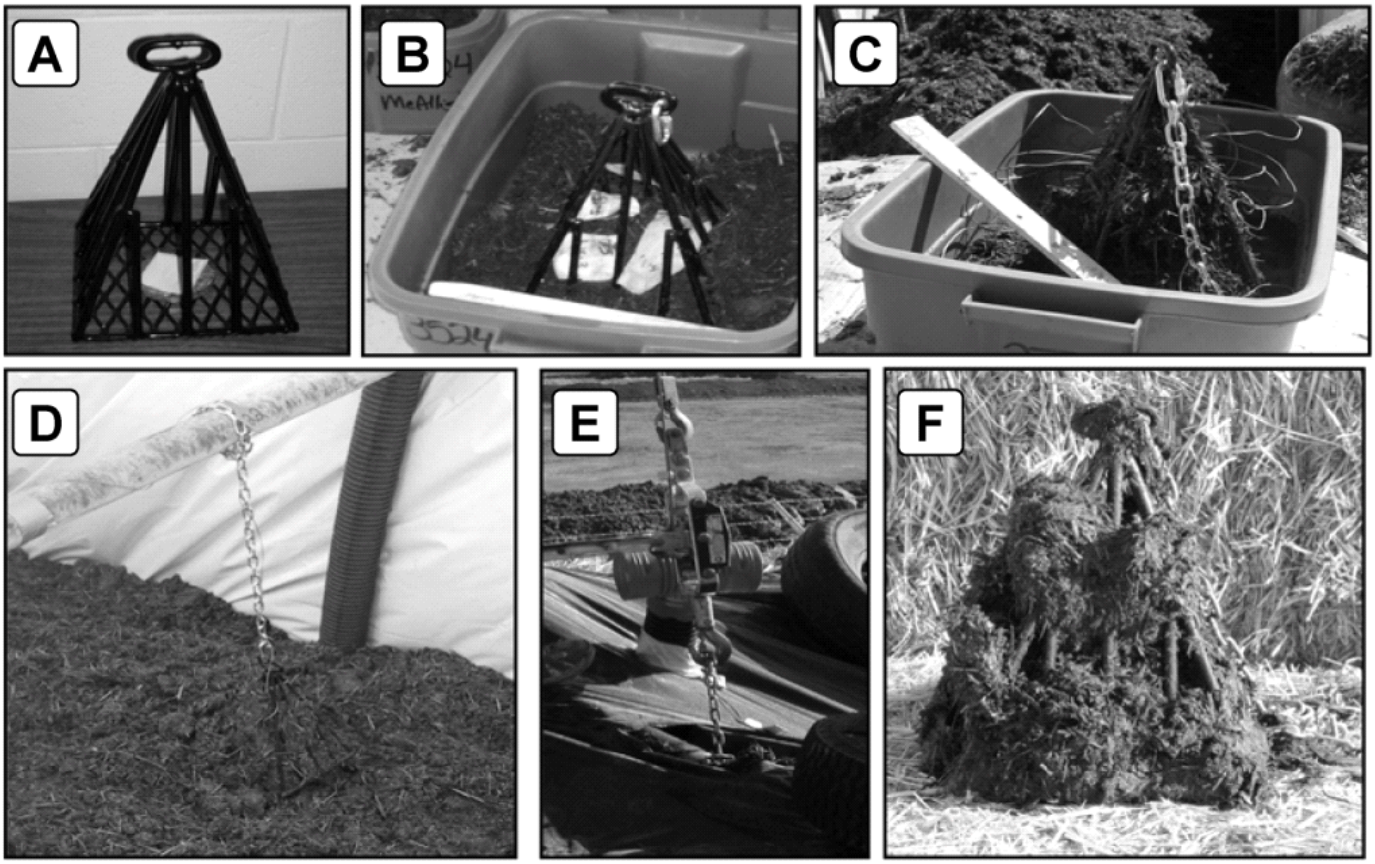


**Fig. 2. Application of sampling device in large scale compost piles: (A) Empty Baker Retrieval pyramid (BRP); (B) Polyester sample bags (50 µm pore size) placed onto a 60-mm layer of loosely packed fresh manure within the BRP; (C) BRP with added thermocouple wires and retrieval chain; (D) BRP being placed into field-scale static composting pile; (E) Retrieval of the BRP using a hand winch attached to a front end loader; (F) BRP after removal from a depth of 1.6 m from the static composting pile.**

thermocouples connected to a Campbell scientific CR10X data logger (Campbell Scientific, Logan, UT). Temperature was recorded hourly and averaged daily over the 147 days of composting. Stainless steel Hobo® Temp Data loggers U12-015 (Onset Computer Corporation, Cape Cod, MA) were placed inside the mouths of carcasses (n = 32; 16 carcasses × 2 structures) and set to record temperature twice daily. Inclusion of the Hobo® Temp Data loggers enabled comparison of temperatures inside and outside of the BRPs at similar depths.

## RESULTS and DISCUSSION

Pyramids were extracted from the compost pile at predetermined weekly intervals for the first month and monthly thereafter over the duration of the 147-day experimental period. BRPs were extracted from both compost piles using a hand winch attached to a front-end loader that was suspended over the composting pile (Fig. 2E). The BRPs were successfully removed from the compost piles at depths of 0.8 and 1.6 m. Although not directly measured, the force required to remove the pyramids became notably higher after two months of composting as the density of the matrix increased as a result of settling and decomposition of organic matter. Electrolysis of the chains was not noticeable, but deterioration of the zinc coated coupler links between the BRPs and chains was evident. The use of stainless steel coupler links with a tensile strength greater than 3.56 kN would likely be advisable if composting periods exceeded 150 days. In more recent experiments, stainless steel cables and clamps with a similar tensile strength have been used instead of chains (Personal communication: Dr. K. Ominski, Assistant Professor, Department of Animal Science, University of Manitoba, Winnipeg, MB). Overall, neither the configuration nor the integrity of the BRPs were affected by exposure to temperatures up to 65°C in either of the two static composting piles.

Temperatures differed ($P<0.05$) between 0.8 and 1.6 m depths with profiles in the 1.6-m BRPs similar to those generated by data loggers in the carcass layer (Xu et al. 2008). Table 1 shows the temperatures monitored at different sites and time points up to 54 days within both compost structures. The measured thermal profiles at carcass level were similar to the temperatures inside the BRPs at 1.6 m are an indication that the environment within the BRP was similar to that of the surrounding compost matrix. Higher temperatures at 0.8 m likely are a reflection of heat transfer within the composting structure.

Thermal and chemical conditions (e.g. pH ranged from 8.3 to 9.5) in the compost did not alter the inert plastisol coating which remained intact at both depths throughout the composting

**Table 1. Average temperatures (°C) from two compost piles at different time points (days) in pyramids positioned at depths of 0.8 and 1.6 m within static composting piles and anchored to cross beams.**

| Pile | Site | Days of composting | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 28 | 56 |
| East | 0.8* | 40 | 57 | 63 | 61 | 54 |
| | 1.6* | 40 | 45 | 48 | 52 | 51 |
| | Carcass** | 36 | 47 | 47 | 48 | 46 |
| West | 0.8* | 40 | 52 | 60 | 60 | 55 |
| | 1.6* | 40 | 44 | 49 | 52 | 50 |
| | Carcass** | 29 | 47 | 46 | 46 | 44 |

* Temperatures shown are averages of n = 12, 10, 8, 6, and 4 at days 1, 7, 14, 28, and 56, respectively.

**Average values are shown (n = 16). Carcasses were at approximately the same depth as the pyramids implanted at 1.6 m.

period (Fig. 2F). The polyester bags also exhibited no signs of deterioration as a result of being exposed to composting conditions for 147 days. The system enabled us to monitor bacterial viability and decomposition of different tissues over the entire experiment and demonstrated that both *E. coli* O157:H7 and Newcastle Disease virus were no longer viable after 21 days of static composting. Over 80% of the keratin and brain tissues placed in the polyester bags had decomposed by the end of the experiment. Sequential measurement of dry matter decomposition over time enabled rates of decomposition to be estimated for these tissues. Whole carcasses also exhibited substantial decomposition over the composting period, suggesting that the degree of microbial activity within the BRPs did not differ from that of the surrounding compost.

## CONCLUSIONS

The present procedure and engineered device enable monitoring of the composting process, decomposition of tissues, and the viability of microbes in a static composting system. The BRP device is inexpensive (≈$40 CAN per unit), simple to construct, and can be retrieved using readily available agricultural implements. Several parameters can be monitored readily within the device as an indicator of composting activity and efficiency. The implantation at 24 different sites within the composting pile provided information of spatial variation in temperature and decomposition within the composting pile. In addition to assessing the decomposition of tissues and pathogen survival, the system could be used to assess the rate of decomposition of other composting matrices such as straw, sawdust, or municipal waste.


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