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Effect of low initial envelope material moisture content on swine tissue degradation in layered livestock mortality composting systems

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**Effect of low initial envelope material moisture content on swine tissue degradation in
layered livestock mortality composting systems**

by

Benjamin Paul Crawford

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Industrial and Agricultural Technology

Program of Study Committee:
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Ames, Iowa

2009

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ABSTRACT

A 12-week laboratory study was conducted to assess the minimum initial moisture content of compost bulking (envelope) materials necessary to sustain desired heat production and completion of carcass decomposition during emergency composting of swine carcasses. During full-scale field testing of a semi-enclosed emergency composting procedure, first developed and used by the Canadian Food Inspection Agency during an avian influenza outbreak in 2004, the ability of potential emergency compost envelope materials were evaluated on their ability to achieve elevated temperatures ($>55^{\circ}\text{C}$) necessary to inactivate pathogens and successfully decompose carcasses under a variety of initial moisture conditions, during cool and warm season trials. Two-way ANOVA modeling of results showed that envelope material type and envelope material initial moisture content had a significant effect on internal temperature production, with silage (52.5°C) having the highest predicted internal temperature production. Counter to these findings, envelope material and initial moisture did not have a significant effect on carcass decomposition, and silage (72%) had the lowest predicted carcass decomposition. To corroborate and better understand these unexpected field test results, laboratory tests were carried out using the same envelope materials, under conditions of identical external temperature and a range of initial moisture contents. Results from the laboratory test showed that, when pre-moistened adequately, total oxygen uptake (and heat production potential) in ground cornstalks and similar materials were significantly higher (48 and 51 mg O_2 respectively) than for moist silage (11 mg O_2). Heat production potential increased significantly when initial moisture was increased from 15 to 35%, and no significant increase was noted when initial moisture content was raised to

60%. Animal tissue decomposition rankings observed in the lab agreed with those from field trials. Decomposition of tissue samples within cornstalks and oat straw exceeded 66% during the 10-day lab study, while decomposition in silage averaged only 54%. Animal tissue decomposition at initial moisture of only 25% was significantly improved over that observed at 15%, and no significant improvement in decomposition was noted when initial moisture was increased to 60%. These results are encouraging as they suggest modest increases in envelope material initial moisture can significantly improve mortality composting system performance. This is particularly important during emergency situations, as moisture addition can be a time consuming process and its practicality during emergency disposal operations will depend on the level of initial moisture necessary to achieve desired results.

Keywords

moisture, swine, carcass, composting, biosecurity, decomposition, OUR

CHAPTER 1. OVERVIEW

In the late 1980s, researchers (Murphy and Handwerker, 1988) successfully composted poultry mortalities in only 30 days by mixing them with straw and litter. Twenty years later, substantial research has been conducted on composting systems to achieve a better understanding of the scientific (Epstein, 1997) and engineering principles associated with the process (Haug, 1993) and how they can be applied to mortality composting systems. Following its success in the poultry industry, composting was adapted to fit disposal needs of the swine industry (Fulhage, 1994; Glanville and Trampel, 1997) in addition to sheep (Stanford et al., 2000) and cattle (Bagley et al., 1999; Glanville et al., 2006a; Looper, 2007). Stanford et al., (2007) were even able to have success composting frozen cattle carcasses in temperatures $< 0^{\circ}\text{C}$ in Canada. This shows the adaptability and versatility of this technology to be successfully applied to many livestock disposal situations.

Composting has become a more favorable carcass disposal option among swine farmers due to growing environmental, biosecurity, and economic concerns associated with other methods (CAST, 2008). Microbial processes drive the process and generate high temperatures ($>55^{\circ}\text{C}$) necessary to inactivate pathogens, making composting a well established pathogen inactivation technology (Kalbasi et al., 2005). During windrow composting of cattle mortalities, Glanville et al., (2005) determined composting was sufficient in containing and inactivating viruses. Research by Glanville et al. (2006c) also showed windrows used for cattle composting had low potential to impact surface or groundwater quality, and pollution risks appeared to be much lower than the potential caused by carcass burial. Other mortality disposal methods such as burial and landfilling only

dispose of the problem and have limited effectiveness in pathogen inactivation (Nutsch et al., 2004). Incineration is a good method for reducing pathogens but fuel costs can be very expensive and the practice raises air pollution concerns. Traditionally, rendering has been the preferred method of carcass disposal, but recent declines in the number of rendering facilities located in the United States has made the rendering of carcasses expensive. There are also concerns of biosecurity issues associated with rendering vehicles traveling from farm to farm collecting carcasses (Auvermann et al., 2004).

Catastrophic losses of livestock caused by natural disasters or infectious disease outbreaks have led to interest in the use of composting for mass mortality disposal. Records of composting used for mass mortality disposal during natural disasters are limited. However, composting was successfully used for disposal of poultry carcasses during avian influenza (AI) outbreaks in Shenandoah Valley, Virginia (2002) and the Delmarva Peninsula of Maryland and Delaware (2004) (Wilkinson, 2007). These instances show the potential of mortality composting during catastrophic events but research is still needed to address biosecurity concerns, particularly associated with the disposal of larger carcasses such as swine and cattle.

During 2004, another outbreak of AI occurred in British Columbia, Canada, leading the Canadian Food Inspection Agency (CFIA) to develop a biosecure composting method to safely dispose of infected birds, in which naturally aerated static piles were wrapped in plastic sheeting to prevent the spread of AI viruses (Spencer et al., 2004). Following the success of these systems, the CFIA contacted researchers at Iowa State University to investigate the effectiveness of a similar system for on-site composting for bio-containment and safe disposal of infectious animal carcasses in the event of a bio-terrorism attack. During

this study, swine carcasses were composted during cool- and warm-season trials using six different co-composting or bulking (hereafter called envelope) materials, under a variety of initial moisture contents, to evaluate the effectiveness of such systems on larger carcasses. At the conclusion of mortality composting field trials, a follow-up laboratory study was conducted to corroborate and better understand field results. The following sections provide a brief overview and background of the field- and lab-scale studies.

1.1 Study Background and Purpose

1.1.1 Field-scale study

Field-scale test platforms were constructed to contain mortality compost piles. These platforms provided a controllable, instrumented, and weighable testing environment to simulate full-scale emergency composting operations, equipped with a passive aeration system and plastic biosecurity barrier (Figure 1). The main function of the platforms was to provide spatial and temporal data on: internal temperature, oxygen (O₂) and carbon dioxide (CO₂) concentrations, moisture content, virus survival, leachate production, carcass decomposition, and mass loss data. Performance of six different co-composting or bulking materials (hereafter envelope materials) were tested during both cool and warm season trials. Trials #1 and #2 tested corn silage, ground oat straw, and ground cornstalks. Trials #3 and #4 tested wood shavings, ground soybean straw, and ground alfalfa hay (2 seasons × 6 materials × 3 replications = 36 test units). At the conclusion of field trials, envelope materials were then evaluated on their ability to:

1. Maintain adequate O₂ levels (>5-10%) throughout the trial period of 8 weeks
2. Achieve elevated temperatures necessary to inactivate pathogenic organisms (>55 °C)
3. Decompose carcass soft tissues (excluding bone)



Figure 1. Field-scale biosecure mortality composting test unit with plastic biosecurity layer.

During these field trials, envelope materials with a wide range of initial moisture contents (w.b.) were used. The differences in initial moisture levels were mainly attributed to different storage practices, with some materials being exposed to more precipitation than others. The optimal recommended moisture content of envelope materials used for composting is approximately 40-65% w.b. (Rynk et al., 1992), but many materials used during mortality composting field trials had moisture levels as low as 10-15% w.b. At these low levels, most microbial activity within the compost pile is expected to stop, and little degradation or heat production will occur (Haug, 1993). However, these compost test piles (hereafter test units) still performed reasonably well in terms of internal temperature production and carcass decomposition when compared with envelope materials starting with 40-60% moisture.

At conclusion of field trials, many carcasses retrieved from compost piles also exhibited excessive drying and desiccation of soft tissues. Swine carcasses are roughly 50-

80% moisture (Georgieskii, et al., 1982) and liquid released (leachate) from ruptured carcasses is absorbed by the surrounding envelope materials. It is thought this leachate may have provided a favorable environment for microbial growth immediately surrounding the carcasses, therefore leading to improved temperatures and carcass degradation, despite having an initial envelope material moisture content which is much lower than the optimal range.

These observations raised questions concerning the amount of initial envelope material moisture needed for mortality composting systems to perform adequately. In the event of an actual emergency, fluctuations in envelope material moisture will naturally occur, so it would be beneficial to know how a variety of envelope materials will perform under a wide range of initial moisture levels. Due to logistical complications during field-scale mortality composting trials, simultaneous testing of all six envelope materials under identical conditions of external temperature and initial moisture was not possible. Therefore it is difficult to draw conclusions regarding the effect of low initial envelope material moisture content on layered mortality composting systems from field-scale trials because of variability caused by other factors, which led to the design and completion of a follow-up laboratory study.

1.1.2 Laboratory-scale study

While conventional complete mix composting systems (i.e. municipal solid waste composting) may require a specific range of moisture contents in order to achieve successful composting, livestock composting systems are different because some moisture is contributed by the carcasses. Based on field-scale data and observations, the follow up lab-

scale study was conducted to further evaluate the influence of moisture on mortality composting systems and answer the following questions:

1. Recognizing carcasses release considerable moisture during decomposition, do mortality composting systems require as much initial moisture (40 to 65% w.b.) as typically recommended for completely mixed systems used for other types of organic wastes?
2. Is there a minimum moisture level needed for successful mortality composting?
3. Do different envelope materials have different minimum moisture requirements?
4. What are the minimum practical envelope material moisture levels which permit acceptable carcass decomposition and temperature development?
5. Is there significant benefit to increasing moisture beyond the minimum practical levels?

To help answer these questions, mortality composting conditions were simulated in a laboratory setting by placing swine tissues and envelope materials in closed vessels (OxiTop® bottles) and incubating at 45 °C for 10 days (Figure 2). The same six materials tested during field-scale trials were also evaluated in the lab study. In addition, materials were adjusted to four different moisture contents to evaluate the impact of both envelope material and envelope material initial moisture content on swine tissue decomposition and oxygen uptake. All 24 treatments (6 materials × 4 moisture levels) were conducted in triplicate (N=3).



Figure 2. OxiTop® bottles used for lab-scale evaluation of swine tissue degradation and oxygen uptake.

This paper is organized with the next section containing a literature review of mortality management systems, composting processes and composting methods, with emphasis on the impact of moisture on mortality composting systems. This is followed by a materials and methods section, which will further detail field-scale activities, observations, and results which led to the development of the lab-scale study. Methods and procedures used during the lab-scale study are also outlined in this section. The results section contains findings and statistical analysis of data collected during lab-scale experiments. Finally, the conclusions section compares key findings from the lab-scale and field-scale studies and offers suggestions for future applications of the results.

CHAPTER 2. LITERATURE REVIEW

2.1 Livestock Mortality Management

The production of livestock, poultry, and their products accounted for sales of \$153.6 billion in the United States (US) during 2007 (US Census, 2007). This included livestock inventories of 96,300,000 cattle and calves, 67,800,000 hogs, and 350,000,000 layers. Iowa is one of the leading livestock production states in the nation, accounting for 3,980,000 cattle and calves (4.1% of the total US inventory, #8 nationally), 19,300,000 hogs (28.5% of the total US inventory, #1 nationally), and 53,800,000 layers (15.4% of the total US inventory, #1 nationally) (US Census, 2007). The swine industry is particularly valuable to the state of Iowa, producing almost half of the state's total revenue from livestock and poultry production (US Census, 2007). Since 2002, swine inventories in Iowa have increased by approximately 3.8 million hogs, while the number of farms raising hogs decreased by 1,875 (US Census, 2007). This trend has been continuing since the 1990s when industrialization and vertical integration of the swine industry started taking place in both the United States and Iowa (Honeyman and Duffy, 2006), leading to an increase in the number of concentrated animal feeding operations (CAFOs). According to the US Environmental Protection Agency (EPA), CAFOs make up approximately 15% of all livestock production facilities, and are defined as operations where high concentrations of animals are raised in confined situations and feed is provided for them (USEPA, 2008a). Large volumes of waste generated by these CAFOs, from both manure and livestock mortalities, must be managed in accordance with EPA nutrient management guidelines to utilize available nutrients and minimize environmental impacts (USEPA, 2008b). This can be a challenge as farm or cropland

surrounding the CAFO may not be sufficient for utilizing all of the waste, and it may therefore need to be transported long distances in order to utilize it appropriately. Having such a large concentration of animals in one location also raises biosecurity concerns, stemming from disease outbreaks or possible bioterrorism events. While everyday management of livestock deaths can be a significant issue for producers, catastrophic death losses will exceed the capacity of routine disposal methods and could potentially cause serious environmental impacts.

2.1.1 Routine vs. emergency mortality disposal

Routine livestock losses are those that occur under normal production conditions. These mortalities are a normal part of operations and need to be disposed of in way which is safe, cost effective, and environmentally friendly (CAST, 2008). Routine death loss generally represents a relatively small percentage of the total herd size and fluctuates throughout the course of production, with highest rates during the weaning and nursery stages (Table 1).

Table 1. Mortality rates of US swine at various production phases (NRCS, 2000).

| Animal type | Mortality rate (%) |
|-------------------------|---------------------------|
| Baby/weaned pigs | 20 |
| Nursery pigs | 2-3 |
| Finishing hogs | 2 |
| Sows | 6 |

Emergency disposal situations occur when mortality losses are much greater in magnitude, caused by events such as natural disasters, animal housing malfunctions,

infectious disease outbreaks, or a bioterrorism event. If such an event occurs, the number of carcasses requiring disposal will most likely exceed the capacity of local disposal methods, and therefore several disposal methods may need to be implemented (CAST, 2009). These emergency or catastrophic mortalities can also be categorized as diseased or non-diseased deaths. If mortalities were caused by disease, special disposal methods may be required to ensure carcasses are disposed of in a manner which contains the disease from being spread further.

2.1.2 Mortality disposal options

Mortality management and disposal technologies are critical components in livestock production systems. Mortalities are managed on the basis of three parameters: hygiene, environmental protection, and aesthetics (Gould et al., 2002), regardless of the production system. Therefore mortalities should be disposed of using methods which: are biologically safe, have minimal environmental impact, and do not raise awareness from the community. Methods utilized for satisfying these parameters when disposing of mortalities will depend upon herd population, size of animals, available resources, local regulations, and personal preference (CAST, 2008). The four predominant methods currently used for swine mortality disposal are burial, incineration, rendering, and composting (CAST, 2008). These methods have advantages and disadvantages in terms of both routine and emergency disposal situations.

2.1.2.1 Burial

Burial has historically been used in the disposal of animal mortalities. Types of burial methods include trench burial, landfills, and mass burial sites (Nutsch et al., 2004).

Burial of routine mortalities is typically done using the trench method, where a shallow trench is excavated and carcasses are placed in a single layer and covered with soil (CAST, 2008). Its use for routine disposal on small livestock operations has been considered to have minimal environmental and groundwater impacts but its use on CAFOs raises groundwater quality concerns because of the number of routine mortalities being accumulated at these sites (CAST, 2008). This causes great potential for groundwater contamination and many states have adopted stricter policies and regulations regarding livestock mortality burial in response to CAFOs (Glanville, 2001). The Iowa Department of Natural Resources (IDNR) limits on-farm burial of 44 hogs on a given acre per year (Glanville, 2001). These types of environmental concerns, along with legislation and negative public perception have all led livestock producers to look elsewhere for routine mortality disposal.

Burial is not well suited for catastrophic or emergency mortality disposal either, due to large volumes of carcasses being placed in one area, causing great potential for groundwater contamination (Nutsch et al., 2004). However, during an emergency situation mass burial may be one of the only options. Careful planning to determine sites with low environmental impact potential should be selected (CAST, 2008). Mass burial in a sanitary landfill is an acceptable method because they have engineered liners to prevent contaminants from leaching into the soil (Nutsch et al., 2004). However, landfill operators may not be willing to allow carcass disposal at their sites. Landfilling of carcasses is also considered to be a form of containment, rather than treatment, so long-term monitoring and management of the waste will be necessary (Nutsch et al., 2004).

2.1.2.2 Incineration

Incineration is one of the safest disposal methods in terms of pathogen destruction. When done correctly, the resultant product is a biologically inactive ash which can be disposed of easily. Types of incineration include open-air burning, fixed-facility incineration, and air-curtain incineration (Kastner et al., 2004). Specially designed fixed-facility incinerators are most common, and can be found at many swine production facilities. These generally use diesel as a fuel source and can be set to automatically turn on and off according to temperature. Incinerators are convenient because they can be used to dispose of carcasses on demand and the remaining ash can be disposed of in a field. Although modern incinerators are equipped with afterburners and other technologies to reduce particulate matter, air quality concerns are a major drawback of their use (CAST, 2008). Some areas may also require special permits in order to operate an incinerator.

Fixed-facility incinerators have limited capacity and would not be well suited for use during a catastrophic or emergency outbreak. Air-curtain incineration was successfully used in limited capacity during the 2001 foot-and-mouth disease (FMD) outbreak in the United Kingdom (UK) (Scudamore et al., 2002). While effective, this process is fuel intensive and would need to be used in conjunction with other disposal options during a catastrophic event. Open-air burning of carcasses is prohibited in most areas but may be allowed during an emergency event (Ellis, 2001). However this can be a fuel and labor intensive process, causes air pollution concerns, and is very undesirable from a public relations standpoint (CAST, 2008).

2.1.2.3 Rendering

Rendering is a process of mixing, cooking, and drying animal and poultry carcasses into value added products, such as meat and bone meal, used for animal feed and various other products (Auvermann et al, 2004). Rendering of animal mortalities and their by-products has been utilized since early 20th century. It is well established as an effective means of routine livestock disposal and provides a valuable, biologically stable final product (CAST, 2008). However, since 1975 the number of rendering plants has decreased substantially (Table 2). This has led to decreased availability and increased costs associated with rendering, making it a less desirable livestock disposal option (CAST, 2008).

Table 2. Decline in US rendering plants since 1921 (CAST, 2009)

| Year | 1921 | 1927 | 1975 | 1997 | 2006 |
|-------------------------|------|------|------|------|------|
| Number of Plants | 823 | 913 | 724 | 282 | 273 |

Rendering alone would not be adequate in handling the large volume of carcasses during an emergency event. While the infrastructure is in place and the technology provides a biosecure final product, it would need to be used in conjunction with other technologies to handle large volumes of mortalities (CAST, 2008).

2.1.2.4 Composting

Livestock mortality composting is the biological decomposition of carcasses and co-composting materials which takes place under aerobic conditions (Mukhtar et al., 2004). Carcass composting can also be thought of as above ground burial of mortalities within a

mound of carbonaceous material with carcasses being decomposed by microorganisms under aerobic conditions (Mukhtar et al., 2004; Kalbasi, 2005).

Using composting as a method of mortality management was first used in the poultry industry during the 1980s when research conducted by Murphy and Handwerker (1988) demonstrated its effectiveness for carcass disposal when poultry carcasses were fully composted in only 30 days. The successful use of this technology in the poultry industry also led to its adoption in the swine industry (Morrow and Ferket, 1993; Fulhage, 1994;). Composting has gained popularity as a routine disposal method for swine mortalities due to growing environmental, biosecurity, and economic concerns associated with other methods (CAST, 2008). Many farmers also have access to materials and equipment needed to compost routine mortalities and therefore it is becoming a very attractive option for them (Glanville, 2001). However, if access to materials and equipment is not readily accessible, capital costs associated with composting can be high and may not be the best option for routine mortality disposal.

The effectiveness of mortality composting systems for the disposal of mass mortalities during catastrophic or emergency situations has been reported in previous papers (Benfeldt et al., 2006; Glanville et al., 2007; Glanville, 2006b; Spencer et al., 2004). These papers outline the ability of mortality composting to be adapted to handle a large volume of mortalities, utilizing a variety of systems and envelope materials. Further details on composting principles, systems, and other factors affecting the composting process are outlined in the remainder of this literature review.

2.2 Composting Principles

2.2.1 The composting process

In a typical sense, composting systems involve stockpiling organic matter or biological waste such as food scraps and municipal solid waste for biological stabilization (Haug, 1993). These systems are often homogeneous in nature and use forced aeration techniques or are frequently turned to re-introduce oxygen into the system. This speeds up the composting process and produces a very stable and uniform end-product which can be marketed as a fertilizer or soil conditioner (Haug, 1993).

Mortality composting systems are very different from conventional systems as they are an inconsistent mixture of high nitrogen content materials (carcasses) enveloped by high carbon content materials (wood by-products or crop residues) (Keener et al., 2000). Mortality composting systems are also more concerned with disposal and mortality management, rather than achieving a stable and uniform end-product. Intensive management of these systems is also not practical for livestock producers, so forced aeration is typically not used and piles are generally only turned once or twice throughout the process.

The time required to decompose swine carcasses using composting is ultimately dependent upon several factors. Considered to be the most critical to the process are temperature, moisture content, oxygen/aeration, and nutrient availability, or the C/N ratio of the system (Haug, 1993). Ultimately, microorganisms drive the entire composting process and therefore these parameters mainly influence the microbial population, which determines the overall performance of the system.

2.2.2 Microbiology

Composting systems are driven by microbiological processes and are used to speed up the decay of organic matter by providing an environment which speeds up the normal process by trying to optimize various parameters (Haug, 1993). The microbial population within a composting system is mostly influenced by temperature, oxygen, moisture, and nutrient availability (Epstein, 1997). The pH of the compost system is also important but is usually not a limiting factor in the process. The process will be maximized with optimal adjustment of these parameters. This helps microorganisms breakdown organic matter in the most efficient manner and also allows for the production of thermophilic temperatures (40 to 71 °C), important for pathogen inactivation (Epstein, 1997).

A wide range of bacteria, actinomycetes (or actinobacteria), and fungi make up the majority of the microbial population within a composting system (Epstein, 1997). During the initial (heating) stages of composting, oxygen-consuming bacteria often dominate the system and metabolize simple carbon compounds first (Bertoldi, 1983; Tebbe, 2002). Fungi and actinomycetes are more predominant during the later stages (maturation) of the composting process when natural long chain polymers, such as cellulose, are primarily degraded (Bertoldi, 1983; Tebbe, 2002). Decomposition of cellulose takes place throughout the entire composting process, but is the primary fraction being degraded during later stages of composting (Bertoldi, 1983). Fungi are more moisture-tolerant than bacteria, and therefore flourish as temperature, pH, and moisture decrease throughout the composting process (Bertoldi, 1983). Actinobacteria also benefit from conditions in later stages of composting and are responsible for the soil-like smell of compost (Epstein, 1997).

2.2.2.1 pH

Compost pH influences the microbial populations of composting systems mainly by affecting the availability of nutrients (Bertoldi, 1983). Bacteria prefer a pH of 6.0-7.5, while fungi and actinomycete flourish when pH is between 5.5 and 8.0. When pH increases above 8.0, excessive amounts of N can be lost from the system due to ammonia volatilization (Bertoldi, 1983).

The pH of a material is the measure of its acidity or alkalinity, and is measured on a scale of 0 to 14, with increasing numbers indicating increasing alkalinity and decreasing numbers indicating increasing acidity. Maintaining a neutral pH (7.0) is ideal for composting, and is mainly dependent on envelope material used. Proper C/N ratio within the pile helps maintain an optimal pH range of 6.5 – 7.2 needed for composting (Carr et al., 1998). Similarly, an optimal pH of 6.5 – 8.0 for swine composting was determined by Langston *et al.* (2002). High carbon materials surrounding high nitrogen content carcasses help maintain a neutral pH around 7.0 by buffering CO₂ and NH₃ released during the composting process (Henry, 2003; Haug, 1993). In order for this to occur effectively, carbon and nitrogen must be present in a suitable ratio.

2.2.2.2 Temperature

The composting process is divided by many researchers (Rynk et al., 1992; Haug, 1993; Epstein, 1997; Keener, 2000) into 2 phases: heating and curing. During the heating phase, microorganisms break down organic matter at a very rapid pace, consuming large amounts of O₂ and generating enough heat to achieve thermophilic (40 to 71 °C) temperatures within the pile (Kalbasi, 2005). The curing phase is characterized by slower

digestion of cellulosic compounds by fungi and actinomycetes (Kalbasi, 2005). When temperatures are in the thermophilic range, the rate of decomposition is much higher than rates measured when temperatures are in the mesophilic (10 to 40 °C) range (Kalbasi, 2005). The most desirable temperatures for composting are between 45 and 55 °C because important microorganisms that play a key role in cellulose degradation are destroyed at levels above this, and fungal activity is also greatly diminished at temperatures greater than 55 °C (Bertoldi, 1983; Kalbasi, 2005).

While temperature is an important factor affecting compost process kinetics, temperatures are also a by-product of organic matter biodegradation (Bertoldi, 1983). Aerobic respiration gives off heat as well as CO₂ and H₂O, which raises internal temperatures of the pile due to self-insulating properties of the envelope materials (Rynk et al., 1992). Heat generated is beneficial as it speeds up decomposition and is needed to inactivate disease causing pathogens within the pile. To meet USEPA Class A and Class B time/temperature requirements for pathogen inactivation, compost must maintain temperatures >55 °C for at least 3 consecutive days, and maintain temperatures >40 °C for at least 5 days with at least 4 hours above 55 °C, respectively (EPA, 2003). Temperatures within the composting pile may become excessive and kill off microbial populations if aeration is not adequate to remove excess heat from the system (Bertoldi, 1983; Rynk et al., 1992).

2.2.2.3 Oxygen / Aeration

Composting is a type of biological oxidation; hence O₂ is critical for this process. Microorganisms use O₂ during aerobic respiration as a terminal electron acceptor and for oxidation of organic substances (Bertoldi, 1983). Oxygen levels should not drop below 18%

otherwise it becomes a limiting factor in the process (Bertoldi, 1983). However, these levels are for optimal conditions, which are not always practical for mortality composting systems, and would also require almost constant aeration (Bertoldi, 1983). As a general rule, oxygen concentrations within the composting matrix should not drop below a more practical level of 5% (Rynk et al., 1992). Reduced oxygen levels will slow microbial processes and eventually lead to anaerobic conditions. If this occurs, degradation will be slowed considerably and offensive and potentially dangerous gasses (sodium hydroxide) may be produced (Rynk et al., 1992). Aeration is also important for removing excess heat and moisture from the system (Rynk et al., 1992).

2.2.2.4 Nutrients

Microorganisms within composting systems require macronutrients such as carbon (C), nitrogen (N), phosphorus (P), and potassium (K) for growth (NEH, 2000). Carbon and nitrogen are the two primary nutrients of importance in composting systems (Epstein, 1997). Nitrogen is utilized by microorganisms for protein synthesis and reproduction, while carbon is used by microorganisms for energy and growth within the cell (Rynk et al., 1992; Haug, 1993; Epstein, 1997). Aerobic degradation uses 15 to 30 parts C for each N (Haug, 1993). A C/N ratio of approximately 25 to 30:1 is recommended for rapid composting of refuse. Levels below this lead to excess N losses due to ammonia volatilization, and ratios above the recommended range cause increases in composting times (Haug, 1993; Epstein, 1997). Nitrogen content of compost decreases during the process due to ammonia volatilization. However, N is recycled in the system when microorganisms die off and loss of C due to

degradation of organic matter into CO₂ and H₂O leads to a decrease in overall C/N ratio (de Bertoldi, 1983).

2.2.2.5 Moisture

Moisture is considered a critical component of composting systems as it is required for biological processes performed by microorganisms within the pile (Rynk et al., 1992; Epstein, 1997). Water is used by microorganisms for locomotion, nutrient transport, and chemical reactions taking place within microorganisms (NRCS, 2000). Initial moisture content of mortality composting systems is also very important from a management standpoint as it is one of the few process variables which could be controlled or manipulated during compost pile construction. The generally accepted range of envelope material moisture content necessary to support successful composting is 40-65% wet basis (w.b) (Rynk et al., 1992). As moisture content decreases below 40%, microbial activity begins to diminish, with most activity stopping when moisture content falls below 15% (Rynk et al., 1992).

2.2.3 Compost Envelope Materials

Composting systems generally contain at least two types of raw materials: relatively moist, high N materials and relatively dry, high C materials (Rynk et al., 1992). Conventional composting systems use the term bulking or co-composting material when describing these high C materials because they are uniformly mixed with other constituents in the compost pile (Haug, 1993). However, mortality composting systems are very heterogeneous in nature and high C materials added to the system surround the carcasses and will therefore be referred to as envelope materials in this paper.

Envelope materials used for mortality composting are typically wood by-products, such as sawdust or wood shavings, or crop residues such as cornstalks or soybean straw. These materials have high C/N ratios but other more biodegradable materials such as silage, alfalfa, and turkey litter may be used to help improve system performance. Envelope materials are added to cover carcasses, filter gasses released during decomposition, provide a more suitable environment for microorganisms, and prevent access to carcasses by insects, birds, or scavengers (Mukhtar, 2004).

Each envelope material has unique characteristics such as C/N ratio, pH, water holding capacity (WHC), particle size, porosity, and mechanical strength which affect the overall performance of the system (Ahn et al., 2008a). Since the composition of swine carcasses is relatively uniform and cannot be modified, the type and preparation of envelope materials used for composting will be the main influence on performance.

2.2.3.1 C/N Ratio

Wood by-products and crop residues used for composting typically have very high C/N ratios, sometimes >400 (Ahn et al., 2008a). Envelope materials with lower C/N ratios have higher degradability and can help high temperature achievement throughout the composting pile, which is beneficial for disposal of diseased carcasses (Haug, 1993; Ahn, et al., 2008b).

2.2.3.2 Mechanical Strength

The mechanical strength or structure of a material is its ability to resist compaction (Rynk et al., 1992). Mortality composting piles can settle considerably after construction, compacting materials and limiting passive aeration throughout the pile by reducing free air

space. Unturned windrows used for cattle composting by Glanville (2006b) settled by as much as 1m in 45 – 60 days. As moisture increases, the mechanical strength of a material decreases, leading to decreased pore space for air transfer through the system (Ahn et al., 2008).

2.2.3.3 Air-filled Porosity

The air-filled porosity or free air space (FAS) of a medium is the ratio of gas or air pore volume to the total pore volume (Rynk et al., 1992). Adequate FAS is necessary to maintain aerobic conditions throughout the compost matrix. Free air space is related to envelope material particle size, mechanical strength, and moisture content. If particle size is reduced too much, FAS will be insufficient to provide oxygen delivery to microorganisms. If the mechanical strength of a material is lacking, it will be unable to maintain FAS under moist or compacted conditions (Rynk et al., 1992). Maintaining air filled porosity of 30% or greater will allow for good aerobic composting conditions, as long as moisture content is less than 65% w.b. (Rynk et al., 1992).

2.2.3.4 Particle Size

Reducing the particle size of envelope materials will help increase performance of the system as the biological oxidation of organic matter is directly proportional to the surface area available for reactions to take place (Bertoldi, 1983). The most effective particle size for a material will vary depending on its physical properties, as particle size should not be reduced to the point where it limits oxygen transfer throughout the matrix (Bertoldi, 1983). Envelope materials with particle size of 0.3 to 5.1 cm in diameter are recommended for composting (Rynk et al., 1992). The particle size of materials should not be reduced too far

as this will create structural issues with the compost pile, leading to limited free air space and reduced performance (Epstein, 1997).

2.3 Mortality Composting Systems

There are several types of composting systems typically used for mortality disposal. Each system has its own advantages and usefulness depending on the type of mortality management scenario to which it is applied. Most on-farm composting procedures will consist of laying out a 30-45 cm base layer of bulking or envelope material, which is typically a wood by-product or crop residue. Carcasses are then placed on the base layer, making sure they do not touch each other, and are at least 30-45 cm from the outer edge of the compost pile (Glanville, 2002). The carcasses are then completely covered with envelope material and additional layers are added, depending on the size of carcasses being placed in the pile.

2.3.1 Bin Systems

Bin composting is typically done in a building or structure with concrete flooring, where compost piles are constructed and contained within 3 sidewalls typically made from treated lumber or concrete, with a roof overhead to protect the piles from precipitation (Keener et al., 2000; Kalbasi et al., 2005). Bin composting structures can be built specifically for the purpose of mortality disposal or existing structures can be retrofitted. An absorptive base layer of 30-45 cm of envelope material is placed on the floor. Carcasses are then placed on the base layer, spaced so they are not touching each other and so they are at least 25-30 cm from sidewalls (Glanville, 2002). Before placing additional layers of

carcasses on the pile, carcasses should be covered with an additional 15-30 cm of material, depending on carcass size (Glanville, 2002). At least three bins are generally required: one for primary carcass loading, one which has completed loading and is used for primary composting, and one for secondary composting (Glanville, 2002; Kalbasi et al, 2005). Additional bins may be required for primary composting, stockpiling of raw materials, or storage of completed compost.

Bin composting methods are recommended for both swine and poultry operations and work very well for routine mortality management conditions (Glanville, 2002). In the event of an emergency, bin composting would not be adequate or efficient for mass disposal, simply because bin composting structures are not large enough to handle mass quantities of carcasses.

2.3.2 Static Windrow / Pile

Compost windrows should be built on a concrete pad or other impervious surface to prevent excessive amounts of nutrients from leaching into the soil (Keener, 2000). Mortality composting windrows are constructed in a similar manner to composting bins but are not constrained by walls. Carcasses are placed on a base layer of envelope material and are covered creating a mound of material typically 1.5 to 2.1 m in height (Keener et al., 2000). This static pile can then be added to, creating a long narrow windrow which can be accessed from all sides for addition of more carcasses or turning (Keener et al., 2000). These types of systems are typically used for larger animals or large operations where routine death losses account for a significant volume of carcasses (Mukhtar et al, 2004). These types of systems

are the most easily adaptable and would therefore be best suited to handle a large quantity of carcasses in the event of an emergency.

2.3.3 Other Systems

2.3.3.1 In-Vessel Composting

In-vessel composting is carried out within a fully enclosed system, usually within some type of synthetic liner. The Ag-Bag™ system, originally designed for ensiling, uses a tractor powered machine to force material into a large plastic tube which is then mechanically aerated (Mukhtar et al., 2004). The United States Department of Agriculture (USDA) and Animal and Plant Health Inspection Service (APHIS) used the Ag-Bag system to successfully compost over 100,000 birds after an avian flu outbreak in West Virginia during 2003 (Mukhtar et al., 2004). Other research has shown poultry mortality composting using the Ag-Bag system is capable of reaching temperatures of 70 to 82 °C (Mukhtar et al., 2004). While these Ag-Bag systems are attractive because they are a closed system and have the ability to produce high temperatures, they would not be the best option for emergency mortality disposal. The loading process can be time consuming and large carcasses would need to be cut into smaller pieces in order to fit into the Ag-Bag, which would be unacceptable from a biosecurity standpoint.

2.3.3.2 Rotary Systems

Rotary composting systems utilize a cylindrical drum to speed up the mortality composting process by continually turning and aerating the system. Carcasses and envelope materials are loaded into the drum and the rotation provides continuous aeration and mixing,

as well as physical break-down of the carcasses. Hogs composted using this method reached maximum temperatures of 60 °C within the first 60 hours of composting (Mukhtar et al., 2004). These systems can decrease composting times and provide a more uniform end-product, making them ideal for routine mortality disposal. Due to size limitations these systems would not be the best choice for emergency disposal situations. Some units are portable but these are often even smaller in size, and these systems are not very common.

2.3.4 Composting as an emergency disposal method

Given the highly integrated structure of the modern animal production industry, the potential for catastrophic livestock losses due to disease outbreaks or other disasters appears to be high. Recent disasters and disease outbreaks at livestock production facilities worldwide, while unwarranted and unwanted, have provided useful insight on how to deal with these types of events in the future.

- In 1998, Texas floods killed livestock resulting in approximately \$11 million in losses (Ellis, 2001).
- Heat waves killed a total of 10,000 cattle in Nebraska and Iowa during the summer of 1995 (USDA, 2002).
- North Carolina contains several of the most densely populated hog production counties in the nation. During Hurricane Floyd in 1999, approximately 2 million chickens, 28,000 hogs, and 1,100 cattle perished (Mukhtar et al., 2004).

- Foot and mouth disease (FMD) outbreaks occurred in Taiwan during 1997 and again in 2001, leading to the disposal of millions of cattle, sheep, and swine (Wilson and Tsuzynski, 1997).
- Several disease outbreaks occurred during 1998, including a Newcastle disease outbreak in New South Wales, FMD outbreak in Asia, Africa, South America, and Middle East, and African swine fever in Madagascar (Pakissan.com, 2001).

During the summer of 2008, heavy rains inundated the state of Iowa causing massive flooding in most parts of the state. Many livestock, particularly from swine operations, had to be relocated to avoid catastrophic losses due to the rising flood waters. Approximately 37,000 animals were moved before flooding but an estimated 3,100 hogs perished in the floods, with an additional 1,000 head considered to be feral (RIO, 2009). This was the second 100-year flood to hit the Midwest in 15 years, which emphasizes the importance of developing an emergency carcass disposal action plan to use in the event of a disease outbreak or natural disaster.

2.3.4.1 Effectiveness

The effectiveness of composting as a method of routine mortality disposal is well documented (Glanville, 2001; Morse, 2001; Mukhtar et al., 2004; Kalbasi et al., 2005). These systems could be easily adapted to handle large quantities of animals in the event catastrophic death losses occurred. Windrows system can be constructed in most open areas with common farm equipment, such as a skid steer or tractor and loader, and can essentially be built as long as required. Glanville et al. (2006b) used windrows to dispose of cattle

mortalities under biosecure conditions (zero turning) and found the systems were successful at containment and inactivating of pathogens. Stanford et al. (2007) also successfully composted cattle using windrows but allowed cattle carcasses to freeze before starting the process. While these systems took longer than routinely turned systems (sometimes >9 months), they were still effective in reaching high temperatures (>55 °C) and decomposing carcasses.

2.3.4.2 Biosecurity

Reliable pathogen inactivation is essential for biosecure disposal of livestock mortalities in the event of a disease outbreak. Composting is a well established pathogen reduction technology (Kalbasi et al., 2005), particularly when applied to routine mortality composting practices. This is due in large part to the envelope materials selected for routine mortality management, as they generally have favorable particle size and may be amended with manure or litter to help improve biodegradation and heat production within the pile. However, pathogen reduction is less certain for emergency mortality composting situations where available envelope materials may be coarse-textured or dry. Furthermore, emergency composting may be done with little or no turning which is a widely accepted practice used in routine composting to introduce oxygen and redistribute moisture and nutrients, thereby stimulating microbial activity and production of heat.

There are few records of composting being applied as a mortality disposal option during actual infectious disease outbreaks.

- Ag-Bag and windrow composting were used in 2002 during an AI outbreak in Virginia's Shenandoah Valley (Bendfeldt et al., 2006)

- In-house composting was used during an AI outbreak in the Delmarva Peninsula of Maryland and Delaware during February of 2004 (Malone et al., 2004).
- Highly pathogenic AI outbreak during 2004 in British Columbia, Canada led to the disposal of 1.25 million birds by burning, burial, and composting (Spencer et al., 2004).

Emergency mortality composting has been successful in disposal of poultry carcasses using in-house windrows. However, poultry carcasses are fairly easy to compost and these systems typically use poultry litter as an envelope material which has very favorable characteristics for composting. While research by Glanville et al., (2006) was not conducted during an actual disease outbreak, it was performed under biosecure conditions and may be some of the most useful emergency mortality composting information for large carcasses to date.

2.4 Moisture Impacts on the Composting Process

Water is essential for all forms of life, including microorganisms. Because moisture affects microbial activity and the physical structure of envelope materials, it has a large influence over the entire composting process (Ahn et al, 2008a). The optimum moisture content for mortality composting systems is a tradeoff between moisture requirements of the microorganisms and their simultaneous need for an adequate supply of O₂ (Haug, 1993). Optimum moisture in mortality composting systems will ultimately depend on carcass loading rates and physical properties associated with envelope materials being used. In addition, optimum moisture will also depend on the disposal circumstance.

Initial moisture content of envelope materials is of particular importance in emergency mortality composting systems because this is likely the only time materials will be moistened and piles will not be turned to redistribute moisture because of biosecurity constraints. During the composting process, moisture is generally lost unless large amounts of precipitation are allowed to fall on unprotected piles. Evaporative loss of moisture increases as temperatures increase and due to passive aeration through the pile. If moisture levels drop too low, microbial processes will slow and may even stop. Minimizing these moisture losses throughout the composting period is very important to help achieve complete degradation of carcasses and to achieve high temperatures necessary to inactivate pathogens.

2.4.1 Microbial Activity

Moisture within the composting system is used by cells for biological processes and it also provides a medium for microorganism mobility (Hamelers and Richard, 2001). Sufficient moisture is needed for microorganisms to flourish, thereby producing high temperatures. Adequate moisture is essential during initial stages of the composting process so microorganisms can freely move about the system (Miller, 1989). During later composting stages, this mobility may not be as important as microbial populations have thoroughly colonized the entire matrix (Miller, 1989).

2.4.1.1 Respiration rate

The respiration rate of a microbial population is directly related to its metabolic activity (Gomez et al., 2006). Therefore increased metabolic activity should lead to an increased respiration rate. Because moisture is critical to microbial respiration, it is expected that increasing moisture should also increase the respiration rate of microorganisms. Based on

oxygen uptake rates (OURs) of materials under various moisture treatment conditions, Ahn et al., (2008b) determined the optimum moisture content of each material to be near 60-80% (w.b.) moisture.

2.4.2 Envelope material physical properties

Moisture affects free airspace of the compost matrix. As envelope material moisture content increases, air voids in the material become filled with water, limiting oxygen transport through the matrix (Haug, 1993). This makes aerobic conditions difficult to maintain and can lead to anaerobic decomposition within the pile, which is considerably slower and can produce excessive leachate and putrescible compounds leading to offensive odors (Haug, 1993). If conditions within the pile are too dry, microbial processes will be slowed, reducing internal temperatures and biodegradation (Schultz, 1961; Nakasaki et al., 1994).

If moisture levels are too high, free air space within the pile will be filled by water, reducing airflow and possibly leading to anaerobic conditions. Moisture also affects mechanical strength of compost envelope materials. As moisture increases, mechanical strength of materials decrease, leading to increased compaction and reduced free air space (Ahn et al., 2008b). If this occurs, degradation will be slowed considerably, excessive leachate may be released, and offensive odors may be produced within the pile (Rynk et al., 1992). More fibrous materials can generally hold more water and still maintain adequate free airspace (Haug 1993) but may lack insulating properties necessary to prevent excessive heat loss from the system.

2.5 Measuring compost metabolic activity

Compost maturity and compost stability are two measures often used to assess the quality and level of microbial activity associated with a compost sample, respectively.

Maturity is a measure of the affect compost will have on plant growth when used as a soil conditioner (Haug, 1993). Mature compost is not phytotoxic and will not adversely affect plant growth when applied as fertilizer (Gomez et al., 2006).

Stability is a measure of compost microbial activity (Haug, 1993; Butler, 2001).

Most readily degradable material has been consumed in stable compost making it biologically inactive (Haug, 1993). If compost is applied to farm or cropland and is not fully stabilized, it could result in a net immobilization of nitrogen from the soil for use for the microbial community in the compost, leading to N deficiencies in crops (Inbar et al., 1993). Because stability is a measure of microbial activity, it can also be applied in monitoring composting process performance (Gomez et al., 2006). Respiration tests are often used to determine compost stability because they provide a reliable and repeatable measure of microbial activity (Gomez et al., 2006).

2.5.1 Respirometric activity

Respirometry tests measure metabolic rates of microorganisms by determining the amount of CO₂ they produce (CO₂ evolution) or the amount of O₂ they consume (O₂ uptake rate, OUR). Both of these measures are considered respiration rates and can be related to microbial activity of compost because C is being converted to CO₂ during the process (Epstein, 1997). Another common method of determining respirometric activity is self-heating tests (Gomez et al., 2006). Microorganisms will have higher metabolic rates in the

presence of large amounts of easily degradable material, but only if other conditions are optimal.

2.5.2 Measurement methods

2.5.2.1 Self-heating test

Compost sample is taken and temperature increases are monitored to determine the amount of heat released from the sample due to biological activity. Other biological and chemical reactions are also exothermic so this test may not be directly correlated to respiration (Gomez et al., 2006).

2.5.2.2 CO₂ production

These systems measure CO₂ production and are directly correlated with aerobic respiration (Gomez et al., 2006). These systems are more complex than self-heating tests and often require more skilled personnel. CO₂ is absorbed by an alkaline substance within the testing environment (Gomez et al., 2006). These systems are unable to distinguish between aerobically and anaerobically produced and can be somewhat pH-dependent.

2.5.2.3 O₂ uptake

These are the most accepted methods of determining biological activity in a compost sample. Several commercial equipments, including the OxiTop® system used in this study, fall into this category of respirometric test. There are two classifications of this method which includes static and dynamic. Dynamic systems have a continuous O₂ supply to prevent limitations from this parameter (Gomez et al., 2006). Static systems, like OxiTop®,

are a sealed system where O₂ has to be replenished on a regular basis. These static systems function by measuring changes in O₂ concentrations in the head space.

2.5.3 Factors influencing respiration rate measurements

Besides temperature, moisture content, O₂ concentration, and free airspace (FAS) are the main factors affecting OUR (Haug, 1993). If all parameters are not provided adequately, the process will become rate limited and fluctuations in oxygen uptake rates will occur.

2.5.3.1 Incubation time

The respiration rate of a compost sample changes over time, with peak values generally occurring at the beginning of the testing period, followed by a gradual decline as organic matter is degraded (Haug, 1993). Recommended incubation times can vary from around 16 hours to up to 3 days (Iannotti et al., 1993), and peak respiration rates are generally achieved within 2-3 days (Lasaridi and Stentiford, 1998).

2.5.3.2 Incubation temperature

Incubation temperature is important as the rate of biodegradation is related to temperature, with higher temperatures generally producing higher rates of degradation. There is no standard incubation temperature for respirometric testing of compost, but many are performed at temperatures between 30 and 37 °C (Gomez et al., 2006). Research by Mari et al., (2003) determined using 48.5 °C for incubation produced more realistic respiration rates from materials and were also more realistic of actual temperatures achieved in a composting system. Haug & Ellsworth (1991) recommended working temperatures of 45 °C to reduce the effect of nitrifying bacteria.

2.5.3.3 Oxygen

If not supplied continuously, O₂ can become rate limiting to the system and it can become anaerobic. If this occurs, the respiration rate may be falsely influenced by the production of other gasses besides CO₂. Oxygen concentration has little impact on microbial activity if concentrations are above 5% (Keener et al., 2002).

2.5.3.4 Moisture

Moisture is critical for microbiological processes. In general, as moisture increases, so does the biodegradation rate of carcasses and envelope materials. Research by Palentski and Young (1995) and Ahn et al. (2008) showed that microbial respiration rates are directly related to the moisture content of compost envelope materials. The optimum moisture content of many envelope materials used for mortality composting is near their water holding capacity (WHC), or approximately 60 to 80% moisture (w.b.) (Ahn et al., 2008). Samples with moisture contents below 35% (w.b.) are considered biologically inactive and will produce “falsely” low O₂ uptake rates (Gomez et al., 2006). It is generally accepted that envelope materials should have 40-65% (w.b.) moisture in order for successful composting to occur (Rynk et al., 1992).

CHAPTER 3. METHODS AND PROCEDURES

3.1 Mortality composting field study

3.1.1 Field-scale study background and procedure

To investigate the effectiveness of on-site composting for bio-containment and safe disposal of infectious animal carcasses in the event of a bioterrorism attack or disease outbreak, swine carcasses were composted using passively-aerated plastic-wrapped composting test units, designed by ISU researchers and the CFIA, to contain possible pathogenic organisms. Test units were 2m × 2m × 1.2m (depth) and are shown in Figure 3. Each test unit consisted of:

- Approximately 225 kg of swine carcasses (4 to 5 carcasses)
- 10cm diameter PVC and drainage tubing used for passive aeration and gas venting
- Plastic biosecurity liner and cover
- Nine sampling ports to collect envelope material from layers beneath (“bottom”), surrounding (“middle”), and above (“top”) carcasses
- Nine data collection clusters, constructed of 3cm diameter PVC, to monitor internal temperature production and O₂/CO₂ concentrations, with monitoring points located in the bottom, middle, and top layers
- Perforated and unperforated plastic vials containing live vaccine strains of Newcastle Disease Virus (NDV) and avian encephalomyelitis (AE) placed in the center of each test unit

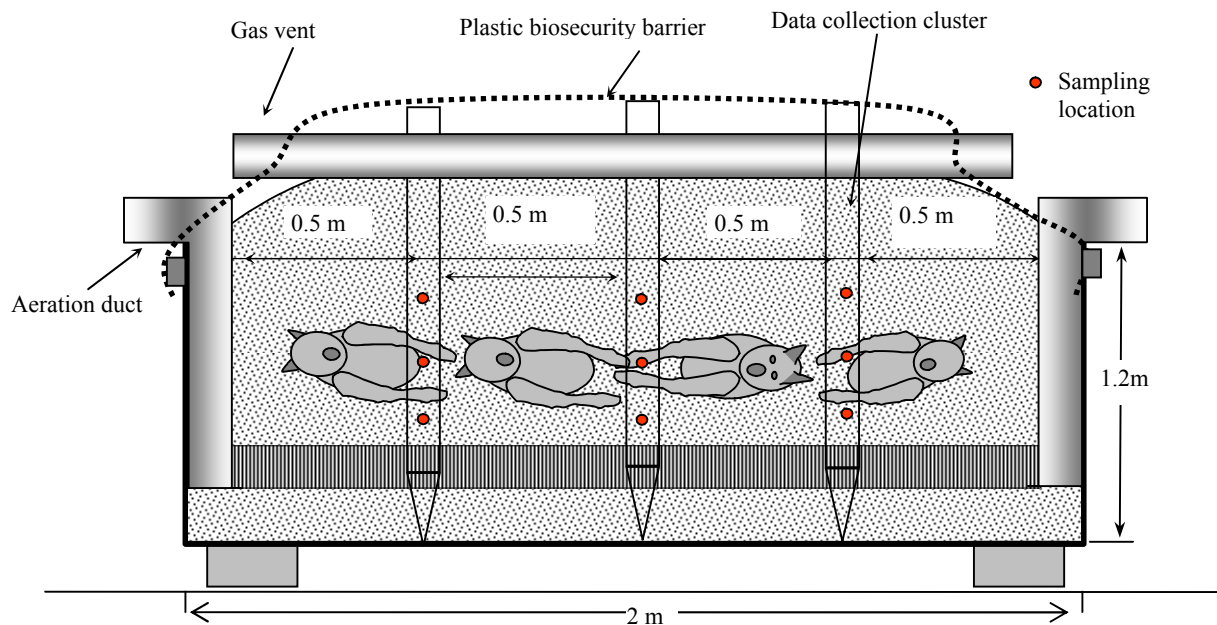


Figure 3. Schematic of field-scale mortality composting test unit.

Upon loading of test units, mortality composting field trials were carried out during cool and warm seasons using six different envelope materials (ground alfalfa, ground cornstalks, ground oat straw, ground soybean straw, silage, and wood shavings) that are likely to be used during livestock disposal emergencies, with each envelope material being tested in triplicate (2 seasons \times 6 envelope materials \times 3 replicates = 36 test units). All trials were conducted for approximately 2 months. Trials #1 and #2 tested corn silage, ground oat straw, and ground cornstalks. Trials #3 and #4 tested wood shavings, ground soybean straw, and ground alfalfa hay. Main performance data collected during these trials included: i) internal temperature production, ii) O_2 and CO_2 concentrations, iii) envelope material moisture content, iv) virus survival, v) leachate production, vi) carcass soft tissue decomposition, and vii) overall compost pile mass loss data. Table 3 summarizes key information from all field-scale trials. Mean initial moisture content (% w.b.) represents

average initial moisture content of materials used during each trial (N=3). T_{30} values are defined as the mean temperature during the first 30 days of the trial.

Table 3. Summary of key information from mortality composting field trials. (N=3)

| Trial Number | Season | Ambient T_{30} ($^{\circ}$C) | Materials Used | Middle layer initial moisture (%w.b.) | Middle Layer T_{30} ($^{\circ}$C) | Mean carcass decomposition (%) |
|---------------------|---------------|---|-----------------------|--|--|---------------------------------------|
| 1 | Cool-season | 4.41 | Cornstalks | 24.9 | 13.8 | 86.0 |
| | | | Oat straw | 24.4 | 11.1 | 79.3 |
| | | | Silage | 75.5 | 48.2 | 66.1 |
| 2 | Warm-season | 22.39 | Cornstalks | 64.0 | 56.1 | 88.5 |
| | | | Oat straw | 58.7 | 43.9 | 85.5 |
| | | | Silage | 56.4 | 62.9 | 78.1 |
| 3 | Warm-season | 20.48 | Alfalfa hay | 17.4 | 37.2 | 78.0 |
| | | | Soybean straw | 17.0 | 34.5 | 84.7 |
| | | | Wood shavings | 10.9 | 41.8 | 77.2 |
| 4 | Cool-season | 13.70 | Alfalfa hay | 38.8 | 40.7 | 84.3 |
| | | | Soybean straw | 53.1 | 44.4 | 86.3 |
| | | | Wood shavings | 58.4 | 27.7 | 85.3 |

3.1.1.1 Field study statistical analysis

The main effect of envelope material and envelope material initial moisture content on swine carcass decomposition and internal temperature production was evaluated using two-way analysis of variance (ANOVA) models (JMP v7.0.2), with replication (trial) used as a block. ANOVA is a statistical test which attributes part of the total variability in a response variable to different levels of the predictor variable(s). The test is used to determine whether a significant relationship exists between the predictor variables and the response variable.

This is important in determining whether the predictor variable actually has an effect on the response or if the effect is not significant and may have happened simply by chance. A p-value is often used to determine if the effect of a predictor variable is significant or not. In this case, treatment means were compared using TUKEY's honestly significant differences (HSD) test at 95% confidence interval. Therefore, if a predictor variable had a p-value less than 0.05, it was considered to have a significant effect on the response variable.

3.1.2 Field study results

At the conclusion of mortality composting field trials, the main performance variables evaluated were swine carcass decomposition and internal temperature production. Internal temperature data were analyzed by middle layer T_{30} values, which represent the mean temperature within the middle layer during the first 30 days of composting. These values were used because they represent temperature production in the carcass decay zone, which is a critical area for high (>55 °C) temperature production necessary to inactivate pathogens.

Carcass decomposition was determined by initially weighing carcasses as they were placed in composting test units and comparing this with the weight of recovered remains at the conclusion of each trial. Carcasses have roughly 12% bone mass (Kuhn et al., 1997) which had minimal decay during the 8 week composting field trials. Therefore, 88% of the total initial weight was used when calculating swine carcass decomposition percentages.

3.1.2.2 Internal temperature data

Based two-way ANOVA modeling, envelope material and middle layer initial moisture content was shown to have a significant effect on internal temperature production within the carcass decay zone ($p < 0.0003$ and $p < 0.0001$, respectively). Predicted least

squares mean values of T_{30} for the six envelope materials are shown in Table 4. Silage had the greatest temperature production, with predicted values over 50 °C. Alfalfa, soybean straw, and cornstalks had predicted temperatures around 40 °C, while oat straw and wood shavings had the lowest temperature production of around 30 °C.

Table 4. Predicted least squares mean of T_{30} for six envelope materials.

| Envelope Material | | Predicted least squares mean T_{30} (°C) | | |
|-------------------|---|--|---|------|
| Silage | A | | | 52.5 |
| Alfalfa | A | B | | 41.4 |
| Soybean straw | A | B | | 41.4 |
| Cornstalks | | B | C | 37.9 |
| Oat straw | | | C | 30.1 |
| Wood Shavings | | | C | 29.7 |

Levels not connected by same letter are significantly different ($p < 0.05$)

3.1.2.3 Swine carcass decomposition

A two-way ANOVA model using the same predictor variables was used to analyze swine carcass decomposition. However, the effect of envelope material and initial moisture content on carcass decomposition was not significant ($p=0.7218$ and $p=0.8199$, respectively) and were shown to have a very weak interaction. These results were somewhat surprising after finding strong interactions in the internal temperature model, as increased internal temperatures are a product of increased microbial activity and therefore an increase in decomposition would be expected.

Upon excavation at the conclusion of field trials, envelope materials sometimes showed excessive drying and carcass remains were desiccated, indicating a lack of moisture in the system. Despite this apparent lack of moisture after only two months of composting, many of these composting test units performed well in terms of temperature production (>50

°C) and carcass decomposition (>80%). Because of limitations in logistics during the field study, which prevented simultaneous testing of all six envelope materials under identical conditions of external temperature and initial moisture content, results from the field study were somewhat inconclusive. Therefore, a follow-up lab-scale study was conducted to further evaluate the influence of initial envelope material moisture content on mortality composting systems. The study was designed to help identify critical envelope material moisture levels that either permit or prohibit successful decomposition of carcasses and achievement of high internal temperatures necessary to inactivate pathogens. These observations are useful for both routine and emergency mortality composting systems by helping determine initial moisture levels at which moisture addition has little benefit. In other words, will adding moisture cause significant improvement in system performance and at what level does adding moisture have little benefit? This is particularly important in the event of an emergency when moisture addition may not be practical or seen as a priority.

3.2 Mortality composting laboratory study

3.2.1 Objectives

- Determine if:
 - Mortality composting systems require as much initial moisture as typically recommended for completely mixed composting systems (40 to 65% w.b.).
 - There is a minimum moisture level needed for successful mortality composting.
 - Different envelope materials have different minimum moisture requirements.
 - There are minimum practical envelope material moisture levels which permit acceptable carcass decomposition and temperature development.
 - There is significant benefit to increasing moisture beyond the minimum practical levels.

3.3 Materials and Methods

3.3.1 Experimental design

In follow-up to field-scale mortality composting trials, a lab-scale study was conducted to further investigate the effect of low initial envelope material moisture content on swine tissue degradation in layered livestock mortality composting systems.

To simulate mortality composting conditions, swine tissue samples – consisting of muscle, fat, hide, and hair cut from the ham muscle of euthanized hogs – were placed between two layers of envelope materials (alfalfa, corn silage, cornstalks, oat straw, soybean straw or wood shavings) that were adjusted to one of four moisture treatment levels (15, 25, 35, or 60% w.b.) (Figure 4). The respiration rate of each material/moisture treatment was then measured to quantify the microbial activity of each sample. The respiration rate was measured using the OxiTop® system, which measures O₂ consumption based on a pressure drop within a closed vessel (Wageningen and NMI, 2003).

The decomposition percentage of swine tissue samples used in each treatment was also calculated by initially weighing each tissue sample and comparing this with the final swine tissue sample weight collected at the end of the testing period.

3.3.1.1 Statistical Design

Lab study replications were performed in triplicate (N=3) using a Randomized Complete Block Design, with 3 replications. Treatments were randomly assigned to OxiTop® pressure sensor heads. A total of 72 units were tested (6 envelope materials × 4 moisture treatments × 3 replications), and the effect of envelope material, moisture treatment,

and their interaction (envelope material \times moisture treatment) on swine tissue decomposition and TOU was evaluated using two-way ANOVA models (JMP v7.0.2). Treatment means were compared using TUKEY's HSD test at 95% confidence interval. Total oxygen uptake data was log transformed (\log_e) to provide a more normally-distributed data set because the raw data was significantly skewed positively (1.30)

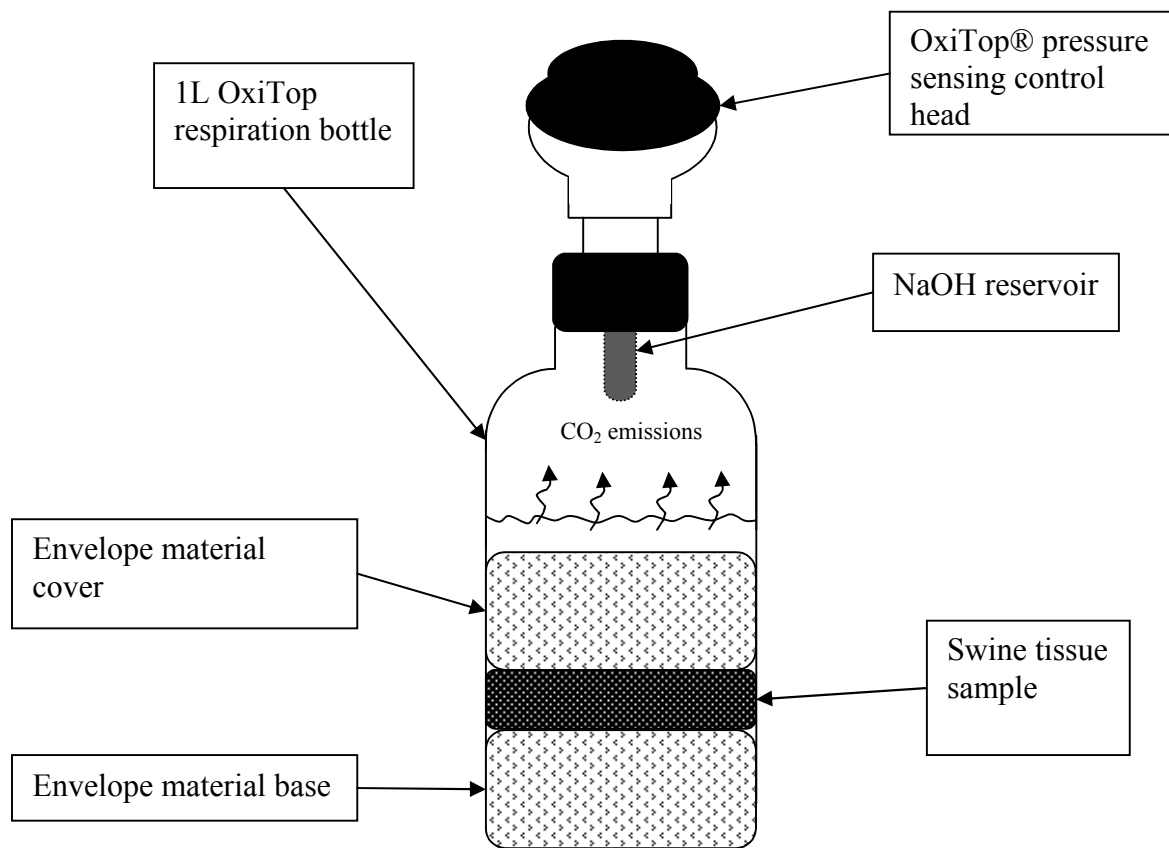


Figure 4. Schematic of OxiTop® respiration bottles used to evaluate swine tissue degradation and oxygen uptake of various envelope materials.

3.3.1.2 Envelope material preparation

All samples were collected within the same 3-5 day period. Corn silage was obtained from a silage bunk at Iowa State University (ISU) Beef Teaching Farm and was

approximately 10-12 months old. Oat straw was also collected from the ISU Beef Teaching Farm was stored under dry conditions to minimize degradation and was estimated to be 1 year old. Alfalfa hay was obtained from ISU Dairy farm and had been in storage for approximately 4 months. Wood shavings were Buchanan brand and kiln dried before packaging. Soybean straw and cornstalks were collected immediately following crop harvest from the ISU Agricultural Engineering Farm. After collection, all envelope materials were placed in a freezer at the ISU Livestock Environment Building Research Complex at -15 °C for approximately 1 month before the lab study was conducted to maintain integrity.

3.3.1.2.1 pH

pH of envelope materials was measured according to Test Methods for the Examination of Composting and Compost (TMECC) Section 04.11 – Electrometric pH Determinations For Compost. Samples of each envelope material were filtered through a 9.5 mm sieve prior to testing. pH meter was calibrated using pH 7.0 and 10.0 buffer solutions. Fisher Scientific AB15 Plus pH meter (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for all pH measurements. pH of envelope materials used for the lab study are shown in Table 5. Ideal pH for swine mortality composting is between 6.5 and 8.0 (Langston et al., 2002). Cornstalks and soybean straw were the only materials falling within the ideal range.

Table 5. pH of envelope materials used during composting lab-scale experiment. (N=3)

| Envelope material | Average |
|--------------------------|--------------------------|
| Alfalfa hay | 5.76 ± 0.01 ^a |
| Cornstalks | 7.64 ± 0.01 |
| Oat straw | 8.52 ± 0.02 |
| Silage | 5.67 ± 0.02 |
| Soybean straw | 6.53 ± 0.01 |
| Wood shavings | 4.83 ± 0.02 |

^a Standard deviation between replicates

3.3.1.2.2 C/N ratio

The C/N ratio of envelope materials was determined by the ISU Soil and Plant Analysis Lab (Table 6). Envelope materials with low C/N ratios generally have higher degradability and can help in achieving high internal temperatures. By varying carcass loading rates, the C/N ratio of mortality composting systems can be altered to achieve better performance.

Table 6. C/N ratio of envelope materials used during mortality composting lab-scale experiment. (N=2)

| Envelope material | Average |
|--------------------------|---------------------------|
| Alfalfa hay | 13.7 ± 148.1 ^a |
| Cornstalks | 69.0 ± 2.5 |
| Oat straw | 96.5 ± 4.2 |
| Silage | 37.4 ± 2.7 |
| Soybean straw | 62.1 ± 6.4 |
| Wood shavings | 286.7 ± 148.1 |

^a Standard deviation between replicates

3.3.1.2.3 Moisture content and volatile solids

Moisture content (% w.b.) and volatile solids (VS) (% d.b.) of envelope materials were determined at the beginning and end of each replication to quantify moisture and nutrient transfer between envelope materials and swine tissue samples. Moisture content of envelope materials was determined by oven drying at 105 °C for 24 hours. Volatile solids (VS) (% d.b.) were determined by combustion at 550 °C for 8 hours (TMECC, 2002).

Initial moisture and VS content of materials was determined upon collection from the field. After determining initial moisture content, materials were evenly split into four groups. Water was added to materials on a mass basis to achieve the desired moisture treatment level of 15, 25, 35, or 60% w.b. Materials wetter than the required moisture were dried at 30 °C for 24 hours to reduce the moisture content. Moisture content of all materials was re-checked after adding water and each time a new replication began. Samples were placed in freezer bags and stored in the freezer between replications to prevent water loss.

Volatile solids content was used to determine respiration rate of samples and was determined at the beginning of each replication. As the VS content of compost increases, microbial activity and therefore respiration rates increase (Sadaka, et al., 2006). The moisture and volatile solids content of envelope materials used during the lab-scale study are shown in Table 7.

Table 7. Initial moisture and volatile solids content of envelope materials used for each moisture treatment during lab-scale study. (N=3)

| Envelope material | Moisture treatment | Initial moisture content (% w.b.) | Initial volatile solids (% d.b.) |
|--------------------------|---------------------------|--|---|
| Alfalfa hay | 15% | 14.8 ± 0.7 ^a | 89.8 ± 0.7 |
| Alfalfa hay | 25% | 26.7 ± 0.7 | 89.3 ± 0.4 |
| Alfalfa hay | 35% | 35.8 ± 0.2 | 89.1 ± 0.6 |
| Alfalfa hay | 60% | 60.0 ± 0.1 | 88.5 ± 0.2 |
| Cornstalks | 15% | 13.9 ± 0.5 | 95.2 ± 0.3 |
| Cornstalks | 25% | 25.3 ± 1.5 | 95.1 ± 0.4 |
| Cornstalks | 35% | 38.9 ± 2.5 | 95.2 ± 0.2 |
| Cornstalks | 60% | 62.0 ± 1.2 | 94.6 ± 0.2 |
| Oat straw | 15% | 16.5 ± 2.0 | 92.4 ± 0.3 |
| Oat straw | 25% | 23.7 ± 0.6 | 92.3 ± 0.1 |
| Oat straw | 35% | 37.0 ± 0.9 | 92.3 ± 0.2 |
| Oat straw | 60% | 57.8 ± 0.9 | 92.2 ± 0.3 |
| Silage | 15% | 15.9 ± 1.0 | 95.2 ± 0.4 |
| Silage | 25% | 27.1 ± 0.9 | 94.2 ± 1.0 |
| Silage | 35% | 36.4 ± 0.5 | 95.0 ± 0.4 |
| Silage | 60% | 55.8 ± 0.6 | 93.2 ± 0.4 |
| Soybean straw | 15% | 14.0 ± 1.0 | 97.4 ± 0.4 |
| Soybean straw | 25% | 26.4 ± 0.7 | 97.1 ± 0.4 |
| Soybean straw | 35% | 35.9 ± 0.9 | 97.2 ± 0.1 |
| Soybean straw | 60% | 55.9 ± 0.1 | 96.4 ± 0.5 |
| Wood shavings | 15% | 14.0 ± 0.4 | 99.5 ± 0.0 |
| Wood shavings | 25% | 26.7 ± 0.5 | 99.5 ± 0.0 |
| Wood shavings | 35% | 37.2 ± 1.2 | 99.5 ± 0.0 |
| Wood shavings | 60% | 55.8 ± 0.4 | 99.5 ± 0.1 |

^a Standard deviation between replicates

3.3.1.2.4 Particle Size

The particle size of long, fibrous envelope materials, such as cornstalks, oat straw, soybean straw, and alfalfa, were reduced by grinding to a size of approximately 1-2 cm. This helped increase the surface area contact between microbes and organic material, leading to better interaction of swine tissues and envelope materials. Figure 5 shows the wood chipper used for particle size reduction of envelope materials.



Figure 5. Troy-Bilt wood chipper used for envelope material particle size reduction.

3.3.1.3 Swine tissue sample preparation

Swine tissue samples were obtained from a central Iowa swine producer. Hogs were euthanized when possible; otherwise only recently deceased carcasses were used to maintain sample integrity. All swine tissues samples were from hogs of relatively the same age and uniform in composition. To maintain consistency, tissues were always collected from the ham muscle. In order to cut and trim swine tissue samples to a uniform and consistent size, entire hams were removed from carcasses, cut flat and frozen solid. Once frozen, hams were trimmed to approximately 1 cm thickness and circular pieces were cut out using a 10 cm diameter hole saw (Figure 6). Each piece weighed approximately 56 grams and was enveloped in approximately 0.7 L of envelope material when placed in OxiTop® bottles. This ratio of swine tissue to envelope material was based on carcass composting research conducted by Glanville (2006b).



Figure 6. Swine tissue samples used during lab-scale experiment cut to uniform size using a hole saw.

3.3.1.3.1 Moisture content and volatile solids

Moisture and VS content of swine tissues were determined by oven drying at 105 °C for 24 hours and combusting in a furnace at 550 °C for 8 hours. As an extra precaution, samples were taken out of the drying oven after 24 hours, weighed, and placed back in the oven for an addition 2 hours to ensure sample mass had stabilized and drying was complete. As with envelope materials, moisture and VS content of swine tissue samples was determined before and after each replication. The moisture and volatile solids content of swine tissues used during the lab-scale study are shown in Table 8.

Table 8. Initial moisture and volatile solids content of swine tissue samples used for each moisture treatment during lab-scale study. (N=3)

| Envelope material | Moisture treatment | Initial moisture content (% w.b.) | Initial volatile solids (% d.b.) |
|--------------------------|---------------------------|--|---|
| Alfalfa hay | 15% | 60.3 ± 6.1 ^a | 97.8 ± 0.5 |
| Alfalfa hay | 25% | 58.9 ± 8.8 | 97.8 ± 0.8 |
| Alfalfa hay | 35% | 51.8 ± 18.3 | 98.1 ± 1.4 |
| Alfalfa hay | 60% | 59.7 ± 8.9 | 97.6 ± 0.7 |
| Cornstalks | 15% | 67.8 ± 8.1 | 96.9 ± 1.2 |
| Cornstalks | 25% | 51.6 ± 5.1 | 98.5 ± 0.3 |
| Cornstalks | 35% | 63.2 ± 6.6 | 97.6 ± 0.8 |
| Cornstalks | 60% | 54.8 ± 7.3 | 98.3 ± 0.4 |
| Oat straw | 15% | 58.5 ± 10.9 | 98.0 ± 1.2 |
| Oat straw | 25% | 68.7 ± 2.9 | 96.9 ± 0.3 |
| Oat straw | 35% | 65.9 ± 5.3 | 97.4 ± 0.7 |
| Oat straw | 60% | 56.6 ± 9.1 | 98.1 ± 0.7 |
| Silage | 15% | 58.8 ± 10.3 | 97.9 ± 0.9 |
| Silage | 25% | 61.9 ± 5.9 | 97.1 ± 0.0 |
| Silage | 35% | 55.9 ± 8.8 | 98.1 ± 0.6 |
| Silage | 60% | 60.8 ± 10.4 | 97.3 ± 1.2 |
| Soybean straw | 15% | 68.6 ± 0.5 | 96.9 ± 0.1 |
| Soybean straw | 25% | 58.7 ± 6.5 | 97.8 ± 0.7 |
| Soybean straw | 35% | 62.1 ± 8.5 | 97.4 ± 0.7 |
| Soybean straw | 60% | 58.1 ± 6.4 | 97.8 ± 0.6 |
| Wood shavings | 15% | 60.4 ± 7.8 | 97.8 ± 0.5 |
| Wood shavings | 25% | 62.8 ± 2.8 | 97.7 ± 0.4 |
| Wood shavings | 35% | 56.6 ± 5.9 | 98.1 ± 0.3 |
| Wood shavings | 60% | 60.5 ± 10.9 | 97.8 ± 0.7 |

^a Standard deviation between replicates

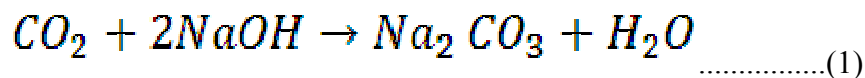
3.3.1.4 Respiration tests – OxiTop® measuring system

The respiration rate of mortality compost samples was determined using the OxiTop® measuring system, using methods similar to those used by Sadaka et al. (2006) and Ahn et al. (2008). The OxiTop measurement system (Figure 7) measures the decline in pressure in a closed vessel to determine the respiration rate of a sample (Wageningen and NMI, 2003).



Figure 7. OxiTop® bottle with pressure sensor head used for respiration tests.

The system consists of a 1.138 L jar, pressure sensor datalogger head (OxiTop-C WTW, Weiheim, Germany), and a plastic chamber used to contain sodium hydroxide (NaOH) pellets. Sodium hydroxide pellets absorb CO₂ produced during aerobic respiration, leading to a pressure drop (hPa) measured several times per hour, which is directly related to O₂ utilization. The chemical reaction in which NaOH absorbs CO₂ is represented in Equation 1 (Sadaka et al., 2006):



During the testing period, changes in pressure are logged by the pressure sensor head several times per hour. Data is collected via a controller (OxiTop® OC 110 WTW, Weiheim, Germany) (Figure 8) and OxiTop® software (Achat OC, PC communication software version 2.03) is used to download the data to a spreadsheet.

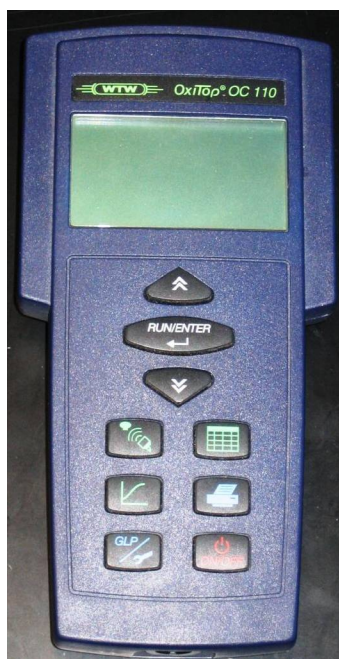


Figure 8. OxiTop® OC 110 Controller used to collect data from OxiTop® pressure sensor heads.

The oxygen uptake rate (OUR) can then be calculated from the recorded changes in pressure according to Equation 2 (Ahn et al., 2008):

$$O_2 = \frac{\Delta P (hPa) \times 100 \left(\frac{Pa}{hPa} \right) \times 1 \left(\frac{N}{m^2} \right) \times V (m^3) \times 32 \left(\frac{g}{mole} \right) \times 1000 \left(\frac{mg}{g} \right)}{8.314 \left(\frac{J}{mole \cdot K} \right) \times 1 \left(\frac{N \cdot m}{J} \right) \times T (K) \times \frac{t (h)}{24 \left(\frac{h}{d} \right)} \times W (g) \times (1 - MC) \times VS_{decimal}} \dots (2)$$

- Where: O_2 = the consumed oxygen [mg/g_{vs}-d]
 ΔP = the difference between the maximum and final pressure (hPa)
 V = the jar volume, 0.00138 (m³)
 T = the incubation temperature, 318.15 (K)
 t = the incubation time, 48 (h)
 W = the weight of sample (g)
 MC = initial moisture content (% w.b.)
 VS = initial volatile solids (% d.b.)

Moisture and VS content used in OUR calculations were determined before each replication.

3.3.1.5 Incubation

3.3.1.5.1 Temperature

OxiTop bottles were incubated at a temperature of 45 °C for this study. This temperature was chosen based on research conducted by Mari et al. (2003), who found that measured respiration rates from composting of olive oil by-products at 48.5 °C provided a better indicator of maximum respiration. Respiration tests done at lower temperatures may underestimate the microbial activity occurring during actual composting (Mari et al., 2003). Haug (1993) also determined that nitrifying bacteria are inhibited at temperatures above 45 °C. Finally, this temperature was decided upon because it was thought to be more representative of temperatures that actually occur during mortality composting. Temperatures were controlled at constant level of 45 °C throughout the 10 day testing period.

3.3.1.5.2 Time

A period of 10 days was chosen for the duration of each replication. Several days are sometimes needed for microbial populations to acclimate to their environment. The testing period duration was chosen based on preliminary respiration tests conducted on swine tissue and envelope material samples which showed respiration rates were starting to slow down and level off for all materials before 10 days had elapsed. Bottles were incubated using a Fisher Scientific Isotemp 228 water bath (Thermo Fisher Scientific, Waltham, Massachusetts, USA) shown in Figure 9.



Figure 9. Fisher Scientific water bath used for OxiTop® bottle incubation.

3.3.2 Respiration measurement procedure

In review, the OxiTop® system measures respiration rates (and therefore levels of microbial activity) based on changes in pressure within the OxiTop® bottles. Pressure within the bottles increases initially due to increased temperatures within the water bath. Once the temperature is equilibrated, pressure begins to decrease as organic matter is degraded, releasing CO₂ which is absorbed by NaOH pellets causing a pressure drop. Respiration rates are influenced by many factors including temperature, moisture, C:N ratio, oxygen, and pH. If optimal conditions are not met for each parameter, measured values may be low because of decreased microbial activity. Because the OxiTop® method is a static system, oxygen must be replenished periodically. For this reason, bottles were opened and oxygen was circulated through the system every 2 days to prevent anaerobic conditions.

Sodium hydroxide pellets were also replaced every 2 days so CO₂ absorption would not be limited. Therefore, respiration data collected during each replication were analyzed to obtain daily average oxygen uptake rates (OURs) every 2 days (Day 2, 4, 6, 8, and 10). Although these precautionary measures were taken, natural fluctuations in pressure occurred in some of the treatments during all replications. These variations can be explained and divided into four distinct phases (Wageningen University & NMI, 2003):

1. Initial pressure increase, due to differences in room and water bath temperature.
2. Lag phase, where microbial population is becoming acclimated to the environment.
3. OUR of the sample is the only limiting factor.
4. O₂ depletion, oxygen uptake is reduced or stops completely due to O₂ depletion.

It is during phase 3 that the true oxygen uptake rate of a material is measured. Therefore, time frames during the laboratory testing period with linear respiration rates were selected to represent the true respiration rate for each 2 day period. Care was taken to provide consistent conditions, but not necessarily optimal, for all replications of this lab experiment. Measured values then are not considered absolute performance indicators of the materials, but can give an indication of performance under these particular conditions, and materials can be ranked based on these conditions.

Daily average OUR for each material and moisture treatment are an average of 3 replications, and are a measure of mg of O₂ consumed per gram of volatile solids per day (O₂ mg/g VS-d). Oxygen uptake rates are calculated based on total initial VS content, which vary for each treatment. The initial total VS content (g) includes VS of envelope material and tissue sample for each treatment.

CHAPTER 4. RESULTS

During lab experiment replications, visual inspection of many OxiTop® bottles revealed moisture transfer between tissue samples and initially dry envelope materials (Figure 10). This phenomenon was not as noticeable in treatments with 60% moisture content because envelope materials were initially very moist. Excessive drying of tissue samples was also noticed in some treatments, particularly low moisture (15 and 25%) silage and alfalfa treatments (Figure 11). This supports observations from composting field trials where desiccated carcasses were uncovered from test units which had low initial moisture content. In general, moisture content of envelope materials increased during the experiment, as high moisture content tissue samples lost moisture to the surrounding envelope materials.

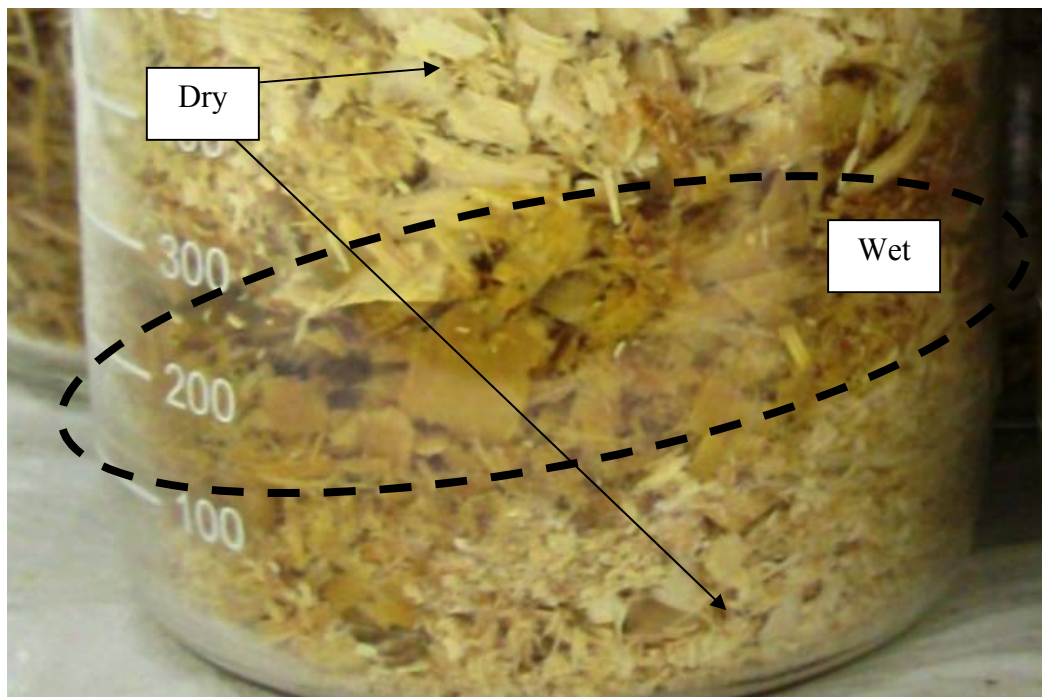


Figure 10. Wood shavings treatment exhibiting moisture transfer from high moisture content swine tissue sample to the surrounding dry envelope material. Area inside dotted line shows where swine tissue sample was located.

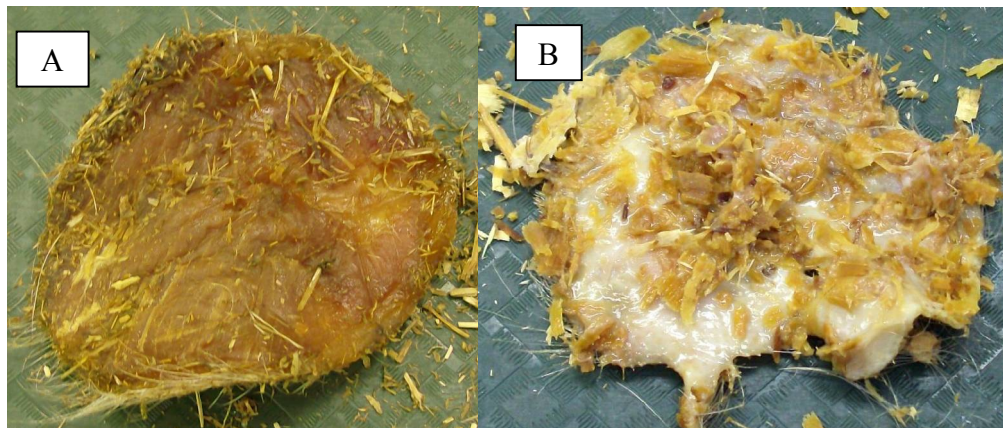


Figure 11. Swine tissue sample removed from 15% moisture alfalfa treatment exhibiting excessive drying (A) and heavily degraded tissue sample removed from 35% moisture wood shavings treatment (B).

4.1 Moisture Interactions

4.1.1 Moisture treatment vs. final envelope material moisture

To evaluate moisture transfer between envelope materials and swine tissues, several one-way ANOVA tests were conducted. A one-way ANOVA of initial envelope material moisture treatment (15, 25, 35, and 60%) was shown to have a strong effect ($p < 0.0001$) on final envelope material moisture (Figure 12). Mean final envelope material moisture content was found to be significantly different across all moisture levels using Tukey HSD significance testing (Table 9). Moisture treatment level also had a strong effect ($p < 0.001$) on final tissue moisture content (Figure 13), but significant differences between moisture levels were not as noticeable (Table 10). Material type had little effect ($p < 0.2651$) on final envelope material moisture content (Figure 14) and there was no significant difference between materials (Table 11). Material type had a strong effect ($p < 0.0002$) on final tissue

moisture, (Figure 15) but few significant differences were noticed between materials (Table 12). Final tissue sample moisture was lowest in silage and alfalfa treatments, which may have implications with decomposition and respiration data discussed in later sections.

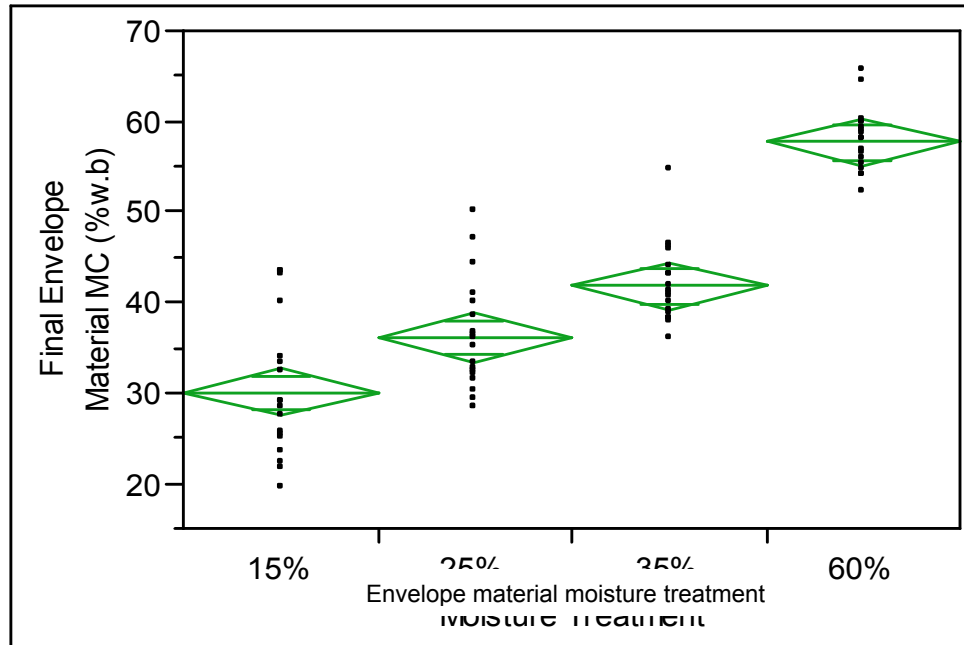


Figure 12. One-way ANOVA of envelope material moisture treatment effect on final envelope material moisture content (MC). Diamonds represent the 95% confidence interval of the mean.

Table 9. Comparison of mean final envelope material moisture content (% w.b.) within four envelope material moisture treatments.

| Envelope material moisture treatment | Mean final envelope material moisture |
|--------------------------------------|---------------------------------------|
| 60% | 57.6 |
| 35% | 41.8 |
| 25% | 36.1 |
| 15% | 30.1 |

Levels not connected by same letter are significantly different. ($p < 0.05$)

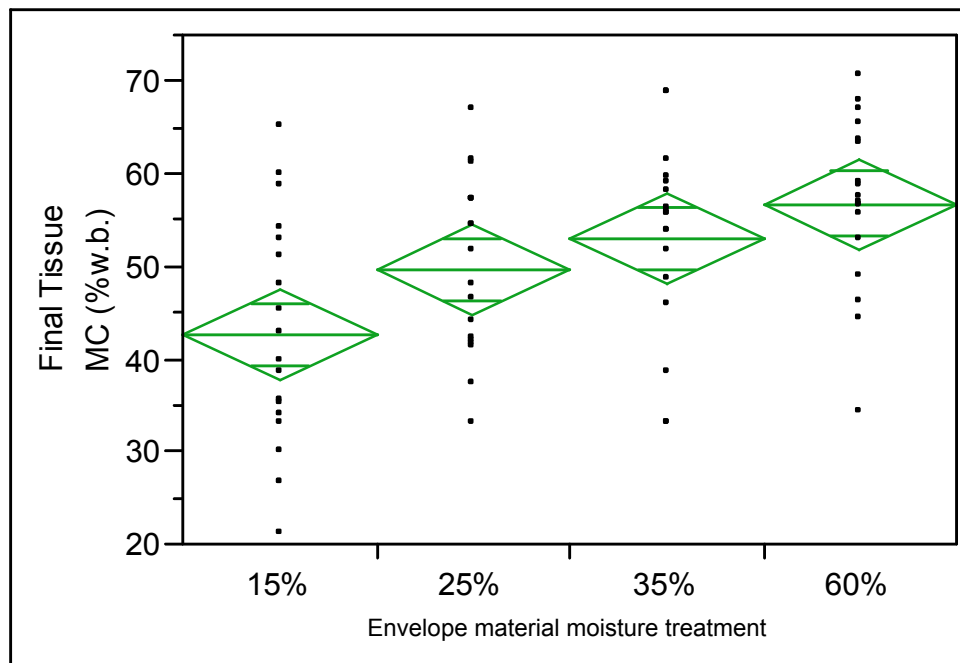


Figure 13. One-way ANOVA of envelope material moisture treatment effect on final swine tissue moisture content (MC). Diamonds represent 95% confidence interval of the mean.

Table 10. Comparison of mean final swine tissue moisture content (% w.b.) within four envelope material moisture treatments.

| Envelope material moisture treatment | | Mean final swine tissue moisture | |
|--------------------------------------|---|----------------------------------|------|
| 60% | A | | 56.7 |
| 35% | A | | 53.0 |
| 25% | A | B | 49.6 |
| 15% | | B | 42.6 |

Levels not connected by same letter are significantly different ($p < 0.05$).

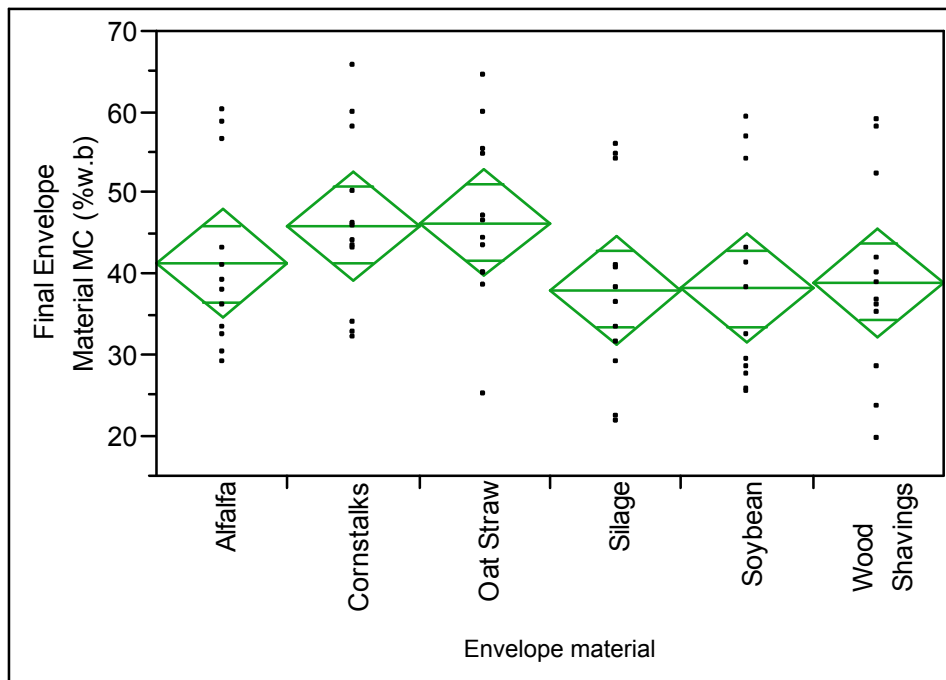


Figure 14. One-way ANOVA of final envelope material moisture content (% w.b.) by envelope material. Diamonds represent 95% confidence interval of the mean.

Table 11. Comparison of mean final envelope material moisture content (% w.b.) within six envelope materials.

| Envelope material | | Mean final envelope material moisture |
|-------------------|---|---------------------------------------|
| Oat straw | A | 46.3 |
| Cornstalks | A | 45.9 |
| Alfalfa hay | A | 41.2 |
| Wood shavings | A | 38.0 |
| Soybean straw | A | 38.1 |
| Silage | A | 37.9 |

Levels not connected by same letter are significantly different ($p < 0.05$).

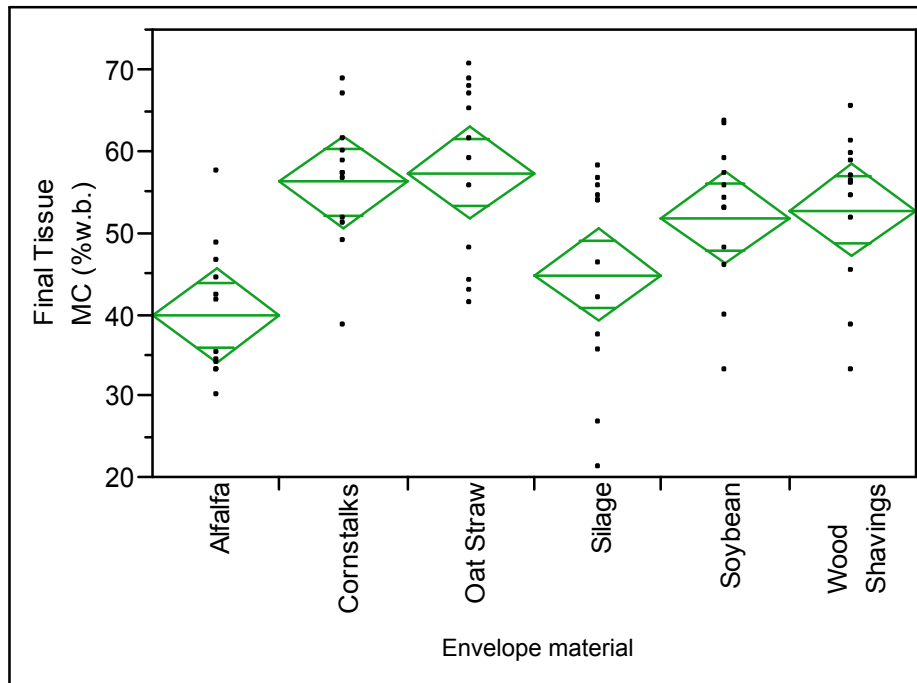


Figure 15. One-way ANOVA of final swine tissue moisture content (% w.b.) by envelope material. Diamonds represent 95% confidence interval of the mean.

Table 12. Comparison of mean final swine tissue moisture content (% w.b.) within six envelope materials.

| Envelope material | | Mean final swine tissue moisture | | |
|-------------------|---|----------------------------------|---|------|
| Oat straw | A | | | 57.4 |
| Cornstalks | A | B | | 56.2 |
| Wood shavings | A | B | | 52.8 |
| Soybean straw | A | B | | 51.9 |
| Silage | | B | C | 44.9 |
| Alfalfa hay | | | C | 39.8 |

Levels not connected by same letter are significantly different ($p < 0.05$).

4.2 Swine Tissue Decomposition

Swine tissue decomposition percentages for all envelope materials within moisture treatments are represented in Figure 16.

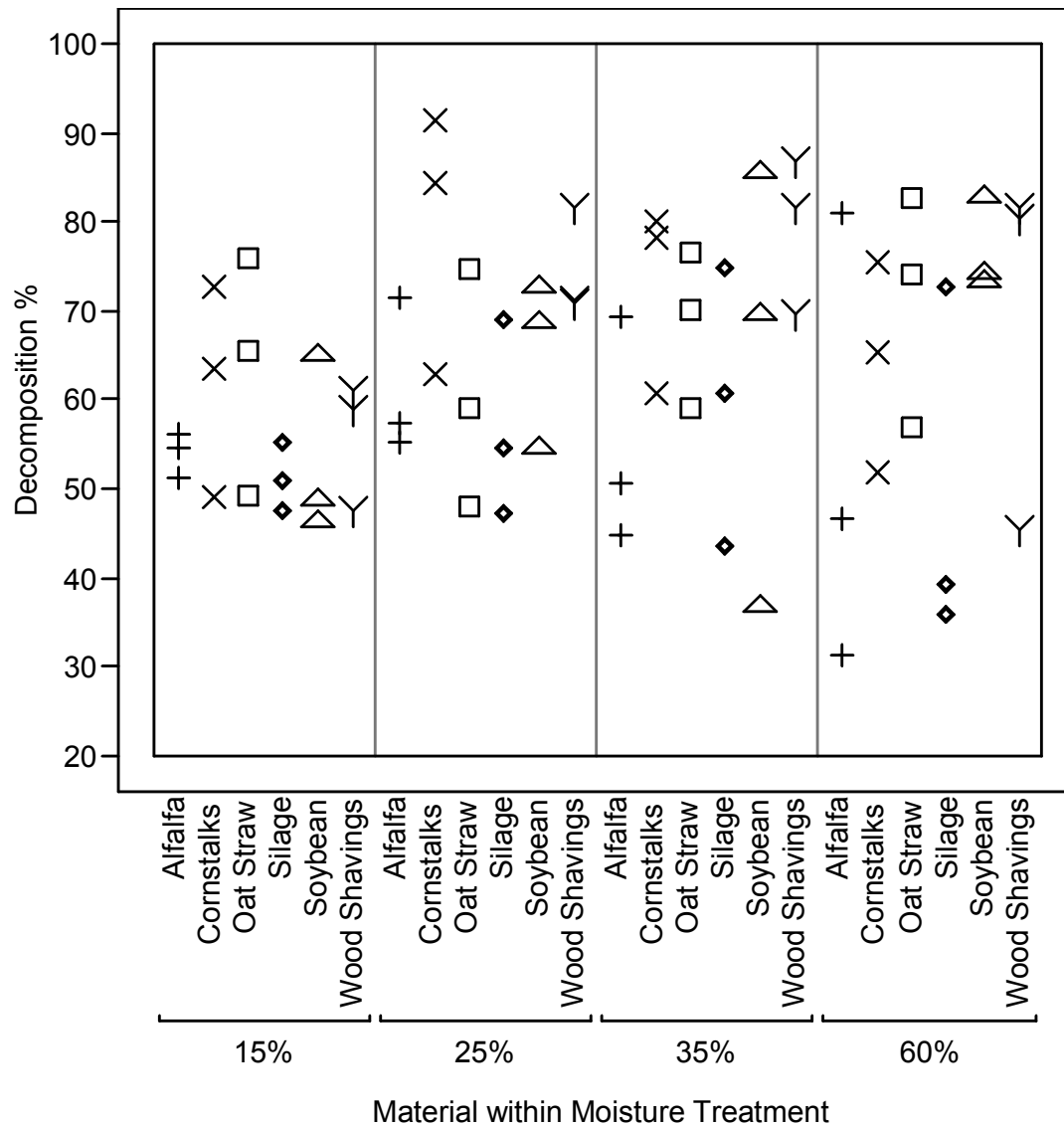


Figure 16. Swine tissue sample decomposition (%) as a function of envelope material within moisture treatment during 10-day lab-scale study. (N=3)

4.2.1 Envelope material effect

Envelope material had a strong effect ($p < 0.0001$) on decomposition percentage and several materials showed significantly different ($p < 0.05$) predicted least squares mean decomposition values (Table 13). Wood shavings treatments had the highest decomposition, followed by cornstalks, oat straw, soybean straw, alfalfa hay, and silage respectively. Wood shavings, cornstalks, oat straw, and soybean straw all had decomposition percentages of approximately 65-70%. Alfalfa hay and silage had decomposition percentages of approximately 55%, and were both significantly lower than wood shavings, cornstalks, and oat straw. Silage was also significantly lower than soybean straw.

Table 13. Comparison of mean decomposition (%) of swine tissue samples within six envelope materials.

| Envelope material | | | | Least squares mean decomposition (%) |
|-------------------|---|---|---|--------------------------------------|
| Wood shavings | A | | | 69.7 |
| Cornstalks | A | | | 69.3 |
| Oat straw | A | | | 65.9 |
| Soybean straw | A | B | | 64.9 |
| Alfalfa hay | | B | C | 55.8 |
| Silage | | | C | 54.3 |

Levels not connected by same letter are significantly different ($p < 0.05$)

4.2.2 Moisture treatment effect

Envelope material moisture treatment also had a strong effect ($p < 0.0009$) on swine tissue decomposition. Predicted least squares mean decomposition percentages for each moisture treatment are shown in Table 14. The 35%, 25%, and 60% moisture treatments had the highest decomposition percentages and were all within 3% of each other. The 15%

moisture treatment had a much lower decomposition percentage of 56.6%, which was significantly lower than the other three treatments.

Table 14. Comparison of predicted mean decomposition (%) of swine tissue samples within four envelope material moisture treatments.

| Envelope material moisture treatment | | Least squares mean decomposition (%) | |
|--------------------------------------|---|--------------------------------------|------|
| 35% | A | | 66.5 |
| 25% | A | | 66.3 |
| 60% | A | | 63.9 |
| 15% | | B | 56.6 |

Levels not connected by same letter are significantly different ($p < 0.05$)

The p-value of the moisture treatment \times envelope material interaction on decomposition percentage was just small enough ($p < 0.0482$) to be considered significant at the 5% level, but the effects of material and moisture treatment individually have a much greater influence on swine tissue decomposition.

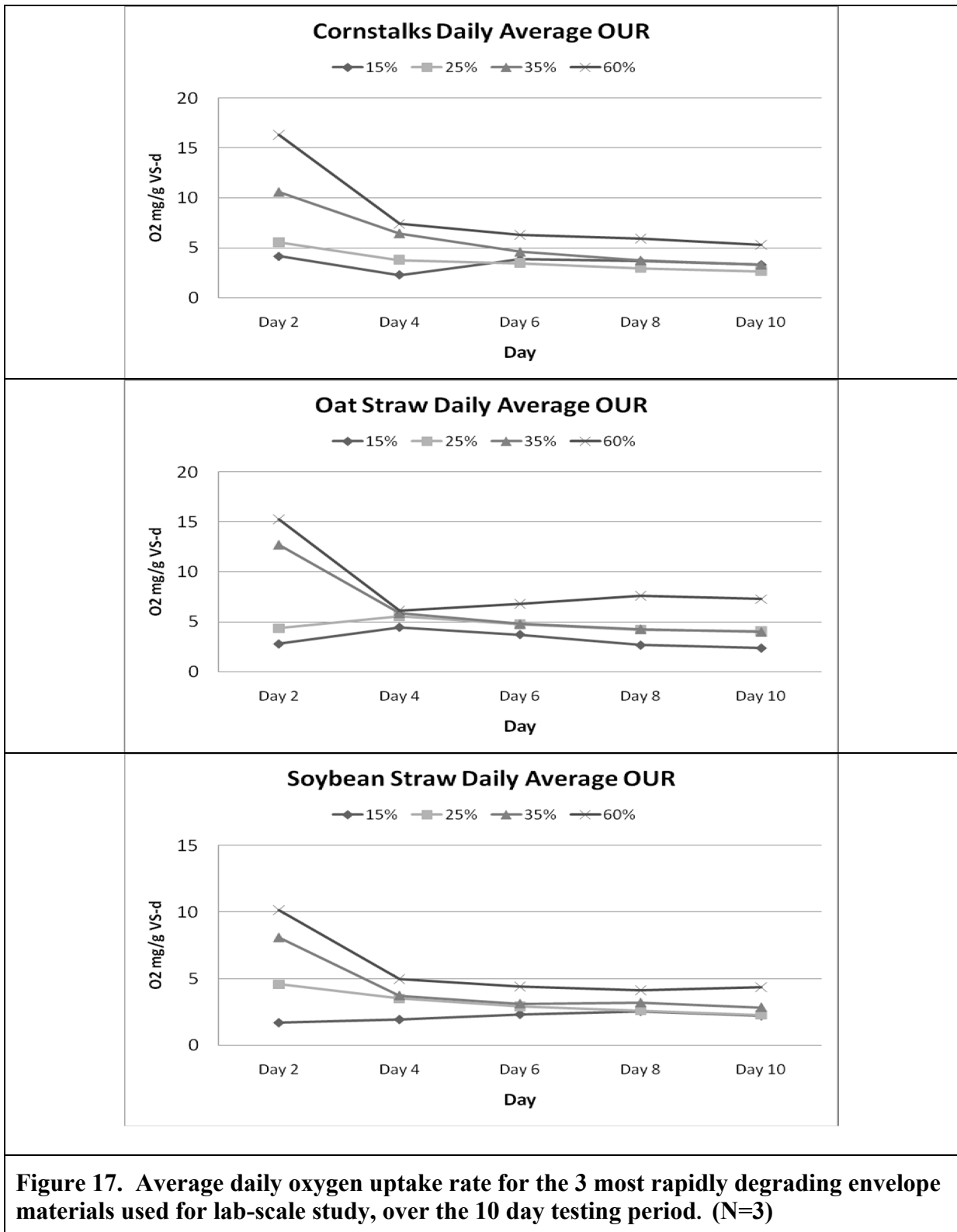
4.3 Respiration / Oxygen Uptake

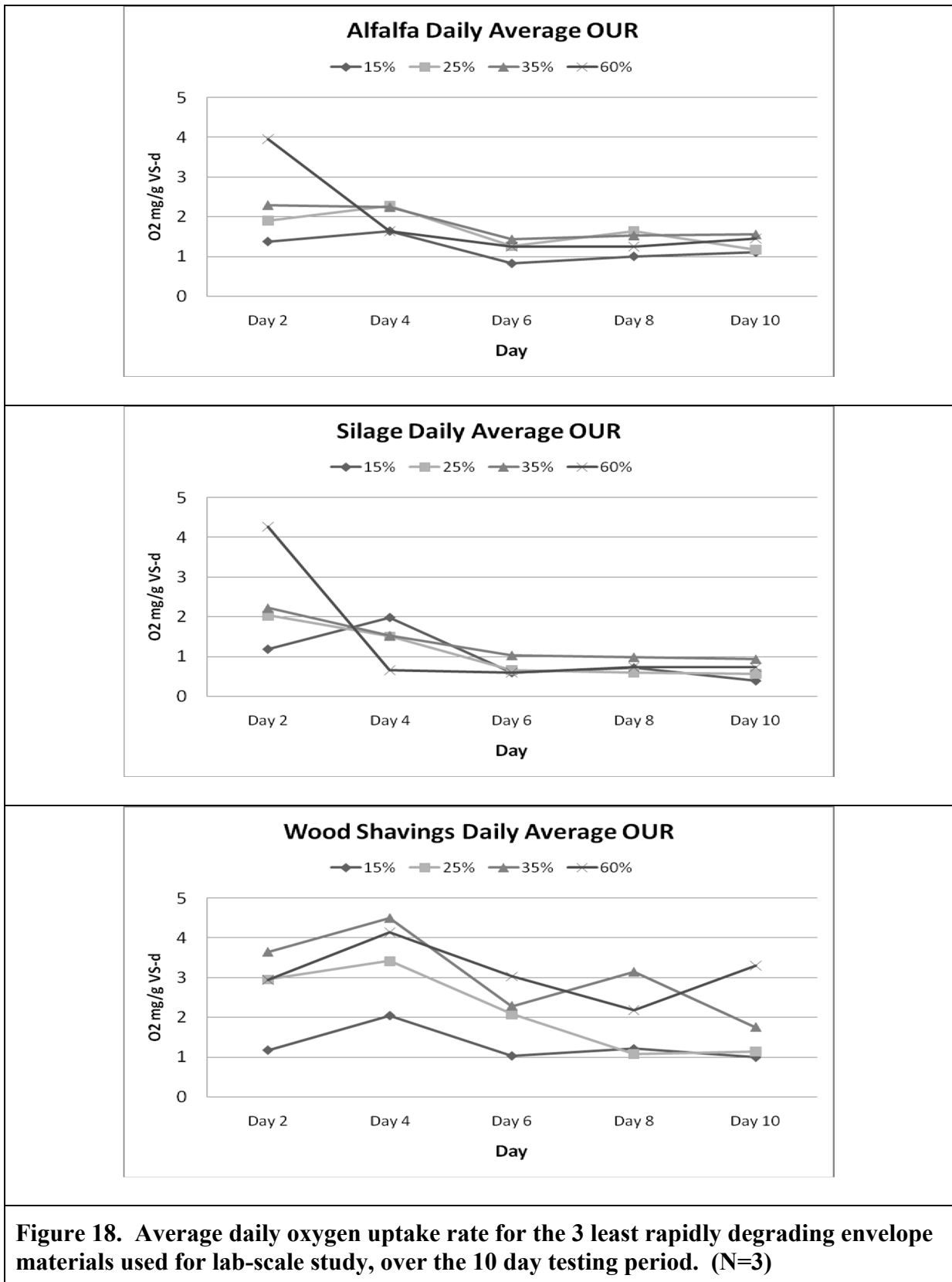
Figure 17 shows the average daily OUR over the 10 day testing period for the 3 MOST rapidly degrading envelope materials (cornstalks, oat straw, and soybean straw) used in the lab study. Figure 18 shows average daily OUR for the 3 LEAST rapidly degrading (alfalfa, silage, and wood shavings) envelope materials used in this study. These graphs show oxygen uptake rate trends over time and across initial moisture treatments for each material. In general, OUR was highest during the first 2 days and gradually decreased. The 60% moisture treatment also appears to have the highest OUR throughout the 10 day testing period, particularly in the top 3 most rapidly degrading materials.

As indicated previously in the methods section, OURs for each treatment vary between days and replications. Therefore it was decided total oxygen uptake (TOU) values would be a better indicator of overall performance from the collected respiration data. Figure 19 and Figure 20 show the natural log of TOU values for the 3 most rapidly degrading and the 3 least rapidly degrading materials used in this study, respectively. The raw total oxygen uptake data were significantly skewed positively (1.30); therefore the natural log of TOU was used to achieve a more symmetrical data set for modeling purposes.

Envelope material type had a strong effect ($p < 0.0001$) on TOU. Comparison of least squares mean values for the natural log of TOU values by material are shown in Table 15 (while statistical analysis was conducted on the natural log of TOU values, actual TOU values are shown for reference only). The 3 most rapidly degrading materials (oat straw, cornstalks, and soybean straw) were statistically different from the 3 least rapidly degrading materials (wood shavings, alfalfa, and silage). This is somewhat surprising as previous research by Ahn et al. (2008) has shown alfalfa and silage to have very high biodegradability compared to other materials tested in this experiment. However, it should be noted that the age differences in silage samples may be responsible for changes in biodegradability.

Initial moisture treatment also had a significant impact ($p < 0.0001$) on TOU. Least squares mean values for the natural log of TOU by moisture treatment (Table 16) shows TOU increases as initial moisture content increases. Total oxygen uptake was highest in 60% moisture treatments, followed by 35, 25, and 15%. The 60 and 35% moisture treatments had similar TOU, and were both significantly different from 25 and 15% moisture treatments.





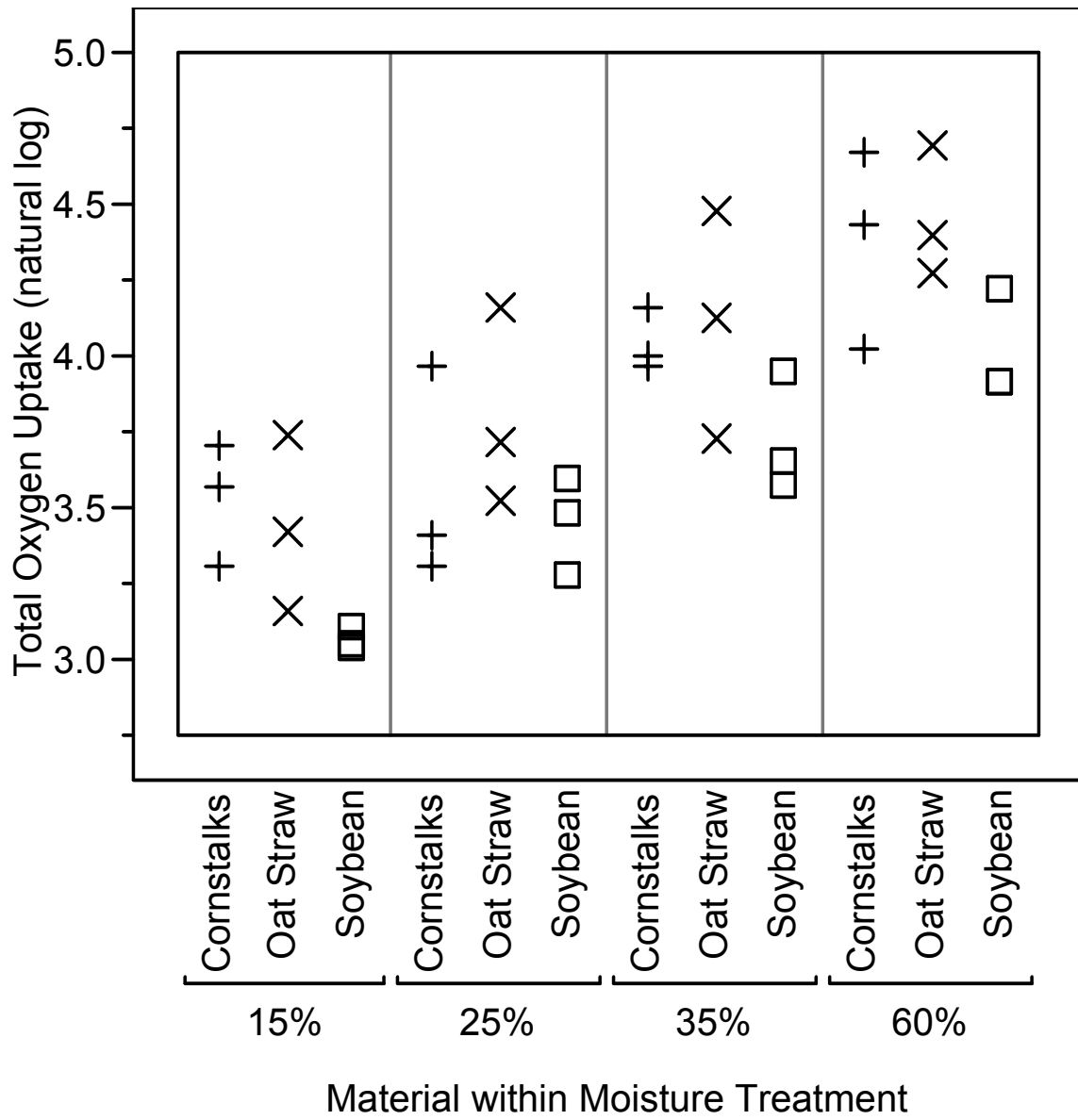


Figure 19. Total oxygen uptake as a function of material within moisture for the three most rapidly degrading materials used in this experiment. (N=3)

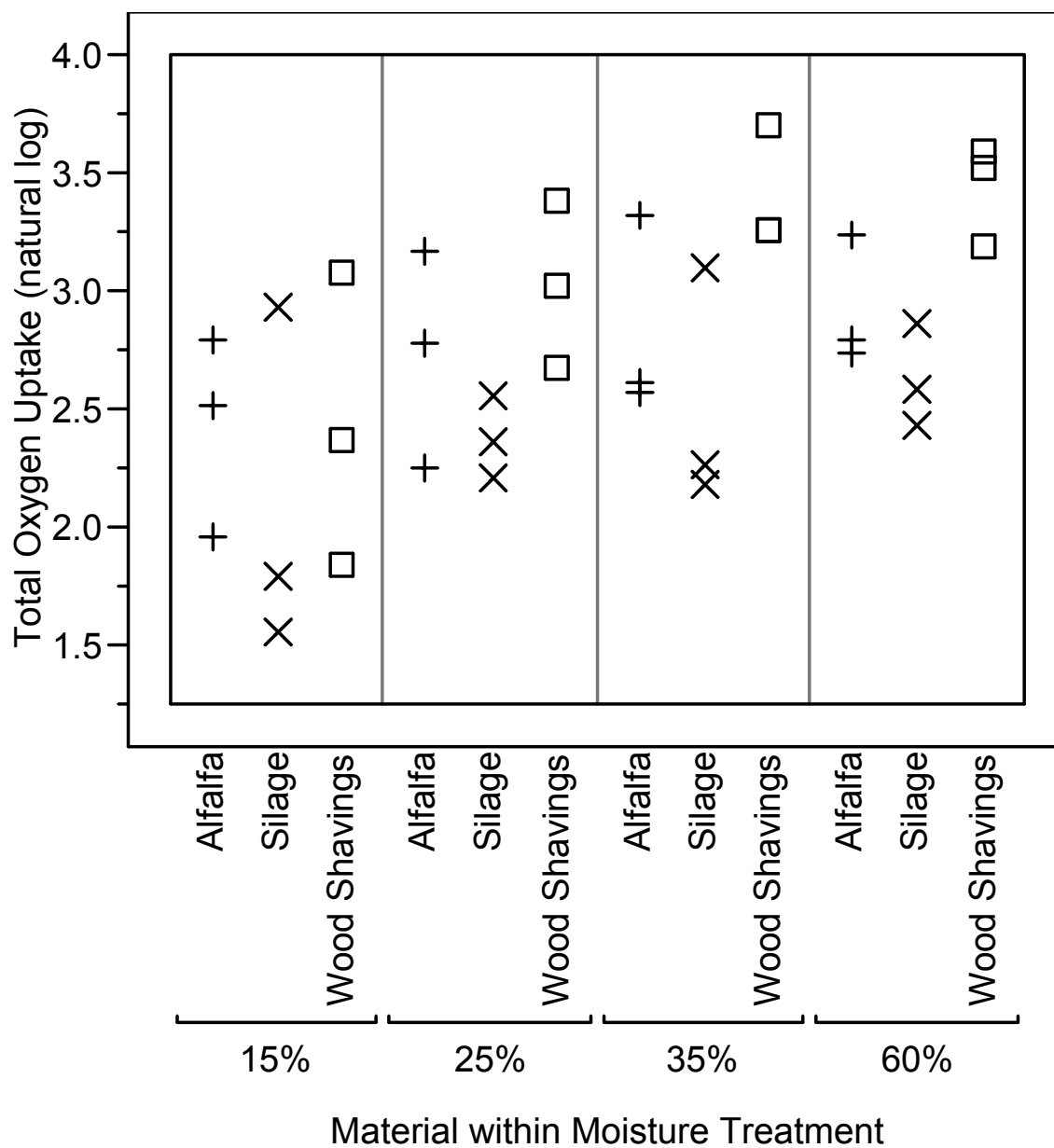


Figure 20. Total oxygen uptake as a function of material within moisture treatment for the 3 least rapidly degrading materials tested in this experiment. (N=3)

Table 15. Comparison of least squares mean of the natural log of total oxygen uptake within six envelope materials. (Total oxygen uptake values are also shown for reference only.)

| Envelope material | | | | | Total oxygen uptake least squares mean (natural log) | Total O ₂ uptake (mg O ₂) |
|-------------------|---|---|---|---|--|--|
| Oat straw | A | | | | 3.9 | (51.4) |
| Cornstalks | A | B | | | 3.9 | (48.2) |
| Soybean straw | | B | | | 3.6 | (35.1) |
| Wood shavings | | | C | | 3.1 | (21.5) |
| Alfalfa hay | | | C | D | 2.7 | (15.3) |
| Silage | | | | D | 2.4 | (10.9) |

Levels not connected by same letter are significantly different ($p < 0.05$).

Table 16. Comparison of least squares mean of the natural log of total oxygen uptake within four moisture treatment levels. Total oxygen uptake values are also shown for reference only.

| Envelope material moisture treatment | | | | Total oxygen uptake least squares mean (natural log) | Total O ₂ uptake (mg O ₂) |
|--------------------------------------|---|---|---|--|--|
| 60% | A | | | 3.6 | (37.8) |
| 35% | A | | | 3.4 | (31.0) |
| 25% | | B | | 3.1 | (23.4) |
| 15% | | | C | 2.8 | (16.8) |

Levels not connected by same letter are significantly different ($p < 0.05$).

4.3 Key Findings

4.3.1 Moisture Interactions

Results from this study suggest envelope material initial moisture content may be a good indicator of final envelope material moisture. This may be useful in determining what initial moisture content envelope materials should be in order to maintain moisture levels within the optimal range throughout the entire composting process. Initial envelope material moisture also has a strong effect on final tissue sample moisture content, which may be useful in determining to what degree carcass desiccation may occur. One-way ANOVA of

final envelope material moisture compared with moisture treatment shows significant differences in final moisture content between all moisture treatment levels ($p < 0.05$), indicating significant moisture transfer between materials.

4.3.2 Moisture effect on decomposition and oxygen uptake

The predicted least squares mean of swine tissue decomposition in 25, 35, and 60% moisture treatments were within two percentage points, and all were significantly higher than the 15% treatment. Total oxygen uptake was highest in 35 and 60% moisture treatments, and both were significantly higher than 15 and 25% treatments. Raising envelope material moisture from 15 to 35% nearly doubled oxygen uptake, while no significant increase was shown when increasing moisture from 35 to 60%. While most composting literature generally recommends moisture levels of 40-65% to achieve optimal conditions, results from this study suggest similar results may be achieved with only modest (10 to 20%) increases in envelope material moisture. These results are promising, particularly in the event of an emergency disposal situation when large amounts of moisture addition may not be practical.

4.3.3 Material effect on decomposition and oxygen uptake

The predicted least squares mean of swine tissue decomposition was highest in wood shavings treatments, followed by cornstalks, oat straw, soybean straw, alfalfa hay, and silage respectively. Alfalfa and silage decomposition percentages were approximately 10% lower than the other four envelope materials, and were significantly lower than wood shavings, cornstalks, and oat straw treatments. These results are similar to carcass decomposition from field studies, as cornstalks and oat straw performed well in both studies, while silage had the lowest decomposition (Table 17). Total oxygen uptake was highest in oat straw treatments,

followed by cornstalks, soybean straw, wood shavings, alfalfa hay, and silage respectively. This lab study suggests materials such as oat straw, cornstalks, and soybean straw have good heat production potential, but based on excessive drying and carcass desiccation observed during field trials, these materials may lack heat retention capabilities due to their larger particle size. Therefore these materials may be effective for mortality composting with ventilation adjustments to reduce airflow through these materials.

Table 17. Mean decomposition of soft tissues from field-scale and lab-scale composting experiments. Different letters in superscript indicate significant differences between materials within a particular type of experiment ($p < 0.05$).

| Envelope Material | Lab-scale mean decomposition (%) | Field-scale mean decomposition (%) |
|--------------------------|---|---|
| Wood shavings | 69.7 ^a | 81.2 ^{ab} |
| Cornstalks | 69.3 ^a | 87.2 ^a |
| Oat straw | 65.9 ^a | 82.3 ^{ab} |
| Soybean straw | 64.9 ^{ab} | 85.4 ^a |
| Alfalfa hay | 55.8 ^{bc} | 81.1 ^{ab} |
| Silage | 54.3 ^c | 72.0 ^b |

Both swine tissue decomposition and TOU values appear to be good indicators of material and moisture effects on composting system performance. Envelope material and moisture treatment both had a strong effect on and total oxygen uptake, their interaction effect was not as significant. This suggests that selection of materials and controlling initial moisture content would be more beneficial to system performance than selecting a combination of the two variables.

CHAPTER 5. CONCLUSIONS

5.1 General conclusions

In answering the specific objectives outlined at the beginning of this study, the following conclusions can be drawn:

1. Results from this study suggest heat production potential (TOU) can significantly increase when raising initial moisture levels from 15 to 25%, but no increases were noticed when increasing from 25 to 60%. This suggests mortality composting systems may not require as much initial moisture, due to liquids and substrate provided from decomposing carcasses.
2. Significant increases in heat production potential were realized when increasing from 15 to 25%, and again when increasing from 25 to 35%. Significant increases in decomposition were noticed when increasing from 15 to 25%, but no other increases were noted. These results indicate that an initial moisture content of 25 to 35% may be sufficient as a minimum level for mortality composting systems.
3. There were no significant interactions between envelope materials and initial moisture content, which suggests that all envelope materials will perform similarly under the same initial moisture content.
4. Results from this study suggest modest increases (10 to 20%) in initial moisture can nearly double the heat production potential of mortality composting systems. These increases should be attainable during emergency disposal situations.
5. Results from this study show there may not be significant benefits to increasing moisture beyond the 25 to 35% range, which is slightly lower than the generally recommended level of 40 to 65%.

It should also be noted that while this laboratory study was developed in support of field-scale mortality composting results, direct comparisons between the two systems would be unrealistic because there are too many external variables associated with full-scale mortality systems. General conclusions from the data offer good indicators of mechanisms happening within full-scale systems:

- Respiration data collected from this study are more directly comparable to field-scale conditions than swine tissue decomposition data because they are representative of microbial activity and heat production potential of different envelope material × moisture treatment combinations, which could be applied to larger field-scale systems.
- The OxiTop measurement system provides a simple, quick, reliable, and repeatable measure of compost sample microbial activity.
- In general, envelope materials with moisture contents falling in the 40-65% range will have greater potential to produce elevated temperatures and increase carcass decomposition percentages. However, this study suggests that moisture levels in the 25-35% range may provide similar performance to those in the optimal range, which would be particularly useful when excessive moisture addition is not practical.
- Based on TOU data and observations from the field study, very porous materials may have very good heat production potential, but lack heat retention capabilities, suggesting ventilation adjustments may be useful to reduce airflow through these materials.

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