Methane and carbon dioxide production from simulated anaerobic degradation of cattle carcasses

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Methane and carbon dioxide production from simulated anaerobic degradation of cattle carcasses

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Abstract
Approximately 2.2 million cattle carcasses require disposal annually in the United States. Land burial is a convenient disposal method that has been widely used in animal production for disposal of both daily mortalities as well as during catastrophic mortality events. To date, greenhouse gas production after mortality burial has not been quantified, and this study represents the first attempt to quantify greenhouse gas emissions from land burial of animal carcasses. In this study, anaerobic decomposition of both homogenized and unhomogenized cattle carcass material was investigated using bench-scale reactors. Maximum yields of methane and carbon dioxide were 0.33 and 0.09 m³/kg dry material, respectively, a higher methane yield than that previously reported for municipal solid waste. Variability in methane production rates were observed over time and between reactors. Based on our laboratory data, annual methane emissions from burial of cattle mortalities in the United States could total 1.6 Tg CO₂ equivalents. Although this represents less than 1% of total emissions produced by the agricultural sector in 2009, greenhouse gas emissions from animal carcass burial may be significant if disposal of swine and poultry carcasses is also considered.

Keywords: cattle carcass, burial, anaerobic decomposition, greenhouse gas production, methane, carbon dioxide

1. Introduction
Cattle and calf production is a significant industry in the United States, with approximately 94 million animals in production in 2010 and an estimated total value of over $77 billion dollars (USDA NASS, 2010a). States with significant cattle and calf production include Texas, with over 13 million animals, Kansas and Nebraska, each with approximately 6 million animals (USDA NASS, 2010a). Although reported routine mortality rates for cattle production facilities are relatively low (approximately 1.3%) (Loneragan et al., 2001), surveys from the United States Department of Agriculture indicated, on average, over 2.2 million deaths per year occur in the US at cattle and calf production facilities (USDA NASS, 2010b). In addition to routine mortalities, mass mortality events may occur due to weather-related stress or outbreaks of infectious disease.

Carcass management methods include on-site burial, composting, landfilling, rendering, and incineration, and these management strategies have been applied to both routine and catastrophic animal mortalities. Mortality composting has been successfully applied in both routine and emergency disposal of poultry and birds (Murphy and Handwerker, 1988; Blake and Donald, 1993; Carter, 1993; Bendfeldt et al., 2005a,b), and has been used for the disposal of livestock carcasses (Xu et al., 2009; Stanford et al., 2009). Although rendering is commonly utilized for disposal of cattle carcasses, Federal Food and Drug Administration rules, which took effect in October 2009, place restrictions on rendering for cattle over 30 months of age (Code of federal regulations, 2010). A lack of available incineration capacity in the United States, coupled with economic and technical limitations (Scudamore et al., 2002) make burial or composting attractive for cattle carcass disposal. Published guidance documents for US states with significant cattle industries typically include burial as a common on-site disposal option (NDEQ, 2009; TCEQ, 2005; KDHE, 2003, 2004). Some states, including Nebraska and Kansas, have implemented carcass weight limitations for composting (Nebraska Administrative Code, 2003) or recommend on-site burial for disposal of cattle carcasses in instances of routine or catastrophic animal mortalities (KDHE, 2003, 2004). Previously, outbreaks of infectious disease have required acute disposal of large numbers of carcasses. The bovine spongiform encephalopathy (BSE or ‘mad cow’) outbreak in the United Kingdom has generated 180,000 confirmed and over 2 million suspected BSE cases since 1985 (Smith and Bradley, 2003), while over 6.5 million animal mortalities were produced in 2001 during a foot and mouth disease (FMD) outbreak, with the majority being disposed of via landfills or land burial (Scudamore et al., 2002).

There have been a limited number of studies evaluating the environmental impacts of various animal mortality disposal options. Soil and groundwater contamination attributable to cat-
tle carcass composting or poultry and cattle carcass burial were reported in previous studies (Glanville et al., 2009; Ritter and Chirnside, 1995; Pratt, 2009). There have been very few studies evaluating air quality impacts from animal mortality management. A field study conducted by Xu et al. (2007) determined that co-composting of 24 cattle mortalities and manure resulted in production of 77.9 kg C Mg⁻¹ (0.145 m³ kg⁻¹) and 3.2 kg Mg⁻¹ (0.006 m³ kg⁻¹) of CO₂ and CH₄, respectively. These data indicate that animal mortality burial can impact air quality due to anaerobic decomposition. Because mortality management is typically conducted on-site with limited regulatory oversight, quantifying the potential environmental impacts of these activities is necessary to assess the risk to environmental health and to develop appropriate strategies to minimize emissions.

In this study, the air quality impacts of land burial of cattle carcasses were investigated using laboratory-scale anaerobic decomposition reactors. The objective of this study was to quantify the methane and carbon dioxide production from decomposition of cattle carcasses after land burial under the most favorable conditions. Leachate quality was also monitored by determining pH and COD throughout the decomposition process.

2. Materials and methods

2.1. Materials

Approximately 20 kg of cattle carcass material was collected downstream of the initial grinder at a rendering facility in Nebraska. The collected material was a mixture of approximately 85% carcass tissue composed of muscles, organs, and other tissues from cattle that had been dead for about 1 day, and around 15% scraps from meat industrial processes such as waste materials left over from butchering. This material was transported to seed the reactors with a source of anaerobic microorganisms. Both bulk and homogenized materials were used in reactor experiments. Homogenized material was ground into particles approximately 6 mm diameter using a food processor. Fat and protein levels in homogenized pre- and post-decomposition materials left over from butchering. This material was transported to seed the reactors with a source of anaerobic microorganisms. For chemical oxygen demand (COD), leachate samples were digested in pre-prepared COD digestion tubes (Hach Company, Loveland, CO.) and then heated to 150 °C for 2 h followed by colorimetric determination at 620 nm. Leachate pH was measured using a pH meter (Oakton pH 510 series) calibrated with standards at pH 4, 7 and 10 before each use.

2.2. Reactor design and operation

The reactor system was constructed of a 2 L polypropylene container (Fisher Scientific), a Tedlar gas collection bag (Pollution Measurement Corporation, Oak Park, IL), and a leachate recycle reservoir (Baxter Healthcare Corporation, Deerfield, IL), which were connected with PVC tubing and nylon fittings. This reactor system has been used in previous studies investigating anaerobic decomposition of solid waste and has been determined to accurately simulate decomposition in a landfill or land burial scenario (Eleazer et al., 1997; Staley et al., 2006). Initially, 860 g of bulk carcass material and 800 mL of deionized water was placed in the reactor (reactor A). The DI water was added to provide sufficient moisture from the start of the experiment to stimulate degradation reactions. Due to operational problems with clogging of leachate tubes and compaction of the carcass material, subsequent reactors were operated with size-reduced carcass material mixed with dry hay (grass) to provide structure. Additionally, less carcass material was used due to the reactor capacity limits. Therefore, in reactor B through D, a 5-cm deep layer of non-carbonate stone was placed in the bottom of the reactors to prevent clogging of the reactor tubing and 380 g of a mixture of homogenized carcass material and dry hay at an average mass ratio of 10:1 was placed in the reactor. Due to the lack of gas production in reactors seeded with deionized water, in reactor B through D, swine lagoon wastewater was diluted 4:1 (v/v) with deionized water to a total volume of 800 mL and was used to seed the reactors with a source of anaerobic microorganisms. Control reactors (reactor C1 to C3) containing only the waste-water seed and corresponding mass of hay were also operated to quantify any gas production due to these components.

Reactors were placed in a temperature-controlled room at 37 °C for up to 630 days. Leachate was recycled through the reactors every 1–2 days for the first 2 months and weekly thereafter. Acid (18.4M H₂SO₄, or 12.1 M HCl) or base (1 M NaOH) was added to each reactor after each pH measurement as needed to maintain pH between 6.8 and 7.5. A 20 mL leachate sample was obtained weekly and frozen at −20 °C for COD analysis. Gas produced in the reactor was collected in gas sampling bags and analyzed for gas composition and volume every 14 days.

2.3. Analytical methods

Gas concentrations (CH₄, CO₂, O₂, and N₂) were measured as described previously (Wang et al., 1997). In brief, gas composition was measured by a gas chromatograph (SRI 8610C) equipped with a CTR-1 double packed column, a thermal conductivity detector (TCD), and an on-column injector. The column oven, TCD, and injector were operated at 37, 142, and 65 °C, respectively. Nitrogen was used as a carrier gas. Chromatographs were quantified using a four-point standard curve. Gas volume was measured by evacuating the gas bag into a 3.85 L cylinder and monitoring pressure changes. Measured gas volumes were adjusted to dry gas at standard temperature and pressure (0 °C and 1 atm). Leachate analysis was performed following standard methods. For chemical oxygen demand (COD), leachate samples were digested in pre-prepared COD digestion tubes (Hach Company, Loveland, CO) and then heated to 150 °C for 2 h followed by colorimetric determination at 620 nm. Leachate pH was measured using a pH meter (Oakton pH 510 series) calibrated with standards at pH 4, 7 and 10 before each use.

3. Results

3.1. Gas production

Methane (CH₄) and carbon dioxide (CO₂) production rates for reactor A through D are shown in Figures 1 and 2, respectively. Variability in production rates for both CH₄ and CO₂ were observed, both over time and between reactors. This variability
may be due to the complex nature of the substrate or incomplete homogenization of the carcass material within each reactor. The average rates of CH$_4$ and CO$_2$ production were approximately 0.58 and 0.17 L kg$^{-1}$ d$^{-1}$ (on a dry weight basis), respectively, for the homogenized carcass material. Approximately five times lower average production rates of CH$_4$ and CO$_2$ were observed in reactor A which contained un-homogenized material and a DI water seed. Observed gas composition in all reactors was approximately 65% methane and 20% carbon dioxide (Figure 3). Minimal gas production was observed in the control reactors over time and the average ultimate yields of CH$_4$ and CO$_2$ in these reactors were measured as 6 mL and 10 mL, respectively (data not shown). Average concentrations of oxygen (O$_2$) and nitrogen (N$_2$) were approximately 5% and 15%, respectively, in reactor B through D (Figure S1). The presence of O$_2$ in the reactors is likely due to a small degree of air infiltration into the reactor systems during leachate sampling and gas bag replacement. Ultimate yields of methane and carbon dioxide were 0.05 m$^3$ kg$^{-1}$ and 0.02 m$^3$ kg$^{-1}$ for reactor A (un-homogenized material), compared with an average yield of 0.33 m$^3$ kg$^{-1}$ and 0.09 m$^3$ kg$^{-1}$ on a dry weight basis for reactor B through D (homogenized material) (Figure 4). The extent of degradation which occurred in the reactor can be demonstrated by the protein, fat, and carbohydrate–content before and after degradation. On average, a total of 212 g fat and 22 g protein were utilized in the degradation reactions with an average starting mass of 350 g (Table 1).

3.2. Leachate characteristics

pH and chemical oxygen demand (COD) content of the leachate is shown in Figures 5 and 6, respectively. The pH of the leachate production rates of control reactors were correspondingly subtracted from those of reactors B to D.

in the reactors was relatively acidic for the first 50 days, but remained consistently between 7.2 and 8.0 prior to neutralization after 80 days of operation (Figure 5) whereas leachate pH of control reactors remained neutral after 350 days (Figure S2). COD concentrations were consistent between reactors containing bulk (un-homogenized) and homogenized carcass materials. Maximum leachate COD concentrations were observed between 20 and 70 days, and ranged from 40 to 65 g L$^{-1}$ (Figure 6). COD concentrations began to decrease around 80 day of operation and were $<$1500 mg L$^{-1}$ by 300 days (430 days for reactor A).

4. Discussion

4.1. Effect of material treatment on gas production

Both un-homogenized and homogenized carcass material were used in these bench reactor studies. Homogenized materials were utilized due to observed issues with compaction of un-homogenized materials and difficulty with leachate recirculation. Homogenization of solid materials and seeding reactors with an anaerobic inoculum (swine wastewater in this case) resulted in increased gas production rate and yield. Intact cattle mortalities will initially provide less surface area for decomposition than the homogenized materials used here. Reactor A (initially seeded with DI water) produced less methane and CO$_2$ compared with reactor B through D. Gas production observed in reactor A is likely due to the existing bacterial population within the material. The source of the bacterial seed also likely influenced gas production. Methane production rates differed substantially over time in individual reactors and between reactors. This variability may be due to the heterogeneous nature of the starting carcass material or inconsistent size reduction between samples. Multiple peaks in gas production rates were observed, consistent with previous reports of anaerobic degradation in food waste (Eleazer et al., 1997). This is not surprising given that multiple substrates are likely present in the heterogeneous carcass material with varying degrees of degradability.

4.2. Comparison of gas production between cattle carcasses and municipal solid waste (MSW)

Unlike municipal solid waste (MSW) that contains mainly cellulose and hemicellulose (Eleazer et al., 1997; Wu et al., 2001), the carcass material utilized in this study was predominantly fat and protein (Table 1). Gas production rates and gas composition observed in this study were consistent with those observed previously in studies of MSW degradation (Eleazer et al., 1997; Barlaz et al., 1989). However, peak methane production rates...
Table 1. Composition of fresh and degraded carcass material of reactors B to D.

<table>
<thead>
<tr>
<th>Component</th>
<th>Average mass of starting material (g)</th>
<th>Average mass of final material (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>89a ± 8.4b</td>
<td>42 ± 2.6</td>
</tr>
<tr>
<td>Protein</td>
<td>23 ± 2.1</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td>Fat</td>
<td>217 ± 20.4</td>
<td>5 ± 3.0</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4 ± 0.3</td>
<td>2 ± 2.8</td>
</tr>
<tr>
<td>Ash</td>
<td>1 ± 0.1</td>
<td>0 ± 0.0</td>
</tr>
</tbody>
</table>

a. Average component weight of reactors B to D.
b. Standard deviation of component weight of reactors B to D.

Using the results of this study, the methane and carbon dioxide yields of a typical cattle carcass can be estimated. Starting with an average live weight of 500 kg, and subtracting moisture (approximately 70% by weight) yields a final dry weight of 150 kg. The average ultimate yields of CH₄ and CO₂ obtained in this study were 0.33 and 0.09 m³ kg⁻¹ dry weight, respectively. Thus, estimated ultimate gas production for a single carcass of average weight is 50 m³ (36 kg) methane and 14 m³ (28 kg) carbon dioxide at standard temperature and pressure. This corresponds to 720 kg CO₂ equivalents. Based on these calculations, an estimated annual production of carbon dioxide emissions contributed by land disposal of 2.2 million mortalities would be approximately 1.6 Tg CO₂ equivalents. In 2009, the US EPA reported emissions of 419.3 Tg CO₂ equivalents from the agricultural sector (EPA, 2011), therefore emissions from cattle carcass burial accounts for less than 1% of total emissions due to agricultural operations. It should be noted that under typical disposal scenarios, carcasses will be covered with a layer of soil, and partial oxidation of methane to carbon dioxide would occur in this soil layer (Park et al., 2008; Hilger et al., 2000). Any conversion of methane to carbon dioxide via methane oxidation in cover soils is not considered in the results presented here. Although the emissions from cattle carcass disposal is small relative to other sources of emissions from the agricultural sector, greenhouse gas emissions from animal carcass burial may be considerable if other types of animal production (poultry, swine) are also considered. Although CH₄ and CO₂ emissions from cattle burial were specifically quantified in this study, there may be other fugitive emissions of N₂O or NMVOCs resulting from animal burial, which could be of additional concern. Emissions of other gases were not specifically evaluated in this study, but may warrant further investigation. This study represents the first attempt we are aware of to quantify greenhouse gas emissions from animal carcass burial. Data generated in this study can be used to inform environmental risk assessments or other studies of the environmental impacts of animal production.

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**Appendix A. Supplementary data** — Supplementary data associated with this article follows the References.

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Supporting Information

**Figure S1.** N$_2$ and O$_2$ composition of gas produced by reactors B to D

**Figure S2.** Leachate pH of control reactors prior to neutralization
Figure S1. $N_2$ and $O_2$ composition of gas produced by reactors B to D

Figure S2. Leachate pH of control reactors prior to neutralization