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Identification, evaluation and quantification of VOCs as biosecure markers of swine carcass degradation

Neslihan Akdeniz

Iowa State University

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<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aerobic</td>
</tr>
<tr>
<td>AA</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>AES</td>
<td>Atomic emission spectroscopy</td>
</tr>
<tr>
<td>AH</td>
<td>Alfalfa hay</td>
</tr>
<tr>
<td>AI</td>
<td>Avian influenza</td>
</tr>
<tr>
<td>An</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>APCI-MS</td>
<td>Atmospheric pressure chemical ionization spectroscopy</td>
</tr>
<tr>
<td>CAR</td>
<td>Carboxen</td>
</tr>
<tr>
<td>CSi</td>
<td>Corn silage</td>
</tr>
<tr>
<td>CST</td>
<td>Corn stalks</td>
</tr>
<tr>
<td>DMDS</td>
<td>Dimethyl disulfide</td>
</tr>
<tr>
<td>DMTS</td>
<td>Dimethyl trisulfide</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinyl benzene</td>
</tr>
<tr>
<td>ECD</td>
<td>Electron capture detector</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HA</td>
<td>Hexanoic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>IC</td>
<td>Ion chromatography</td>
</tr>
<tr>
<td>MBA</td>
<td>3-Methyl butanoic acid</td>
</tr>
<tr>
<td>MDL</td>
<td>Method detection limit</td>
</tr>
<tr>
<td>MIMS</td>
<td>Membrane inlet mass spectrometry</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>OS</td>
<td>Oat straw</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEA</td>
<td>Pentanoic acid</td>
</tr>
<tr>
<td>PR</td>
<td>Pyrimidine</td>
</tr>
<tr>
<td>PRA</td>
<td>Propanoic acid</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PTR-MS</td>
<td>Proton transfer reaction mass spectroscopy</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid phase microextraction</td>
</tr>
<tr>
<td>SS</td>
<td>Soybean straw</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile organic compounds</td>
</tr>
<tr>
<td>WS</td>
<td>Wood shavings</td>
</tr>
</tbody>
</table>
Swine carcasses were composted using passively-aerated composting system designed by the Canadian Food Inspection Agency during the 2004 Avian influenza outbreak in British Columbia. In this system, swine carcasses were fully covered by plastic sheets due to biosecurity concerns and visual inspection of the swine carcasses was impossible. Monitoring volatile organic compounds (VOCs) released from carcasses was a promising approach to assess progress and completion of the carcass degradation. In this study, VOCs were sampled using solid phase microextraction (SPME). Samples were analyzed using multidimensional gas chromatography-mass spectrometry (MDGC-MS) (a) to develop a comprehensive chemical library of volatile organic compounds emitted during carcass composting, (b) to determine if specific compounds could be correlated with various phases of the composting process and therefore be used to determine completion of composting and, (c) to determine the effects of compost operating parameters on the chemical make-up of gaseous emissions.

A completely new quantification method of measuring VOCs was developed with accuracy ranging from 79.04 to 98.53 % and method detection limits ranging from 0.01 to 580 ppbv. Eighty five µm CAR/PDMS was shown to extract the highest amount of analytes at one hour sampling time. Dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be produced during degradation of swine carcass tissues but not produced from decaying plant (envelope) materials. These compounds could serve as marker compounds of swine carcass degradation process. Laboratory studies showed that marker compounds cannot be detected in the headspace when the respiration rates of carcasses decrease to a level of 3.25 mg CO₂-C/g VS*d (stable compost). Field studies showed that when carcass degradation was incomplete, detection of marker compounds was still possible in the eighth
week of the process. After eight week composting time, the highest concentrations of marker compounds were detected for the carcass samples with the highest respiration rates (least stabilization). No relation was observed between temperature data and degradation rates of carcasses. A better estimate of carcass degradation was made by measuring concentrations of the marker compounds. The highest concentrations of the compounds were detected from the swine carcasses with the highest respiration rates and lowest decomposition. Dimethyl disulfide, dimethyl trisulfide, and pyrimidine were produced from all compost units under various conditions regardless of the plant material, moisture content, porosity and temperature. These compounds were reliable marker compounds that could be followed to test completion of a swine mortality composting process when the carcasses were fully covered by plant materials and plastic sheets due to biosecurity reasons.

**Keywords**

Compost, Dimethyl disulfide, Dimethyl trisulfide, GC-MS, Mortality, Pyrimidine, SPME, Swine, VFA, VOC
CHAPTER 1. GENERAL INTRODUCTION

1. Introduction

Composting is gaining acceptance as a disposal method for animal mortalities. Improper disposal of carcasses, such as illegal dumping, has become a problem due to limited number of licensed landfills, incineration and rendering services (Bonhotal et al., 2002). Biosecurity agencies in USA, Canada, Australia, and New Zealand have recognized the potential benefits of composting as an emergency management of mortalities (Wilkinson et al., 2007). Diseased mortality composting was first developed for the poultry industry (Spencer et al., 2004; Bendfeldt et al., 2006). During the 2004 Avian influenza outbreak in British Columbia (Canada), 1.25 million infected birds were disposed by burial, burning, and composting. At the beginning of the outbreak, carcasses were transported to other locations where they were buried and burnt. In the middle of the outbreak, the disposal strategy switched to on-farm composting. The first stage of the composting was conducted in the poultry houses. After 5 days, windrows were re-formed on a layer of heavy black plastic over wood shavings. Piles were passively aerated by black agricultural drainage pipe aligned across the plastic. The windrows were covered with vapor barriers, wood shavings, and finally black plastic. Studies have been conducted to adapt mortality composting for swine and cattle emergency disposal (Glanville et al., 2006a, 2006b; Ahn et al., 2007; Glanville et al., 2007).

In biosecure composting applications, mortalities are fully covered and degradation of mortalities cannot be monitored by visual inspection. Thus, assessment methods for the completion of decay process are needed. One promising approach is to monitor gases released by decaying mortalities. Composting operations generate VOCs including
oxygenated compounds (e.g., aldehydes, ketones, alcohols), nitrogen-containing compounds (e.g., pyrimidine), sulfur-containing compounds (e.g., mercaptans, sulfides), alkanes (e.g., pentane, hexane), alicyclic hydrocarbons (e.g., terpenes), and aromatic compounds (Golueke et al., 1954; Chanysak et al., 1982; Fisher et al., 1986; Koe and Ng, 1987; Derikx et al., 1990; Miller, 1993; Williams, and Miller, 1993; Day et al., 1998; Krzymien et al. 1999; Kim et al., 2005a). An understanding of the chemical components released from animal mortality composting processes can give scientists insights into the completion of the degradation. Moreover, the composition of the exhaust air indicates the aeration status of the process and quality of the compost (Day et al., 1999; Romain et al., 2005).

It can be challenging to analyze VOCs in air samples. Since air has a low density and VOCs have low concentrations (pg/L to µg/L), sample preparation for VOCs in air requires pre-concentration. SPME offers many advantages for air sampling such as high precision and sensitivity, applicability to high moisture samples, reusability, and compatibility with conventional analytical equipment (Pawliszyn, 1993; Mani, 1999; Wercinski, 1999; Jia et al., 2000; Augusto et al., 2001; Pacolay et al., 2006). With SPME, there is no need for Tedlar bags or Summa canisters for sampling or extraction (Kim et al., 2005a). Kim et al (2002) described a method that utilizes two different SPME fiber coatings for the analysis of propanoic acid, butyric acid, and sulfur compounds from waste treatment systems. Davoli et al (2003) and Kim et al. (2005b) characterized volatile organic compound emissions (e.g., propanoic acid, butanoic acid, hexanoic acid and dimethyl disulfide) from landfills using SPME and GC-MS. Kim et al (2005a) identified headspace odorants (e.g., propanoic acid, butanoic acid, and dimethyl disulfide) from commercial composts of 14 different producers using SPME method. Akdeniz et al. (2007a and 2007b) identified VOCs (e.g., volatile fatty acids, dimethyl disulfide, dimethyl trisulfide, and nitrogen containing compounds) during biological decomposition of plant and animal materials utilizing SPME and GC-MS. Syringe pump injection is a convenient quantification method, which does not require preparing a
high number of standard analyte solutions (Koziel et al., 2004). Syringe pumps inject liquid standards into an air stream at a delivery rate ranging from 0.0010 µl/h to 8800 ml/h, depending on the syringe volume.

Objectives of this study are (1) to develop an air sampling and analysis method for the quantification of VOCs from composting operations, (2) to test the applicability of the quantification method for a full scale biosecure swine mortality composting operation, (3) to identify characteristic VOCs evolved from decaying envelope materials and swine tissues in order to find marker compounds of animal tissue biodegradation, (4) to develop calibration curves to quantify marker VOC emissions from decaying swine tissues, (5) to evaluate a potential usage of VOCs to test completion of the carcass degradation in laboratory scale composting operations, (6) to develop a comprehensive chemical library of VOCs emitted from full scale swine mortality compost materials surrounded by plastic bio-security barriers, (7) to determine if specific compounds can be correlated with different phases of the composting process and be used to determine completion of the process in full scale composting operations, and (8) to determine the effects of compost operating parameters on the chemical make-up of gases.

References


2. Thesis organization

This thesis is consisted of a literature review followed by three journal papers: (a) Method for air sampling and analysis of VOCs from full scale mortality composting operations using SPME and GC-MS, (b) Evaluation of VOC emissions as biosecure markers of swine carcass degradation, (c) Identification, evaluation, and quantification of VOCs from full scale biosecure swine mortality composting operations, and a short communication paper: Sampling method of VOCs for biosecure composting operations. In addition, it contains a conclusion section where general conclusions and recommendations for future studies are discussed. Additional details on methods and results are presented as an appendix to the journal paper to which it applies.
CHAPTER 2. LITERATURE REVIEW

1. Animal mortality disposal

Iowa has led the US in swine production and pig inventory for one hundred twenty years (Honeyman and Duffy, 2006). In 2005, Iowa produced 28.4% (8 billion pounds) of all the swine in the U.S (Iowa Agricultural Statistics Bulletin). This intensive production of swine in one area inevitably results in production of high amount of piggery waste including animal carcasses. Animal carcasses, like all animal waste, contain useful nutrients which can be recycled into agricultural land. However, this recycling must be done in an environmentally sound, economically feasible and socially acceptable manner especially when carcasses are capable of spreading diseases in soil, plants, animals and humans (Imbeah, 1997, Kalbasi et al., 2005). Methods for disposal of swine carcasses include on-farm disposal (i.e., burial, incineration, and composting) and off-farm utilization (i.e., rendering and sanitary landfills). Animal mortality disposal methods are compared in table 1.

Burial is the most basic method for the disposal of animal carcasses. In cold climates burial is not a practical option due to frozen ground (Gould et al., 2002). A burial pit can be fabricated from concrete block, monolithic concrete, or treated lumber. Pre-cast, open-bottom septic tanks can be delivered to the site. These offer the best way of developing a concrete disposal pit at relatively low cost. Burial has a limited usage due to the decline in ground water quality in the area of an open-bottom pit and the fact that residue remains after years of use (Blake et al., 2004). It is important to note that burial is not acceptable for disposal of animal by-products generated during butchering. Burial of large volumes of dead animals is not appropriate unless conducted under a Catastrophic Animal Mortality Management Plan (Gamroth et al., 2008). In Arkansas, legislation prohibited the use of burial pits as a method to dispose poultry carcasses beginning July 1, 1994. In Alabama, the State Veterinarian’s
Office banned burial pits for the disposal of poultry carcasses after July 1, 2000 (Blake et al., 2004).

Incineration is recognized as one of the biologically safest methods of animal carcass disposal. It eliminates the threat of disease and does not cause water quality problems. On the other hand, it can be slow, requires fuel and expensive equipment and can generate nuisance complaints from particulate air pollution and odors (Gould et al., 2002). Incineration uses forced air combustion, petroleum fuel and an insulated fire box to achieve the high temperatures needed to burn diseased carcasses without producing serious air pollution. In the event of widespread animal disease, the unavailability of these equipments and high costs of burning can limit the use of this method (Glanville, 2006).

Composting is controlled and natural decomposition of organic material into a stable and useful end-product. Composting is a viable alternative for the disposal of animal mortalities (Fulhage and Ellis, 1994; Glanville and Trampel, 1997). Mortality composting can be described as aboveground burial in a biomass filter where most of the pathogens are killed by high temperatures. As the microorganisms consume the most readily degradable material and grows in numbers, the temperature of the compost piles increases (Gould et al., 2002). Properly designed composting operation will operate in all seasons and handle daily management of mortalities on farms as well as carcass disposal in emergency animal disease outbreak (Glanville, 2006). It has more recently been accepted as an option for dead animal management (Blake et al., 1992; Sims et al., 1992; Cummins et al., 1994; Stanford et al., 2000; Fonstad, 2003).

Rendering converts the nutrients contained in animal carcasses into a protein by-product, which is included in pet food (Blake et al., 2004). The outbreak of Bovine Spongiform Encephalopathy (BSE) in the United Kingdom (1986) led to restrictions in rendering. Animal carcasses should be transported to a rendering facility within 24 hours if carcasses are not preserved. Freezing and fermentation are used as preservation techniques.
Preservation allows mortalities to be stored on the farm until amounts are sufficient to warrant the cost of transportation. Bio-security measures are required to minimize the spread of disease from farm to farm by rendering vehicles and personnel (Gould et al., 2002).

In some areas, disposal of dead animals in a sanitary landfill is permitted. Some states require special licenses to transport dead animals (Gould et al., 2002). One of the major concerns with this method is possibility of disease transmission. Sound biosecurity at sanitary landfills is essential to prevent disease transmission (Blake et al., 2004).

**Table 1. Comparison of animal mortality disposal methods**

<table>
<thead>
<tr>
<th>Disposal method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burial</td>
<td>Capital limited to land and excavating equipment</td>
<td>Nutrients contained in the dead animals are wasted, increases sanitary precautions to prevent disease transmission, land area becomes significant for large operations, not practical when ground is frozen</td>
</tr>
<tr>
<td>Incineration</td>
<td>Sanitary and safe</td>
<td>Nutrients contained in the dead animals are wasted, initial cost, fuel and maintenance costs, potential air quality impairment</td>
</tr>
<tr>
<td>Composting</td>
<td>Conserves nutrients contained in the dead animals, low odor, environmentally safe</td>
<td>High initial cost, labor intensive, regular monitoring and maintenance is required, cropland required</td>
</tr>
<tr>
<td>Rendering</td>
<td>Conserves nutrients contained in the dead animals, minimal capital interest, low maintenance</td>
<td>Increases sanitary precautions to prevent disease transmission, storage of animal is required until pick-up, charges for pick-up, rendering service may not be available</td>
</tr>
<tr>
<td>Sanitary landfills</td>
<td>Simple and no capital cost</td>
<td>Nutrients contained in the dead animals are wasted, few landfills accept dead animals, transportation is costly, no permit in many states</td>
</tr>
</tbody>
</table>

(Source: Gould et al., 2002; Blake et al., 2004; Gamroth et al., 2008)
2. The composting process

The process of carcass composting can be described as (a) temporarily burying dead animals above ground in a mound of supplemental carbon and (b) allowing decomposition by thermophilic microorganisms to (c) heat up the pile, (d) kill most of the pathogens and (e) digest the carcass tissues under predominantly aerobic conditions (Kalbasi et. al., 2005).

The main products of aerobic decomposition are CO$_2$, water vapor and heat. Metabolic end products of anaerobic decomposition are CH$_4$, CO$_2$ and numerous low molecular weight intermediates such as organic acids and alcohols. Anaerobic composting releases significantly less energy per weight of organic decomposed compared to aerobic composting. It has a higher odor potential because of the nature of many intermediate metabolites. For these reasons almost all engineered compost systems are operated under predominantly aerobic conditions (Haug, 1993).

The first microorganisms to colonize the compost heap are mesophilic such as mesophilic bacteria, actinomycetes, fungi and protozoa. They grow between 10 and 50 °C (Cooperband, 2000) and break down easily degradable components such as sugars and amino acids (Hellmann et al., 1997). Organic fresh material degradation starts as soon as the compost heap is made and temperature increases due to the oxidative action of microorganisms. Despite a drop in pH at the very beginning of composting, the degradation of acids brings about a pH increase (Peigne and Girardin, 2004). Thermophilic microorganisms replace mesophilic ones when the temperature reaches 45-50 °C (Hellmann et al., 1997). The second phase is called the thermophilic phase and can last several weeks. It is the active phase of composting where, most of the organic material is degraded and consequently most oxygen is consumed (Peigne and Girardin, 2004). Lignin degradation starts during this phase. Above 60 °C, thermophilic micro-fungi and actinomycetes cannot grow and lignin degradation is slow (Hellman et al., 1997). Important hydrolytic enzymes involved in the composting process include cellulases, hemicellulases, proteases, lipases,
phosphatases and arylsulphatases. High levels of proteases, lipase and cellulose activities have been detected throughout this phase of composting (Herrman et al., 1993; Cunha Queda et al., 2002; Mondini et al., 2004). After the thermophilic phase that corresponds to a peak of degradation of fresh organic matter, the microbial activity and temperature decrease. This is called the cooling phase. The maturation phase begins when the compost temperature falls to that of the ambient temperature. More specifically mesophilic microorganisms continue to degrade complex organic compounds. This last phase is important because humus-like substances are produced to form mature compost (Cooperband, 2000).

3. Factors affecting the composting process

Factors affecting the composting process can be listed as aeration, nutrients (C: N ratio), moisture, porosity, structure, texture, particle size, and pH.

3.1 Aeration

Aerobic composting consumes large amounts of oxygen and rapidly metabolizes easily degradable organic materials during the initial days of composting. The need for oxygen and heat production is greatest at early stages and then decreases as the process ages. A minimum oxygen concentration of 5% within the pore spaces of the composting pile is necessary (Rynk et al., 1992). Proper aeration may be achieved by forcing air through compost materials, passive air exchange, mechanical turning, and combinations of these methods (Henry, 2003). Five days of aeration by turning the compost piles containing cow and horse carcasses coupled with a series of rainfall events resulted in temperature rise to 74 ºC. Temperature remained above 55 ºC during three months (Mukhtar et al., 2003). Insufficient aeration causes anaerobic decomposition. Anaerobic decomposition is slower and less efficient than aerobic processes. Sufficient heat is not generated to evaporate water from organic materials. Anaerobic process causes offensive compound formation including methane, ammonia, hydrogen sulfide and organic acids. These compounds have strong odors.
and present safety concerns. Maintaining aerobic conditions is important to avoid the offensive odors associated with anaerobic decomposition. In addition to providing oxygen, aeration is needed to remove extra heat, water vapor, and gases trapped inside the compost materials (Rynk et al., 1992, Haug 1993, Epstein 1997).

3.2 Nutrients

The primary goals of selecting materials for composting are to provide (a) the desired ratio of carbon and nitrogen, (b) a balanced diet of all the other essential nutrients, (c) the greatest possible biological diversity to inoculate the pile, (d) a mix of particle sizes that favors aeration in the pile, (e) materials that favor adequate but not excessive moisture retention (Biernbaum and Fogiel, 2004). Carbon (C), nitrogen (N), phosphorus (P), and potassium (K) are the primary nutrients required by microorganisms. Microorganisms use carbon for energy and growth. Nitrogen is essential for protein and reproduction. As a rule of thumb, biological organisms need about 25 times more carbon than nitrogen. Thus, raw materials are blended to provide a C: N ratio of 25:1 or 30:1 for active phase (Rynk et al., 1992). Different carbon feedstocks have different degradation rates. For instance, wood shavings and wood chips decompose very slowly due to the presence of lignin. Leaves are decomposed very quickly. Alfalfa hay is a good source of nitrogen, which is also rich in other essential nutrients. Animal manure and food wastes are also higher in nitrogen. It is preferable to have a higher C: N ratio than lower. The higher the C: N ratio, the slower degradation occurred and the less likelihood for the process to go anaerobic. This will prevent offensive odor formation. Available envelope materials can be listed as legume and grass hay, leaves, farm and garden plant residues, vegetable and fruit processing residue, corn silage, soybean stems, wood shavings, animal (sheep, horse, poultry, beef, dairy, swine) manure. Leaves, sawdust, straw, wood shavings, and wood chips are good sources of carbon (Biernbaum and Fogiel, 2004).
Envelope materials serve as biofilters, which (a) deodorizes the gases released from carcasses, (b) prevents access by insects and birds and thus minimizes transmission of disease agents from mortalities to livestock or human (Mukhtar, 2004).

3.3 Moisture

Water provides the medium for chemical reactions, nutrient transportation, and microbiological activity. During composting, moisture level changes as water evaporates and added by rain and snow. Generally more water evaporates than is added. Thus, moisture content of compost materials tends to decrease as the process proceeds. Moisture levels should be kept at a level that materials are thoroughly wetted without dripping excessive water. As a rule of thumb, materials are too wet if water can be squeezed out by hand and too dry if moist is not felt when touched (Rynk et al., 1992). Moisture content also affects the physical structure. It is relatively easy to measure moisture content so it often serves as a proxy for other critical factors such as particle size, porosity, permeability, all of which limit oxygen transport in the high moisture range (Miller et al., 1993, Richard et al., 2002). Generally recommended values are in 50 to 70 % range (Bishop et al., 1983; Haug, 1993; Imbeah, 1997; Richard et al., 2002). As is evident from this relatively wide range of reported values, there is no universally applicable optimum moisture content for composting materials. Each material has unique physical, chemical and biological characteristics and these affect the relationship between moisture content and its water availability, particle size, porosity and permeability (Ahn et al., 2005).

3.4 Porosity, structure, texture and particle size

Porosity, structure, and texture are related to the physical properties of the materials including particle size, and shape (Rynk et al., 1992). They affect availability of oxygen, temperature, microbial activity, composting time, and bulk and packed densities. They can be adjusted by selecting raw materials and grinding or mixing. Materials added to adjust these
properties are called envelope materials. The porosity should be around 35% (volume) to facilitate the air penetration inside the pile and maintain microbial growth (Keener et al., 2001; Looper 2002). In a composting process, aeration and degradability can be improved by reducing the particle size while increasing the surface area, as long as porosity remains 30% (Rynk, 1992).

3.5 pH

Alkaline or acidic environments are not well suited for carcass composting. A large amount of free carbon blended with the nitrogenous materials of carcasses helps nitrogen immobilization and prevents its loss by ammonification, maintains the pH of carcass pile at neutral (7.0) or slightly lower (Henry, 2003). Since the biochemical reactions release CO₂ (a weak acid) and NH₃ (a weak base), the compost process can buffer pH near the neutral range as composting proceeds (Haug, 1993).

4. Production of gases

Mineralization and humification occur simultaneously during composting and are the main processes of fresh organic material degradation. Many gases are emitted during composting including CO₂, ammonia (NH₃), nitrous oxide (N₂O), methane (CH₄), hydrogen sulfide (H₂S), nitrogen oxides (NOₓ), and volatile organic compounds (VOCs) (Hellmann et al., 1997; Mahimairaja et al., 1995; Tamura et al., 1999). The number of volatile organic compounds that could be potentially produced during composting is virtually limitless. Composting operations generate VOCs including oxygenated compounds (e.g., aldehydes, ketones, alcohols), nitrogen-containing compounds (e.g., pyrimidine), sulfur-containing compounds (e.g., mercaptans, sulfides), alkanes (e.g., pentane, hexane), alicyclic hydrocarbons (e.g., terpenes), and aromatic compounds (Golueke et al., 1954; Chanysak et al., 1982; Fisher et al., 1986; Koe and Ng, 1987; Derikx et al., 1990; Miller, 1993; Williams, and Miller, 1993; Day et al., 1998; Krzymien et al. 1999; Kim et al., 2005a).
Carbon is transformed into CO$_2$ and integrated into humus-like substances as a result of huminification (Peigne and Girardin, 2004). Depending on the oxygen concentration within the heap, CH$_4$ can be formed (Lopez-Real and Baptista, 1996). During thermophilic phase, maximum O$_2$ is consumed by aerobic microorganisms, so reduction of the O$_2$ concentration favors anaerobic conditions methanogenic bacteria to produce CH$_4$ (Ott, 1990).

Fatty acids are relatively long chained, moncarboxylic acids that occur in nature as constituents of fats, oils and waxes. The longer chained acids can be hydrolyzed to a lower molecular weight, volatile acids such as, acetic, propionic and butyric acids. Acetic acid, commonly known as vinegar, has an obvious and recognizable odor (Haug, 1993). They are produced in the environmental from bacterial decomposition of organic materials commonly under anaerobic conditions (Kaplovsky, 1951). Lynch et al (1980) has reported degradation of straw under anaerobic conditions forms as products acetic acid and smaller amounts of propanoic and butanoic acid. Problems with odor can arise when acidogenic bacteria produce VFAs faster than acetogenic/ methanogenic species can consume the acids (Zhu et al., 1999).

Aromatic organics are all based on the benzene ring and may contain one or more cyclic groups. Aromatic compounds can be produced during aerobic composting from the breakdown of lignin which is usually abundant. Indole and skatole are examples of heterocyclic compounds that contain a benzene ring condensed with a second five-member ring containing nitrogen. Both have a very unpleasant odor and are produced during the anaerobic decomposition of protein matter (Haug, 1993).

Hydrogen sulfide (H$_2$S) produces the characteristic rotten egg smell and can be detected at a concentration of only 2 parts per billion (ppb) in air. H$_2$S is produced by two principle pathways. First, it can be produced from the anaerobic decomposition of proteins or other S-containing organics. Second, under anoxic conditions with organics and sulfate present, the sulfate can be used as an electron acceptor and reduced to H$_2$S. Hydrogen sulfide can be formed during composting if anaerobic conditions exit such as in the interior of poorly
aerated clumps of material (Haug, 1993). Mercaptans are the sulfur analog of alcohols, having the general formula R-SH. Their distinguishing physical characteristic is their vile and repulsive odor, which diminishes with increasing molecular weight. The nose can detect one part ethyl mercaptan in about 3 billion parts of air. The odor of skunks is largely due to butyl mercaptan. Alkyl sulfides with a formula R-S-R are the sulfur analog of the corresponding ethers. The nose can detect one part dimethyl sulfide in 1 billion parts of air. Mercaptans can be formed from S-containing amino acids under both aerobic and anaerobic conditions, both production under anaerobic conditions is greater. If oxygen becomes available, such as within a compost clump with some anaerobic zones, the mercaptan can be oxidized to dimethyl sulfide, dimethyldisulfide, and dimethyl trisulfide. Thus, it is likely that each of these compounds is being formed and degraded within the compost pile (Wilber and Murray, 1990; Shurson et al., 1998). Terpenes are cycloalkane derivatives having one or more carbon rings and are a class of naturally occurring, organic compounds. Limonene, α-pinene and cineol are examples of terpenes. They are important for any compost process that uses wood chips or sawdust (Wilber and Murray, 1990).

Ammonia (NH₃) is produced from either aerobic or anaerobic decomposition of proteins and amino acids. Any substrate with a low C/N ratio (high protein substrates) will likely release excess ammonia into the vapor phase. Fortunately has a high threshold concentration and considered to be a relatively minor odorant (Haug, 1993).

5. Mortality composting options

The main goals of animal mortality composting are (a) to prevent the transmission and dissemination of infection, (b) to minimize infectious materials to contaminate air, water, soil, and vegetation, (c) to convert carcasses to beneficial end products. Mortalities are usually layered into a pile with no mixing occurring until after the high rate sub-phase of composting has occurred and the carcasses are fully decomposed (Kalbasi et al., 2005).
5.1 Windrow composting

In windrow composting system, a pile is constructed on a compacted soil with low liquid permeability or concrete pads. This technique is most popular for composting large carcasses. Carcasses and envelope materials are placed in specific orders and turned periodically ((Mukhtar et al., 2004). The required oxygen is supplied by natural ventilation resulting from the buoyancy of hot gases in the window to a lesser extent by gas change during turning (Haug, 1993). Glanville (2000) reported that during emergencies, a windrow system that remains unturned for 90 to 120 days can be used for cattle carcasses with an average weight of 1000 lb. Compost piles are usually located in open spaces and not protected from rain, wind, and sun. Aligning the uncovered stacks of carcass piles north to south and maintaining windrow with moderate side slopes maximizes solar warming and avoids accumulation of precipitation (Henry, 2003).

5.2 Bin composting

Bin composting is used for small (e.g., poultry) and medium (e.g., swine) sized mortalities. In this system, carcasses and plant (envelope) materials are filled in a container built by wooden and slatted walls with or without a roof (Kalbasi et al., 2005). Roofed bins are more expensive but have some advantages including reduced weather effects, better moisture control, lower leaching potential and better working conditions for operators (Fulhage, 1997). Except the installation cost, bin systems have advantages over windrow systems. Bin composting allows higher stacking of compost materials, better use of floor space, elimination of weather problems when a roof is used, control of odors, and better temperature control (Rynk et al., 1992). Temporary bins, which are constructed from large hay bales can be used for large (e.g., cattle) carcasses (Fulhage 1997; Looper, 2002; Mukhtar et al., 2003).
5.3 Rotating vessel

Rotating vessels can be used for the first phase of carcass composting to minimize the time and management requirements (Kalbasi et al., 2005). This method (a) isolates mortalities from the surrounding environment, (b) provides protection from weather effects, (c) decreases the risk of odor production, and (d) produces a more uniform product allowing a better control over composting parameters such as temperature, moisture content, pH, and particle size (Rynk, 2003). Cawthon (2000) showed that when a blended mixture of poultry carcasses and sawdust are loaded into a rotating vessel and turned at a rate of 4 revolutions per hour, compost temperatures inside the vessel reaches to 60ºC within hours and stays for three days. Rynk (2003) indicated that rotating vessel followed by windrow composting reduces the composting time of swine carcasses by 60%.

5.4 Other systems

A decrease in the particle size of carcasses increased surface area of the degradable material and composting process takes place much faster, particularly if particle sizes of carcass and envelope materials are similar (Bagley, 1999; Looper, 2002). Kube (2002) studied two composting processes in a windrow system. The first system was ground Holstein steers (approximately 100 lbs) mixed with sawdust and the second one was whole carcasses covered with sawdust. The grinding process reduced the number of turns and decreased the composting time from twelve to six months.

An in vessel system of composting organics using aerated synthetic tubes called EcoPOD (preferred organic digester) has been available for 10 years. These tubes are equipped with an air distribution system connected to a blower (Kalbasi et al., 2005). Cawthon (1998) used a blower to transfer and compost a mixture of hay, litter, and poultry carcasses. It is reported that temperatures inside the tube ranged from 70 ºC to 82 ºC within 5 to 7 days of composting. Haywood (2003) indicated that the decomposition of medium to
large size carcasses inside tubes had gone anaerobic and end product was separated to solid and liquid portion.

The Ag Bag system has been successfully used to compost bio-solids and mortalities. The finished product can remain in the bags long after composting is completed. Farrell (2002) used the Ag Bag system to compost bio-solids with grass clippings and chipped brush. The materials were composted in the bags for eight to ten weeks and temperatures reached to 70 °C. Ag-Bag Environmental managed to aerobically compost over 100,000 avian flu virus infected birds in West Virginia (Mukhtar et al., 2004).

6. Diseased mortality composting

6.1 Biosecurity of mortality composting

Multiple mechanisms are known to be involved in the inactivation of pathogens during composting including (a) exposure to heat, (b) microbial antagonism, (c) production of organic acids and ammonia, (d) competition for nutrients (Epstein, 1997). Among these, temperature is considered as the most important factor in pathogen inactivation and is relatively easy to measure.

Pathogenic bacteria are inactivated by high thermophilic temperatures in the active phase of the composting process. Inactivation is a function of temperature and length of exposure (Mukhtar et al., 2004). Haug (1993) reported that (a) clumping of solids, which can isolate material from the temperature effects, (b) non-uniform temperature distribution, which can allow pathogens to survive in colder regions, (c) re-introduction of pathogens after the high temperature phase can reduce actual pathogen inactivation during the composting process. The inactivation energy is between 50 and 100 kcal/mol for many spores and vegetative cells. Based on this, Haug (1993) calculated the heat inactivation of enteric pathogens by considering the conditions common to composting and reported that an average
temperature of 55 to 60 °C for a day or two will provide this energy and is sufficient to reduce the pathogenic spores, viruses, bacteria, and protozoa to an acceptable level.

Senne et al. (1994) investigated the effects of poultry carcass composting on the survival of HPAIV (highly pathogenic avian influenza virus) and reported that 10 days of composting completely eliminates both viruses. Glanville et al. (2006) investigated survival of vaccine strains of poultry viruses placed inside cattle compost piles. Survival times of the viruses were much shorter when they expose to the full range of environmental conditions compared to placing them inside vials. Mukhtar et al. (2004) and Keener et al. (2005) discussed the effect of site selection and layout on biosecurity of mortality composting.

6.2 Use of mortality composting in outbreaks

The avian influenza (AI) outbreak (H7N2) in the central Shenandoah Valley (USA) in 2002 affected 197 poultry farms and cost US $ 211 million to eradicate (Bendfeldt et al., 2005). Five different methods including on-farm burial, landfilling, incineration, slaughter, and composting were used to dispose AI infected carcasses. Initially carcasses were buried in the farm but soon stopped as adjoining landowners complained about the risk of contamination. Approximately 65 % of carcasses were landfilled. Landfilling was showed to be expensive and problematic due to the transport distance and lack of available trucks. Forty-three thousand birds were composted using Ag-Bag and windrow systems. The in house composting method reported to be more convenient due to logistical problems associated with Ag-Bag system.

In AI outbreak in British Columbia (Canada) in 2004, 1.25 million infected birds were disposed by burial, burning, and composting. At the beginning of the outbreak, carcasses were transported to other locations and they were buried and burnt in these locations. In the middle of the outbreak, the disposal strategy switched to on-farm composting. The first stage of the composting was conducted in the poultry houses. After 5
days, the windrows were re-formed on a layer of heavy black plastic over wood shavings. Piles were passively aerated by black agricultural drainage pipe aligned across the plastic. The windrows were covered with vapor barriers, wood shavings, and finally black plastic (Spencer et al., 2004). Glanville et al. (2007) and Ahn et al. (2007) investigated application of the same composting system to diseased swine carcasses in case of an outbreak.

7. Stability and maturity of animal composts

Two key criteria in the determination of compost quality are stability and maturity. Maturity is related to phytotoxicity (Iannotti et al., 1993). Stability is associated with compost microbial activity. Stabilization of composts affects the response of plants to compost applications, potential for microbial activity, odor generation, and pathogen regrowth (Zucconi et al., 1985; Miller 1993). Also, the degree of stability achieved within a certain time can be used to evaluate process performance (Stentiford, 1993). Stability is generally defined as a function of microbial activity and it can be determined by $O_2$ uptake rate, $CO_2$ production rate or heat release as a result of microbial activity (Chen and Inbar, 1993; Wu et al., 2000). Respirometric techniques are currently widely used as stability indicators (Sadaka et al., 2006). The basis of these methods is that immature compost material has a higher $O_2$ consumption and $CO_2$ production rates due to rapid development of microorganisms as a consequence of the easily biodegradable compound (Gomez et al., 2005). Thompson (2002) classified compost stability based on respiration rates as very stable (1 mg CO$_2$-C/ g VS. d), stable (2-4 mg CO$_2$-C/ g VS. d), moderately unstable (5-7 mg CO$_2$-C/ g VS. d), unstable raw compost (8-9 mg CO$_2$-C/ g VS. d), raw compost (10-11 mg CO$_2$-C/ g VS. d), and raw feedstock (>11 mg CO$_2$-C/ g VS. d).

8. Monitoring composting process

Five parameters, temperature, moisture content, $O_2$, $CO_2$, and VOC production can be monitored to assess composting process. Temperature can be easily monitored during
composting. Thermocouples with 12” probes are available for under $20. The recommended location to test the internal temperature is 2/3rd down and 1/3rd in from the side. If temperature of a pile is not increasing, nutrient supply, moisture level or aeration may not be sufficient. Other reason of it may be over aeration of the pile which causes removal of the produced heat. Thus, a decline in temperature does not necessarily show the composting process is completely finished (Biernbaum and Fogiel, 2004).

The moisture content of a compost material during active phase should be at a level that some water can be squeezed out by hand. However there is not any easy and affordable way to collect representative samples and accurately measure moisture content (Biernbaum and Fogiel, 2004).

Aeration can be measured with O₂ and CO₂ probes. Oxygen levels should be above 5% if compost is aerated properly. Oxygen and CO₂ levels can be measured with a probe to test aeration status of a compost pile. However, O₂ and CO₂ levels do not indicate stabilization of the compost materials (Biernbaum and Fogiel, 2004).

Measurement of VOC emissions is another alternative to test aeration and stabilization of the process (Kim et al., 2005a). Akdeniz et al. (2007a, 2007b) investigated VOCs evolved from decaying envelope materials and swine tissues using SPME and GC-MS. Further studies are needed to test VOCs as marker compounds of the carcass degradation process.

9. Volatile organic compound sampling and analysis

Volatile organic compounds (VOCs) receive great attention of scientists in different disciplines such as (a) food, flavor, and fragrances, (b) medical, pharmaceutical and forensic sciences, and (c) environmental sciences (Demeestere et al., 2007). In the literature a wide range of definitions can be found for VOCs. There is no universally accepted definition of VOC (Wang and Austin, 2006). Kennes and Veiga (2001) define VOC as organic
compounds containing carbon atoms and having a boiling temperature below 373.15 K at 101 kPa. The World Health Organization (WHO) defines VOCs as organic compounds sampled in a solid sorbent and having a boiling point range of 50-100 °C to 240-260 °C. VOCs cover a broad range of organic compounds including alkanes, alkenes, saturated/unsaturated alkyl halides, carbonyls, alcohols, aromatic and halogenated aromatic hydrocarbons. Methane is usually considered separately because of its stability in the troposphere. The acronym VOC often means “non-methane volatile organic compounds (NMVOC) (Wauters et al. 2000). In the dissertation, VOC is used to refer NMVOC.

To gain knowledge about the VOCs in all fields of interest, precise and accurate analytical techniques are necessary. The most common analytical methods include separation by gas chromatography (GC) followed by on-line mass spectrometry (MS), flame ionization detector (FID), or electron capture detection (ECD). More recently, atomic emission spectroscopy (AES) has been recognized as a sensitive and highly selective detection system. In a limited number of cases high pressure liquid chromatography (HPLC) or ion chromatography (IC) is used for the analysis of derivatized compounds. Direct MS techniques such as membrane inlet MS (MIMS), atmospheric pressure chemical ionization MS (APCI-MS), and proton transfer reaction MS (PTR-MS) can be also used (Demeestere et al., 2007).

Conventional VOC sampling methods use sorbent tubes, impingers, vacuum canisters, and gravimetric filters. A wide variety of organic and inorganic sorbents are available for VOC sampling. Inorganic sorbents include silica gel, alumina, and molecular sieves. These sorbents are considered more polar than organic porous polymeric adsorbents such as Tenax. However, they are rapidly inactivated in the presence of water, making sampling in humid conditions impractical (Wang and Austin, 2006). Collection of a sample of air into a container is an alternative to sorbent sampling. This is referred as “whole air sampling” (EPA, 1988). Advantages of container sampling over sorbent methods include (a) whole-air
sampling, (b) no breakthrough of target compounds, (c) no thermal or solvent desorption requirement, (d) no need for field calibration. Potential problems associated with container sampling (a) possible sample instability by adsorption onto the walls of the container, (b) sample permeation in or out of the container in the case of bags, (c) no exclusion of non-target compounds which may lead to sampling matrix effects or analytical interferences (Wang and Austin, 2006).

There are difficulties in the analysis of VOCs particularly in environmental matrices, where VOC concentrations are at the level of pg/L to µg/L. In these cases, appropriate sampling and pre-concentration techniques are necessary to comply with the sensitivity of the analytical instruments. These methods require costly equipment, lengthy sample collection and preparation periods, and complicated cleaning and extraction procedures. SPME offers many advantages for air sampling such as high precision and sensitivity, applicability to high moist samples, reusability, and compatibility with conventional analytical equipment (Pawliszyn, 1997; Mani, 1999; Wercinski, 1999; Jia et al., 2000; Augusto et al., 2001; Pacolay et al., 2006).

10. Solid phase microextraction

10.1 SPME fiber coatings

Solid phase microextraction (SPME) combines sampling, pre-concentration and the direct transfer of the analytes into a standard GC system (Pawliszyn, 1997). It is based on partitioning between the polymeric phase and the sample matrix.

The common homogenous polymer coatings are polydimethylsiloxane (PDMS) and polyacrylate (PA). PDMS is a nonpolar phase and extracts nonpolar analytes very well. However, it can be applied to polar compounds after optimizing extraction conditions. The PA phase is suitable for polar compounds. It is a low density solid polymer, which allows analytes to diffuse into the coating. Diffusion coefficients of PA are lower compared to
PDMS, resulting in longer extraction times for volatile analytes in the headspace. Mixed phase coatings such as Carboxen/PDMS and PDMS/DVB (divinylbenzene) have complementary properties compared to PDMS and PA. Since the majority of interaction is determined by adsorption process, they are more suitable for more volatile organic compounds (Pawliszyn, 1993). DVB can be classified as mesoporous with some macropores and micropores. DVB micropores are fairly large, relative to micropores in Carboxen particles. DVB pores are ideal for trapping C6-C15 VOCs. CAR/PDMS can be used to trap C2-C6 VOCs (Mani, 1999).

10.2 SPME time

Sampling time for SPME is typically determined by detector sensitivity and quantification requirements. Sampling times can change from a few seconds to days for assessment of short-term and long-term exposures (Koziel and Novak; 2002). In general, the objective of the SPME experiments is to reach distribution equilibrium in the system. At this condition, the system is stationary and a variation in the mass transfer does not affect the final results. The equilibration time is defined as the time after which the amount of extracted analytes remains constant. For adsorptive type fibers, after a certain time displacement of the adsorbed analytes can be observed. In these cases, shorter extraction times can be used. When using a shorter extraction time compared to equilibration time. Care must be taken to control the exposure time and the longest extraction time must be applied (Pawlizsyn, 1993).

10.3 Quantification by SPME

SPME has been showed as a useful air sampling technique for agricultural operations. Kim et al (2002) described a method that utilizes two different SPME fiber coatings for the analysis of propanoic acid, butyric acid, and sulfur compounds from waste treatment systems. Davoli et al (2003) and Kim et al. (2005b) characterized volatile organic compound emissions (e.g., propanoic acid, butanoic acid, hexanoic acid and dimethyl disulfide) from
landfills using SPME and GC-MS. Kim et al. (2005a) identified headspace odorants (e.g., propanoic acid, butanoic acid, and dimethyl disulfide) from commercial composts of 14 different producers using SPME method. Akdeniz et al. (2007a and 2007b) identified VOCs (e.g., volatile fatty acids, dimethyl disulfide, dimethyl trisulfide, and nitrogen containing compounds) during biological decomposition of plant and animal materials utilizing SPME and GC-MS.

Development of quantification methods for SPME requires the use of reliable gas standards and accurate simulation of the sampling conditions. There are many ways to generate gas standards. In batch gas generation systems, standard liquids are injected and evaporate into a defined air volume (Koziel et al., 2004). Lee et al. (2002) utilized 1 L Tedlar bag to load a known amount of standard gas mixtures. Van Durme et al. (2007) used 118 mL glass bottles to insert liquid standards of the compounds. Continuous gas generation and flow through systems have advantages over batch systems, such as minimization of the effects of adsorption to surfaces of the sampling system and continuous range dilution (Koziel et al., 2004). The common methods for continuous generation of VOCs can be listed as: (a) permeation method, (b) diffusion method, and (c) syringe pump injection. To quantitatively analyze VOCs in field air samples, Jia et al. (2000) prepared a standard gas generating device, which lets the flow pass through permeation tubes. Spinhirne and Koziel (2003) used permeation tubes to generate standard gases of volatile fatty acids (acetic, propanoic, 3-methyl butyric, butyric, isovaleric, valeric, and hexanoic acids). The disadvantage associated with the permeation method is the need for periodic weight measurements of the tubes. Augusto et al. (2001) developed two portable devices to perform diffusion based SPME. Diffusion based standard gas generator provides a simple mean to quantify VOCs. The disadvantage of the diffusion method is that temperature has to be controlled within ±1 % accuracy. Syringe pumps are used for generation of standard gases in many applications including validation of direct reading air samplers and generation of pollution standards.
(Koziel et al., 2004). Syringe pumps inject liquid standards into an air stream at a delivery rate ranging from 0.001 µl/h to 8824 ml/h, depending on the syringe volume. Syringe pump injection is a convenient quantification method, which does not require preparing a high number of standard analyte solutions. In this method, concentrations of the solutions are controlled by adjusting air flow rate of the system.

11. References


Mukhtar, S., Auvermann, B.W., Heflin, K., and Boriack, C.N. 2003. A low maintenance approach to large carcass composting. ASABE paper No 032263. ASABE annual international meeting. Las Vegas, Nevada, USA.


CHAPTER 3. SPME AND GC-MS METHOD FOR AIR SAMPLING AND ANALYSIS OF VOCS FROM FULL SCALE MORTALITY COMPOSTING OPERATIONS

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ABSTRACT

In biosecure composting, animal mortalities are fully isolated during the degradation process. Its completion cannot be monitored by visual inspection. One promising approach is to monitor volatile organic compounds including sulfur and nitrogen containing compounds released by decaying mortalities and to use them as biomarkers of the process status. A new method has been developed to quantitatively analyze dimethyl disulfide, dimethyl trisulfide, pyrimidine, acetic acid, propanoic acid, 3-methyl butanoic acid, pentanoic acid, and hexanoic acid from full scale mortality composting operations. Among the four SPME fiber coatings, 85 five µm CAR/PDMS is shown to extract the highest amount of analytes at 1 h sampling time. Calibration curves with high correlation coefficients ranging from 96 to 99 % were obtained. The differences between theoretical and calculated concentrations were found to be ranging from 1.47 to 20.96 %. The applicability of the developed sampling method was tested to analyze samples prepared from a full scale swine mortality composting unit. It was showed that the calibration curves were valid for the highest possible concentration (in the headspace) that could be measured from a full scale composting operation.

Keywords. Compost gas, GC-MS, Dimethyl disulfide, Dimethyl trisulfide, SPME.
Volatile fatty acids
1. Introduction

The development of new analytical techniques for volatile organic compounds evolved from agricultural operations receives great attention. Composting is gaining acceptance as a disposal method for animal mortalities. It can be used for the day-to-day management of mortalities on farms as well as carcass disposal in emergency animal disease outbreak (Wilkinson, 2007). In biosecure composting applications (Spencer et al., 2004; Bendfeldt et al., 2006), mortalities are fully covered and degradation of mortalities cannot be monitored by visual inspection. Thus, assessment methods for the completion of decay process are needed. One promising approach is to monitor gases released by decaying mortalities. Composting operations generate VOCs including oxygenated compounds (e.g., aldehydes, ketones, alcohols), nitrogen-containing compounds (e.g., pyrimidine), sulfur-containing compounds (e.g., mercaptans, sulfides), alkanes (e.g., pentane, hexane), alicyclic hydrocarbons (e.g., terpenes), and aromatic compounds (Golueke et al., 1954; Chanysak et al., 1982; Fisher et al., 1986; Koe and Ng, 1987; Derikx et al., 1990; Miller, 1993; Williams, and Miller, 1993; Day et al., 1998; Krzymien et al. 1999; Kim et al., 2005a). An understanding of the chemical components released from animal mortality composting processes can give scientists insights into the completion of the degradation. Moreover, the composition of the exhaust air indicates the aeration status of the process and quality of the compost (Day et al., 1999; Romain et al., 2005). Under aerobic conditions, specific compounds are released such as carboxylic acids and NH$_3$ (Beck-Friis et al., 2001). One of the most common compounds that contributes to odors (approximately 90%) during composting is dimethyl disulfide (Chiumenti et al., 2005). Other significant compounds are dimethyl trisulfide, pyrimidine, and volatile fatty acids (VFAs). VFAs are associated with anaerobic degradation during composting and produced from carbohydrate fermentation (Epstein, 1997).
There are difficulties in sampling and analysis of air samples from composting operations. Conventional air sampling methods use sorbent tubes, impingers, vacuum canisters, and gravimetric filters. These methods require costly equipment, lengthy sample collection and preparation periods, and complicated cleaning and extraction procedures. SPME offers many advantages for air sampling such as high precision and sensitivity, applicability to high moist samples, reusability, and compatibility with conventional analytical equipment (Wercinski, 1999; Jia et al., 2000; Augusto et al., 2001; Pacolay et al., 2006). SPME-GC-MS has been showed as a useful air sampling technique for agricultural operations. Kim et al (2002) described a method that utilizes two different SPME fiber coatings for the analysis of propanoic acid, butyric acid, and sulfur compounds from waste treatment systems. Davoli et al (2003) and Kim et al. (2005b) characterized volatile organic compound emissions (e.g., propanoic acid, butanoic acid, hexanoic acid and dimethyl disulfide) from landfills using SPME and GC-MS. Kim et al (2005a) identified headspace odorants (e.g., propanoic acid, butanoic acid, and dimethyl disulfide) from commercial composts of 14 different producers using SPME method. Akdeniz et al. (2007a and 2007b) identified VOCs (e.g., volatile fatty acids, dimethyl disulfide, dimethyl trisulfide, and nitrogen containing compounds) during biological decomposition of plant and animal materials utilizing SPME and GC-MS.

Development of quantification methods for SPME requires the use of reliable gas standards and accurate simulation of the sampling conditions. There are many ways to generate gas standards. In batch gas generation systems, standard liquids are injected and evaporated into a defined air volume (Koziel et al., 2004). Lee et al. (2002) utilized 1 L Tedlar bag to load a known amount of standard gas mixtures. Van Durme et al. (2007) used 118 mL glass bottles to insert liquid standards of the compounds. Continuous gas generation and flow through systems have advantages over batch systems, such as minimization of the effects of adsorption to surfaces of the sampling system and continuous range dilution.
The common methods for continuous generation of VOCs can be listed as: (a) permeation method, (b) diffusion method, and (c) syringe pump injection. To quantitatively analyze VOCs in field air samples, Jia et al. (2000) and Ouyang et al. (2006) prepared a standard gas generating device, which lets the flow pass through permeation tubes. Spinhirne and Koziel (2003) used permeation tubes to generate standard gases of volatile fatty acids (acetic, propanoic, 3-methyl butyric, butyric, isovaleric, valeric, and hexanoic acids). The disadvantage associated with the permeation method is the need for periodic weight measurements of the tubes. Augusto et al. (2001) developed two portable devices to perform diffusion based SPME. Diffusion based standard gas generator provides a simple mean to quantify VOCs. The disadvantage of the diffusion method is that temperature has to be controlled within ±1% accuracy. Syringe pumps are used for generation of standard gases in many applications including validation of direct reading air samplers and generation of pollution standards (Koziel et al., 2004). Syringe pumps inject liquid standards into an air stream at a delivery rate ranging from 0.001 µl/h to 8800 ml/h, depending on the syringe volume. Syringe pump injection is a convenient quantification method, which does not require preparing a high number of standard analyte solutions. In this method, concentrations of the solutions are controlled by adjusting air flow rate of the system.

In this study, a method that utilizes syringe pump injection and SPME is described to simultaneously quantify dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), pyrimidine (PR), acetic (AA), propanoic (PRA), 3-methyl butanoic (MBA), pentanoic (PEA) and hexanoic (HA) acids from biosecure mortality composting operations. Physical properties of the compounds that are used in the development of the method are presented in table 2.

The number of organic compounds that could be potentially detected during a mortality composting process is virtually limitless (Haug, 1993). Although other chemicals from the sulfur and volatile fatty acid classes or from other compound classes such as mercaptans, cresols, and indoles may be required to fully evaluate the status and completion
of a mortality composting process, this study focuses on the quantification of DMDS, DMTS, PR, AA, PRA, MBA, PEA, and HA. It introduces a method for rapid measurement of the compounds from composting operations. The main objectives of the study are (a) to develop an air sampling and analysis method for the quantification of VOCs from mortality composting operations, and (b) to test the applicability of the quantification method for a full scale biosecure swine mortality composting operation.

2. METHODOLOGY

2.1 Standards and reagents

High pressure liquid chromatography grade standards of DMDS, DMTS, PR and VFAs were purchased from Sigma-Aldrich (Milwaukee, WI). Air cylinder (99.995%) and ethanol (100 %) were purchased from Chemistry Store of Iowa State University. Standard solutions were daily prepared in ethanol. After preparation, the vial with standard mixture was manually agitated. Before using, glass sampling bulbs and other glassware were carefully washed and rinsed and then baked at 110 °C overnight.

2.2 Air flow and air relative humidity

Air flow rates were controlled by using a mass flow controller and a mass flow meter (Aalborg, Oangeburg, NY). To test the humid conditions on the extraction efficiency of SPME fiber, a 15 ml humidifier (Supelco, Bellefonte, PA) was used (figure 1). The results were reported for both dry air and the maximum humid air that could be reached with the system (97 % relative humidity).
2.3 Sampling bulbs for SPME

Two hundred fifty mL glass sampling bulbs (Supelco, Bellofonte, Pa) were used for SPME. Three sampling bulbs were connected in series to provide enough volume to homogenously mix the analytes before sampling. After each concentration change, the system was allowed to reach steady state and then PTFE stopcocks on the 3rd glass bulb were closed and air sample was captured in the bulb. The air sample was extracted from the 3rd sampling bulb using a SPME fiber (figure 1).

2.4 Syringe pump injection

A KD Scientific syringe pump (Model 200, Holliston, MA) and 100 µl gastight Hamilton syringe (Reno, NV) were used to deliver the solution of standard analytes through a Thermogreen LB-2 septum into a Swagelok mixing tee (Koziel et al., 2004) (figure 1). The injection rate was chosen as 0.1 µl/min. The standard gas method detection limit (MDL) was calculated for a signal to noise ratio of five from the standard deviation of 10 measurements and at 99 % confidence level (Wisconsin Department of Natural Resources). The theoretical analyte concentration for each analyte (in ppmv) was calculated using the following equation (Koziel et al., 2004):

\[
C_{\text{analyte}} = \frac{Q_{\text{analyte}}}{Q_{\text{air}}} \times \frac{m_{\text{analyte}}}{m_{\text{total}}} \times 8.3144 \left[ \frac{L \cdot kPa}{mol \cdot K} \right] \times \frac{293 K}{101.32 kPa} \times \frac{1}{MW_{\text{analyte}}}
\]

\[\text{(1)}\]

Where \(Q_{\text{analyte}}\) is the total mixture delivery rate (µg/min), \(Q_{\text{air}}\) the air flow rate (L/min), \(m_{\text{analyte}}\) the mass of analyte of interest (µg), and \(m_{\text{total}}\) is the total mass of mixture injected (µg), \(MW_{\text{analyte}}\) is the molecular weight of the analyte of interest.

The syringe pump system was tested before starting experiments. In this study, injection tee was not heated. Since the injection tee was not heated, the possibility of condensation in the tee was checked. For this purpose, the mixing tee was replaced with a
glass sampling bulb, letting air with standard analytes pass through for 2 hours. Then, glass bulb was washed with ethanol. This washing ethanol was directly injected into GC-MS. None of the compounds were found in the washing ethanol. It is concluded that there is no condensation in the system. One of the other concerns was the accuracy of the syringe pump delivery rate, which was confirmed through two approaches. In the first approach, the pre-weighed syringe was used to deliver the mixture for a specific period of time and then weighed again. In the second approach, the volume difference is recorded and delivery rate was calculated based on this. Only 0.01% difference was found between the theoretical delivery rate and the delivery rates calculated from these two approaches. Another concern was homogenous mixing of air and standards before reaching the 3rd glass sampling bulb. The preliminary experiments showed that when 3 glass sampling bulbs were used, homogenous mixing was not achieved before reaching the 3rd glass bulb. Thus, pyrex 6 mm diameter glass beads (0.25 lb, Fisher Scientific) were used to provide homogenous mixing in the 3rd sampling bulb (figure 1). The last concern was a possible reaction between the mixed analytes. This was tested by direct injection of the mix into GC-MS. No new compounds were detected.

2.5 SPME fiber selection

Four commercially available SPME fibers were compared at 1 hour extraction time in terms of extraction efficiency by evaluating the peak area values (i.e. the amount of the analyte extracted by the fiber) (Juan et al., 2007). The tested fibers were 85 µm carboxen/polydimethylsiloxane (CAR/PDMS), 100 µm polydimethylsiloxane (PDMS), 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 85 µm polyacrylate (PA) (Supelco, Bellefonte, PA). New SPME fibers were first conditioned according to the manufacturer directions. In addition, SPME fibers were inserted into the injection port of GC for five minutes to thermally desorb impurities on the fiber immediately before sampling.
2.6 Extraction time selection

The extraction time was tested. For this purpose the extraction times of 1, 3, 10, 60, 360 and 720 minutes were tested. Since the fiber reaches equilibrium after 60 min extraction time, the linearity was checked for 1, 3, 10, and 60 min extraction times.

2.7 Demonstration of the quantitatively analysis

An example of the quantitative analysis is demonstrated for the air samples collected from full scale swine mortality composting units. Details of the composting units are described in Ahn et al., 2007 and Glanville et al., 2007. Air samples were drawn from the center location of corn stalks units using SKC pumps (224-PCXR4, PA). Samples were collected in the 10th day of the process. It is known that VOC production is the most intense in the first 10 days of a composting process (Haug, 1993). This intense VOC production period was considered to collect VOC samples to be able to show the applicability of the prepared calibration curves for the possible highest concentrations of a full scale composting unit. The applicability of the calibration curves for the lowest concentrations are shown by calculating method detection limits. Air samples were captured inside 250 mL glass sampling bulbs. VOCs were sampled using the selected SPME fiber and time.

2.8 Sample analysis

All gas analyses were performed using integrated multi dimensional 6890N GC and 5973 MS (Agilent Inc., Wilmington, DE) system. Ultrahigh pure (99.995 %) helium was used as the carrier gas at constant pressure. The injector and SPME fiber desorption temperature was 260 ºC. The initial temperatures of the GC oven were 40 ºC with 3 min holding time, followed by a ramp of 10 ºC/min until reaching 220 ºC, where it was held for 10 min. Two capillary columns, connected in series, were used to separate compounds. The pre column was BP5 12 m × 0.53 mm I.D × 0.25 µm and the analytical column was BP20 25
m× 0.53 mm I.D. × 0.25 µm (SGE, Austin, TX). The heart-cut valve between the pre-column, and analytical column was opened between 0.05-28 min, and backflush of the pre-column was activated between 28-31 min to prepare the system for the following run. The MS mass/charge (m/z) ratio was set between 33 and 150 for the first eight minutes. After the first eight minutes, MS detection was between 34 and 280 m/z. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 ºC, respectively.

2.9 Data analysis

Chromatography data acquisition software consisted of MSD ChemStation (Agilent) and BenchTop/PBM™ V. 3.2.4 (Palisade Corporation, Ithaca, NY) was used to analyze data. Separated compounds were identified using mass spectral matches with ChemStation’s NIST MS Library and PBM Benchtop MS libraries. Spectral matches and column retention times were compared with those of standard analytes.

2.10 Statistical analysis

Experiments were performed in triplicate (N=3). JMP v 6.0.2 package was used to run statistical tests. Data were subjected to one-way analysis of variance (ANOVA). Treatment means were compared using TUKEY’s honestly significant differences (HSD) test at 95 % confidence level.

3. Results and discussion

3.1 Fiber selection

In order to select the most suitable fiber, SPME fibers were compared according to the amount of analytes they extracted at one hour sampling time (figure 2). The relative standard deviation of the data was ranging from 1 to 9 %. 85 µm CAR/PDMS provided the highest extraction efficiency and was found statistically significantly different than the other
fibers. Based on the amount of analytes extracted, efficiencies of the fibers can be ranked from the highest to the lowest as: CAR/PDMS > PDMS/DVB > PDMS ≈ PA (figure 2). These results can be explained by the characteristics of the fiber coatings. Mixed phase coatings (CAR/PDMS and PDMS/DVB) have complementary properties compared to homogenous phase coatings (PDMS and PA). Since the majority of interaction is determined by the adsorption process on porous surface, CAR/PDMS and PDMS/DVB are suitable for more volatile organic compounds than PDMS and PA fiber coatings (Pawliszyn, 1997). Thus, DMDS, DMTS, PR and VFAs were extracted at higher amounts by CAR/PDMS and PDMS/DVB than PDMS and PA (figure 2). DVB is mainly mesoporous and ideal for trapping C_6-C_{15} analytes. Unlike DVB, CAR is microporous and traps C_2-C_6 analytes (Mani, 1999). This explains the better extraction efficiency of CAR/PDMS compared to PDMS/DVB for the compounds ranging from C_2-C_6 (figure 2 and table 2).

3.2 Extraction time selection

Carboxen/PDMS is an adsorptive fiber coating. During fiber selection, the extraction time was chosen as 1 h. This extraction time might cause replacement of the compounds from the micropores of the CAR/PDMS coating. Six different extraction times were tested and the amounts of analytes extracted were shown in figure 3. The relative standard deviations of the data were ranging from 1 to 14%. In 6 and 12 h extraction times, an increase in extraction time did not lead to an increase in the amount of analyte extracted. This is caused due to displacement of analytes. If an extraction time that corresponds to the linear range of the data is used, there will not be any risk of displacement. For this reason, graphs were plotted for 1, 3, 10, and 60 mins. Extraction times and linearity of the graphs was checked (figure 4). Correlation coefficients of the graphs were ranging from 96 to 99%. Since the linearity of the graphs were high, it was concluded that at one hour extraction time
there is no displacement of the compounds and one hour is a proper extraction time for CAR/PDMS fiber coating to sample DMDS, DMTS, PR, AA, PRA, MBA, PEA, and HA.

3.3 Calibration curves and method detection limits

The quantification of target VOCs was based on the calibration curves obtained under non-equilibrium conditions and 1 h sampling time. Calibration curves were prepared for both dry and humid (97% relative humidity) air conditions (table 3). The points have relative standard deviations ranging from 0.32 to 5.01 %.

A comparison of theoretical concentrations with the concentrations calculated from the calibration curves of the dry and humid conditions is presented in table 4. The data is presented using ppbv units. The theoretical concentrations were chosen arbitrary but at low concentrations considering difficulties in analysis at low concentrations. The concentrations calculated were all above method detection limits and thus reliable (table 4). Measured concentrations were found to be different than the theoretical concentrations at a level ranging from 1.47 to 20.96 %. These differences are close to the ones reported in Koziel et al. (2004) and believed to be in an acceptable range. Koziel et al. (2004) compared theoretical n-alkane concentrations with those measured using 100 µm PDMS fiber and reported differences in the concentrations ranging from 2 to 17 %.

No significant difference was detected between dry and humid conditions for DMDS, DMTS, PR, and HA. However, significant differences were detected for AA, PRA, MBA, and PEA. Lower concentrations of acids (AA, PRA, MBA and PEA) were detected under 97% humid conditions. In a swine mortality composting operation, if air samples are collected in an early stage of the process, air samples are expected to have a very high relative humidity (approximately 100 %). However, in the following stages, compost materials will lose some of their moisture and air samples will have a relative humidity ranging from 0 to
100%. Thus, by using calibration curves prepared for dry and humid conditions a range of concentrations can be reported.

3.4 Demonstration of the quantitatively analysis

An example of total ion chromatogram of the air samples drawn from full scale swine mortality composting units is presented in figure 5. Concentrations of the VOC’s were calculated using the calibration curves of dry and humid (97 % relative humidity) conditions (table 5). Concentrations of the compounds were ranging from 0.06 to 7.39 ppmv in the headspace. It is shown that the prepared calibration curves are useful for the concentrations that can be detected from a full scale composting unit. The developed method considers both the dry and humid air conditions of the composting process and is applicable to quantitatively analyze VOC’s from full scale swine mortality composting operations.

4. CONCLUSIONS

A completely new method is developed to quantitatively analyze DMDS, DMTS, PR, AA, PRA, MBA, PEA, and HA from full scale mortality composting operations. Eighty five µm CAR/PDMS is shown to extract the highest amount of analytes at 1 h sampling time. It is observed that at this sampling time, there is no risk of analyte replacement. The prepared calibration curves have high correlation coefficients ranging from 96 to 99 %. The differences between theoretical and calculated concentrations are found to be ranging from 1.47 to 20.96 %. These differences are close to the ones reported in other studies and believed to be acceptable. No significant difference is found for DMDS, DMTS, PR and HA concentrations extracted under dry and humid conditions. However, lower concentrations of AA, PRA, MBA, and PEA were detected under 97 % humid conditions compared to dry conditions. Some amounts of the compounds are lost with the humid air due to their high water solubility. In quantification of these compounds, if the relative humidity of the sample is not know, a range of concentrations can be reported using calibration curves of dry and
humid air conditions. The applicability of the prepared calibration curves is tested for air samples drawn from a full scale swine mortality composting unit. It is showed that the calibration curves are valid for the highest possible concentration (in the headspace) that can be measured from a full scale composting operation. The method detection limits are found to be ranging from $10^{-5}$ to $5.8 \times 10^{-1}$ ppmv. SPME and standard gas generation using syringe pump injection are found to give good estimates of the concentrations of DMDS, DMTS, PR, AA, PRA, MBA, PEA, and HA. These techniques can be used for rapid quantitative analysis of volatile organic compounds from composting operations. This study will lead to other scientists to develop sampling and quantification method for the other volatile organic compounds of the mortality composting operations.

Acknowledgment

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References


Utilization Aspects, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, 1993; pp: 219-242.


Table 2. Physical properties of the quantified compounds

<table>
<thead>
<tr>
<th>Compound name</th>
<th>CAS #</th>
<th>MW*</th>
<th>Formula</th>
<th>Density (g/mL)**</th>
<th>Solubility in water</th>
<th>Solubility in ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMDS</td>
<td>624-92-0</td>
<td>94.20</td>
<td>C₂H₆S₂</td>
<td>1.062</td>
<td>insoluble</td>
<td>very soluble</td>
</tr>
<tr>
<td>DMTS</td>
<td>3658-80-8</td>
<td>126.26</td>
<td>C₂H₆S₃</td>
<td>1.202</td>
<td>insoluble</td>
<td>very soluble</td>
</tr>
<tr>
<td>PR</td>
<td>289-95-2</td>
<td>80.08</td>
<td>C₃H₆N₂</td>
<td>1.016</td>
<td>miscible</td>
<td>soluble</td>
</tr>
<tr>
<td>AA</td>
<td>64-19-7</td>
<td>60.05</td>
<td>C₂H₄O₂</td>
<td>1.049</td>
<td>soluble</td>
<td>miscible</td>
</tr>
<tr>
<td>PRA</td>
<td>79-09-4</td>
<td>74.07</td>
<td>C₃H₆O₂</td>
<td>0.980</td>
<td>soluble</td>
<td>miscible</td>
</tr>
<tr>
<td>MBA</td>
<td>503-74-2</td>
<td>102.13</td>
<td>C₅H₁₀O₂</td>
<td>0.931</td>
<td>soluble</td>
<td>miscible</td>
</tr>
<tr>
<td>PEA</td>
<td>109-52-4</td>
<td>102.13</td>
<td>C₅H₁₀O₂</td>
<td>0.933</td>
<td>soluble</td>
<td>soluble</td>
</tr>
<tr>
<td>HA</td>
<td>142-62-1</td>
<td>116.15</td>
<td>C₆H₁₃O₂</td>
<td>0.920</td>
<td>soluble</td>
<td>soluble</td>
</tr>
</tbody>
</table>

(Source: Lide, 2004; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: Pyrimidine, AA: acetic acid, PRA: propanoic acid, MBA: 3-methyl butanoic acid, PEA: pentanoic acid, HA: hexanoic acid, MW: molecular weight, density at 20°C)
Table 3. Calibration curves with correlation coefficients, concentration ranges of the calibration curves and relative standard deviations (RSDs) of the points

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Dry conditions</th>
<th>Humid conditions</th>
<th>Concentration range (ppmv)</th>
<th>RSD range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMDS</td>
<td>$y=1.78E+07x-6.17E+04$</td>
<td>$y=1.74E+07x-8.75+04$</td>
<td>0.01-6.85</td>
<td>1.20-4.15</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.998$</td>
<td>$R^2=0.998$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMTS</td>
<td>$y=1.51E+07x-3.33E+06$</td>
<td>$y=1.46E+07x-2.86E+06$</td>
<td>0.02-5.95</td>
<td>1.07-4.73</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.990$</td>
<td>$R^2=0.990$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>$y=2.08E+07x+4.47E+06$</td>
<td>$y=2.03E+07x+3.49E+06$</td>
<td>0.03-6.25</td>
<td>0.24-7.16</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.976$</td>
<td>$R^2=0.979$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>$y=5.17E+06x-1.54E+06$</td>
<td>$y=4.78E+06x-2.46E+06$</td>
<td>0.2-15.64</td>
<td>0.96-11.40</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.993$</td>
<td>$R^2=0.986$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA</td>
<td>$y=9.92E+06x-4.87E+06$</td>
<td>$y=8.50E+06x-3.20E+06$</td>
<td>0.16-13.25</td>
<td>0.72-14.77</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.967$</td>
<td>$R^2=0.972$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBA</td>
<td>$y=2.24E+07x-9.09E+05$</td>
<td>$y=2.07E+07x-3.56E+06$</td>
<td>0.11-6.12</td>
<td>0.57-11.22</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.985$</td>
<td>$R^2=0.984$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEA</td>
<td>$y=2.51E+07x-2.81E+05$</td>
<td>$y=2.30E+07x-8.03E+05$</td>
<td>0.11-7.24</td>
<td>0.40-7.26</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.995$</td>
<td>$R^2=0.990$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>$y=2.32E+07x-1.94E+06$</td>
<td>$y=2.28E+07x-2.55E+06$</td>
<td>0.09-5.54</td>
<td>0.47-12.43</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.991$</td>
<td>$R^2=0.991$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Dry condition $\approx 0\%$ relative humidity and humid condition $\approx 97\%$ relative humidity)

Table 4. Comparison of theoretical and measured concentrations and method detection limits for dry and humid air conditions

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Theory (ppbv)</th>
<th>SPME (ppbv) at dry conditions</th>
<th>SPME (ppbv) at humid conditions</th>
<th>MDL (ppbv)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>% difference**</td>
<td>Average</td>
<td>% difference</td>
</tr>
<tr>
<td>DMDS</td>
<td>28</td>
<td>30$^{A}$</td>
<td>7.14</td>
<td>27$^{A}$</td>
</tr>
<tr>
<td>DMTS</td>
<td>23</td>
<td>20$^{A}$</td>
<td>13.04</td>
<td>19$^{A}$</td>
</tr>
<tr>
<td>PR</td>
<td>2</td>
<td>2.1$^{A}$</td>
<td>0.5</td>
<td>2$^{A}$</td>
</tr>
<tr>
<td>AA</td>
<td>430</td>
<td>789$^{A}$</td>
<td>9.30</td>
<td>765$^{B}$</td>
</tr>
<tr>
<td>PRA</td>
<td>610</td>
<td>631$^{A}$</td>
<td>3.44</td>
<td>601$^{B}$</td>
</tr>
<tr>
<td>MBA</td>
<td>225</td>
<td>251$^{A}$</td>
<td>11.55</td>
<td>233$^{B}$</td>
</tr>
<tr>
<td>PEA</td>
<td>229</td>
<td>195$^{A}$</td>
<td>14.87</td>
<td>181$^{B}$</td>
</tr>
<tr>
<td>HA</td>
<td>197</td>
<td>210$^{A}$</td>
<td>6.59</td>
<td>202$^{A}$</td>
</tr>
</tbody>
</table>

(MDL: method detection limit at dry (left) and humid (right) conditions; % difference: % difference between the measured (average) and theoretical concentrations; average concentrations at dry and humid conditions are compared; means that are not connected with the same letter are significantly different)
Table 5. Calculated concentrations of the VOCs samples from a full scale swine composting system

<table>
<thead>
<tr>
<th>Retention time (min)*</th>
<th>Compound name</th>
<th>MS area counts (arbitrary units)</th>
<th>Concentrations (ppmv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>dry conditions</td>
</tr>
<tr>
<td>5.55</td>
<td>DMDS</td>
<td>1.1E+07</td>
<td>0.593</td>
</tr>
<tr>
<td>7.86</td>
<td>PR</td>
<td>4.7E+06</td>
<td>0.066</td>
</tr>
<tr>
<td>12.10</td>
<td>AA</td>
<td>3.3E+07</td>
<td>6.84</td>
</tr>
<tr>
<td>12.27</td>
<td>DMTS</td>
<td>9.9E+06</td>
<td>0.432</td>
</tr>
<tr>
<td>13.78</td>
<td>PA</td>
<td>2.3E+07</td>
<td>2.76</td>
</tr>
<tr>
<td>16.17</td>
<td>MBA</td>
<td>1.9E+07</td>
<td>0.88</td>
</tr>
<tr>
<td>17.34</td>
<td>VA</td>
<td>8.5E+06</td>
<td>0.35</td>
</tr>
<tr>
<td>19.10</td>
<td>HA</td>
<td>1.2E+07</td>
<td>0.64</td>
</tr>
</tbody>
</table>

(Retention times of the compounds were matched with the standard analytes; dry condition ≈ 0 % relative humidity and humid condition ≈ 97 % relative humidity)

Figure 1. Schematic of the standard gas generation using syringe pump injection and SPME

(MFC: Mass flow controller, MFM: mass flow meter)
Figure 2. Extraction efficiencies of different SPME fibers

(SPME at room temperature at 60 min extraction time; means that are not connected with the same letter are significantly different; N=3; p≤0.05)
Figure 3. Extraction efficiencies at 1, 3, 10, 60, 360, and 720 minute extraction times (SPME at room temperature with 85 µm CAR/PDMS; N=3)
Figure 4. Extraction efficiencies at 1, 3, 10, and 60 minute extraction times (SPME at room temperature with 85 µm CAR/PDMS; N=3)
Figure 5. Total ion chromatogram of VOC sampled from full scale swine mortality composting units
(SPME at room temperature with 85 µm CAR/PDMS at 1 h sampling time)
Appendix A. List of compounds identified from preliminary laboratory scale composting experiments

(+ detected and – not detected from headspace)

<table>
<thead>
<tr>
<th>Compound name</th>
<th>CAS #</th>
<th>MW</th>
<th>Corn silage composts</th>
<th>Swine tissue composts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volatile fatty acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>64-19-7</td>
<td>60.05</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>79-09-4</td>
<td>74.07</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Propanoic acid, 2-methyl</td>
<td>79-31-2</td>
<td>88.10</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>107-92-6</td>
<td>88.10</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Butanoic acid, 3-methyl-</td>
<td>503-74-2</td>
<td>102.13</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pentanoic acid</td>
<td>109-52-4</td>
<td>102.13</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>142-62-1</td>
<td>116.15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Phenolics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol, 2-methoxy-</td>
<td>90-05-1</td>
<td>124.13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzeneethanol</td>
<td>60-12-8</td>
<td>122.16</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>108-95-2</td>
<td>94.11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol, 4-methyl-</td>
<td>106-44-5</td>
<td>108.13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol, 4-ethyl</td>
<td>123-07-9</td>
<td>122.16</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Esters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butanoic acid, ethyl ester</td>
<td>105-54-4</td>
<td>116.15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Butanoic acid, propyl ester</td>
<td>105-66-8</td>
<td>130.18</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hexanoic acid, ethyl ester</td>
<td>123-66-0</td>
<td>144.21</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Butanoic acid, 3-methylbutyl ester</td>
<td>106-27-4</td>
<td>158.23</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>111-27-3</td>
<td>102.17</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Butanone</td>
<td>78-93-3</td>
<td>72.10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>110-43-0</td>
<td>114.18</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Sulfur-containing compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disulfide, dimethyl</td>
<td>624-92-0</td>
<td>94.20</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Trisulfide, dimethyl</td>
<td>3658-80-8</td>
<td>126.26</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methanethiol</td>
<td>74-93-1</td>
<td>48.10</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tetrasulfide, dimethyl</td>
<td>5756-24-1</td>
<td>158.33</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Nitroge-containing compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Piperidinone</td>
<td>675-20-7</td>
<td>99.13</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>120-72-9</td>
<td>117.14</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1,3-Diazine</td>
<td>289-95-2</td>
<td>80.08</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Appendix B. Preliminary experiments to compare SPME fiber coatings

**Corn silage**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>CAR/PDMS</th>
<th>PDMS</th>
<th>PDMS/DVB</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFAs</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolics</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esters</td>
<td>B</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohols</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketones</td>
<td>C</td>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Swine tissues**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>CAR/PDMS</th>
<th>PDMS</th>
<th>PDMS/DVB</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur-containing</td>
<td>A</td>
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<td></td>
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</tr>
<tr>
<td>compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen-containing</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketones</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolics</td>
<td>C</td>
<td>C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Average total peak area of each chemical group is shown; N=3)
Appendix C. Preliminary experiments to compare SPME time

Corn silage

Swine tissues

(Average total peak area of each chemical group is shown; N=3)
Appendix D. Calibration curves at dry (=0% relative humidity) air conditions

**DMDS**

\[ y = 1.78 \times 10^{7}x + 6.17 \times 10^{4} \]

\[ R^2 = 9.98 \times 10^{-1} \]

**DMTS**

\[ y = 1.51 \times 10^{7}x + 3.33 \times 10^{6} \]

\[ R^2 = 9.93 \times 10^{-1} \]

**PR**

\[ y = 2.08 \times 10^{7}x + 4.47 \times 10^{6} \]

\[ R^2 = 9.76 \times 10^{-1} \]

**AA**

\[ y = 5.17 \times 10^{6}x - 1.54 \times 10^{6} \]

\[ R^2 = 9.93 \times 10^{-1} \]

**PRA**

\[ y = 9.92 \times 10^{6}x - 4.87 \times 10^{6} \]

\[ R^2 = 9.67 \times 10^{-1} \]

**MBA**

\[ y = 2.24 \times 10^{7}x - 9.09 \times 10^{5} \]

\[ R^2 = 9.85 \times 10^{-1} \]

**PEA**

\[ y = 2.51 \times 10^{7}x - 2.81 \times 10^{5} \]

\[ R^2 = 9.95 \times 10^{-1} \]

**HA**

\[ y = 2.32 \times 10^{7}x - 1.94 \times 10^{6} \]

\[ R^2 = 9.91 \times 10^{-1} \]
Appendix E. Calibration curves at humid (97% relative humidity) conditions

**DMDS**

\[ y = 1.74 \times 10^7 x + 8.75 \times 10^4 \]

\[ R^2 = 9.99 \times 10^{-1} \]

**DMTS**

\[ y = 1.46 \times 10^7 x + 2.86 \times 10^6 \]

\[ R^2 = 9.96 \times 10^{-1} \]

**PR**

\[ y = 2.03 \times 10^7 x + 3.49 \times 10^6 \]

\[ R^2 = 9.79 \times 10^{-1} \]

**AA**

\[ y = 4.78 \times 10^6 x - 2.46 \times 10^6 \]

\[ R^2 = 9.86 \times 10^{-1} \]

**PRA**

\[ y = 8.50 \times 10^6 x - 3.20 \times 10^6 \]

\[ R^2 = 9.72 \times 10^{-1} \]

**MBA**

\[ y = 2.07 \times 10^7 x - 3.56 \times 10^6 \]

\[ R^2 = 9.84 \times 10^{-1} \]

**PEA**

\[ y = 2.30 \times 10^7 x - 8.03 \times 10^5 \]

\[ R^2 = 9.90 \times 10^{-1} \]

**HA**

\[ y = 2.28 \times 10^7 x - 2.55 \times 10^6 \]

\[ R^2 = 9.91 \times 10^{-1} \]
Appendix F. Sampling low molecular weight sulfur-containing compounds at different extraction times

(Methanethiol and ethanethiol sampled at 1, 10, 60, 360, 720 min extraction times; N=3)
CHAPTER 4. LABORATORY SCALE EVALUATION OF VOC EMISSIONS AS MARKERS OF BIOSECURE SWINE CARCASS DEGRADATION

A paper to be submitted to Bioresource Technology

Neslihan Akdeniz, Jacek A. Koziel, Hee-Kwon Ahn, Thomas D. Glanville, Benjamin P. Crawford, D. Raj Raman, Anthony L. Pometto III, Sam Beattie

ABSTRACT

In case of an emergency disposal, to reduce the potential of spreading live viruses to the environment, a passively-aerated plastic wrapped mortality composting system has been designed. In this type of system visual inspection of the carcasses is not possible. Monitoring volatile organic compounds (VOCs) released by carcasses is a promising approach to assess progress and completion of the degradation process. In this study, to better understand VOC emissions from field scale mortality composting systems swine carcass tissues were composted using a laboratory set-up. The objectives of the study were to identify characteristic VOCs evolved from decaying swine tissues and to evaluate VOC emissions to test completion of the carcass degradation. Among forty three compounds, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be produced by decaying swine tissues and named as biosecure marker compounds of the degradation process. The highest emissions rates of the marker compounds were measured for the first three weeks and after the fifth week of the process they were not detected from the headspace. It was concluded that degradation process was completed and swine composts were completely stabilized. This finding was supported by respiration rates. Based on respirometric tests, swine tissues are categorized as stable compost with respiration rates of 3.25±0.12 mg CO$_2$-C/ g VS*d.

Keywords. Compost, Dimethyl disulfide, Dimethyl trisulfide, Pyrimidine, SPME, VOC
1. Introduction

The safe disposal of carcasses during emergency animal disease (EAD) outbreaks is a significant issue (Wilkinson, 2007). Improper disposal of carcasses, such as illegal dumping, has become a problem due to limited number of licensed landfills, incineration and rendering services (Bonhotal et al., 2002). Biosecurity agencies in USA, Canada, Australia, and New Zealand have recognized the potential benefits of composting as an emergency management of mortalities (Wilkinson et al., 2007). Diseased mortality composting was first developed for the poultry industry (Spencer et al., 2004; Bendfeldt et al., 2006). Studies have been conducted to adapt mortality composting for swine and cattle emergency disposal (Glanville et al., 2006a, 2006b; Ahn et al., 2007; Glanville et al., 2007).

In case of an emergency disposal, to reduce the potential of spreading live viruses to the environment, a passively-aerated plastic wrapped mortality composting system has been designed (Spencer et al., 2004; Ahn et al., 2007; Glanville et al., 2007). In this type of biosecure composting applications, carcasses are fully covered with envelope materials and plastic sheets. It is not safe to turn, mix, or expose the diseased carcasses to the environment until the carcass degradation is completed. Since visual inspection of the carcasses is not possible, a new method is needed to assess progress and completion of the degradation process. A promising approach is to monitor VOCs released by decaying mortalities.

VOCs are a wide group of organic compounds including sulfur-containing compounds, nitrogen-containing compounds, alcohols, phenols, ketones, esters, volatile fatty acids, and terpenes (Pagans, et al., 2006). Since air has a low density and VOCs have low concentrations (pg/L to µg/L), sample preparation for VOCs in air requires pre-concentration (Dewulf and Van Langenhove, 2002; Clemitshaw et al., 2004). Ultraviolet differential absorption spectroscopy (UV-DOAS), Fourier transform infrared spectroscopy (FT-IR), and colorimetric method are the techniques that allow measurement of VOCs in air without sample preparation but the number of analytes that can be detected is limited (Cheng and Lee,
Gas phase SPME coupled with GC-MS is a convenient method for measurement of VOCs in air. The analysis of VOCs can be done directly from SPME fibers without further sample handling. With SPME, there is no need for Tedlar bags or Summa canisters for sampling or extraction (Kim et al., 2005a). SPME has been reported for the analysis of VOCs in indoor air (Wei et al., 2004; Larroque et al., 2006; Hippelein, 2006), gaseous industrial effluents (Domeno et al., 2004), lower troposphere (Mangani et al., 2003; Barro et al., 2004;) with low detection limits (LODs) below 1 ng/L. It has been used for identification of VOCs from landfills, commercial composts and decaying plant and animal materials (Davoli et al., 2003; Kim et al., 2005a, 2005b; Akdeniz et al., 2007a, 2007b; Akdeniz, 2008). During sampling, the major factors affecting SPME efficiency are fiber coating, extraction time and temperature. Mixed phase coatings, CAR/PDMS (carboxen/polydimethylsiloxane) and PDMS/DVB (polydimethylsiloxane/divinylbenzene), have complementary properties compared to homogenous phase coatings PDMS (polydimethylsiloxane) and PA (polyacrylate). Since the majority of interaction is determined by the adsorption process on porous surface, mixed phase coatings are proper for more volatile organic compounds than homogenous fiber coatings (Pawliszyn, 1997). DVB is mainly mesoporous and ideal for trapping C<sub>6</sub>-C<sub>15</sub> analytes. CAR is microporous and traps C<sub>2</sub>-C<sub>6</sub> analytes (Mani, 1999). For mixed phase coatings, headspace extraction is carried out in non-equilibrium conditions. Non-equilibrium conditions are required to avoid saturation of the fiber and competition between analytes (Pawliszyn, 1997; Roberts et al., 2000; Nongonierma et al., 2006). Temperature plays an important role in the way volatiles are released in the vapor phase. Although there are more compounds in the vapor phase when the temperature increases, amounts of volatiles adsorbed can decrease (Song et al., 1998; Jia et al., 1998; Demyttenaere et al., 2003). This can be explained by the fact that adsorption is an exothermic process (Pawliszyn, 1997). Also, adsorption induces heat release, which can
increase the temperature of the adsorbent and decrease the amounts of analyte adsorbed (Nongonierma et al., 2006).

In this study, a laboratory scale composting system is prepared to simulate full scale biosecure composting systems. The objectives of the study are (a) to identify characteristic VOCs evolved from decaying envelope materials and swine tissues in order to find marker compounds of animal tissues, (b) to develop calibration curves to quantify marker VOC emissions from decaying swine tissues, (c) to evaluate a potential usage of emission measurements to test completion of the carcass degradation.

2. Methods

2.1 Experimental set-up

A laboratory-scale composting system with aerobic and anaerobic test units was prepared to collect VOC samples from the headspace of envelope materials and swine tissues (figures 6 and 7). Envelope materials (corn silage, oat straw, alfalfa hay) were collected from central Iowa. Oat straw and alfalfa hay were chopped to approximately 10 cm lengths to prevent an excessively porous structure. A whole pig body (14 lbs) was shredded using an industrial grinder with its bones, internal organs and skin. The initial moisture contents of the corn silage, oat straw, alfalfa hay, and pig tissues were 43.6, 37.7, 44.2, and 65 %, respectively. Moisture contents of the envelope materials were adjusted to 65 % by spraying deionized water to prevent any adverse effect of storage. Envelope materials were incubated at 30 °C for 24 h to let the materials absorb the sprayed water (Ahn et al., 2005). Moisture loss during incubation was compensated.

Approximately 70 dry grams of samples were placed inside 1 L glass jars (Mason, Wal-Mart, IA). Jars were washed with deionized water and baked at 110 °C overnight to drive off volatile impurities. PTFE (polytetrafluoroethylene) liners with 1/32 inch thickness (USP, OH) were placed inside on the lids (inner side) to separate headspace of the jars from
tin lids. VOC sampling ports were made by drilling 5 mm holes in the middle of the lids and Thermogreen half-hole septa (Supelco, PA) were tightly placed in these holes. In all over the system, clean ¼ inch O.D. PTFE tubing (E&S Technologies, MA) and stainless steel fittings (Swagelok, KS) were used to prevent any contamination. All the test units were placed in same brand water baths (Fisher Scientific, IL). Temperatures of the water baths were adjusted every day manually (figure 8). Temperatures were increased up to 67 ºC and then decreased to 22 ºC. This is an average temperature profile of a warm season full scale biosecure swine mortality composting process. After temperature decreased to 22 ºC, it was increased again (figure 8) to check VOC emissions in case of a temperature increase after the composting process is completed.

2.2 Aerobic test units

Two oilless pumps (Combine Fluid Products, IL) were used to provide the necessary aeration for aerobic test units. Air was pumped through hydrocarbon traps (Supelco, PA) to prevent any contamination from the ambient air. Curved PTFE tubings with equal number of holes were used to aerate test units (figure 6). Air flow rate was set at 100±1.66 mL/min by using stainless steel 3-way needle valves (Swagelok, NE). Rynk et al. (1992) suggested air flow rates ranging from 25 to 100 cfm per dry ton. In this study, air flow rate was chosen as 1.4 mL per dry g of compost material (50 cfm per dry ton) and kept constant in all test units. Air flow rate of each test unit was checked weekly from the outlet of the test units (figure 6). Aeration stopped and aerobic test units kept closed 1 h before SPME to let the VOCs reach equilibrium in the headspace.

Humidified air was pumped through aerobic test units. Relative humidity at the outlet of the test units was calculated by measuring dry and wet bulb temperatures. Outlet of the test units were connected to small glass jars and dry/wet bulb temperatures were measured inside these jars using thermocouples (Omega Engineering, CT). Wetted gauze was used to
measure wet bulb temperatures (figure 6). A datalogger (Model CR10X, Campbell Scientific, USA) was utilized to record temperature data.

2.3 Anaerobic test units

Anaerobic conditions were provided by tightly closing the anaerobic test units and passing 99.995 % pure N\textsubscript{2} (Chemistry Store, Iowa State University) through test units for two minutes. The O\textsubscript{2} trap (Alltech Associates, IL) was used to prevent any O\textsubscript{2} contamination. Mass flow of N\textsubscript{2} was set at 1L/min and checked by a mass flow controller (Dry Cal, NJ).

2.4 CO\textsubscript{2} and O\textsubscript{2} measurements

The CO\textsubscript{2} and O\textsubscript{2} concentrations were measured on weekly basis from the outlet of the test units using a CO\textsubscript{2} (Vaisala, CO) and O\textsubscript{2} (Apogee, UT) probe. The CO\textsubscript{2} and O\textsubscript{2} concentrations of the aerobic test units were recorded to test aeration status of the test units. O\textsubscript{2} concentrations of anaerobic test units were measured in order to check a possible O\textsubscript{2} contamination. The CO\textsubscript{2} and O\textsubscript{2} probes were calibrated weekly using clean ambient air and gas mixtures (5% CO\textsubscript{2}-15% O\textsubscript{2} and 15% CO\textsubscript{2}-5% O\textsubscript{2}) prepared by Chemistry Store, Iowa State University.

2.5 Headspace SPME and GC-MS analysis

A 85 μm Carboxen/polydimethylsiloxane SPME fiber and 1 h extraction time was used to collect VOCs from the headspace of the test units. This fiber and extraction time was chosen based on previous studies (Akdeniz, 2008). Samples were run using a 6890N GC and 5975 MS (Agilent Inc., DE). Pure helium (99.995 %) was used as the carrier gas at constant air flow (1.7 m/s). The injector and SPME fiber desorption temperature was 240 °C. The initial temperatures of the GC oven were 40 °C with 3 min holding time, followed by a ramp of 8 °C/min until reaching 220 °C, where it was held for 10 min. A polar capillary column (BP21, 60 m* 0.32 mm ID* 0.25 μm film thickness, SGE, TX) was used to separate analytes.
The MS mass/charge (m/z) ratio was set between 29 and 150 for the first eight minutes. After the first eight minutes, MS detection was between 34 and 280 m/z. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 °C, respectively.

Chromatography data acquisition software including MSD ChemStation (Agilent) and BenchTop/PBM™ V. 3.2.4 (Palisade Corporation, Ithaca, NY) were used to analyze data. Separated compounds were identified using mass spectral matches with ChemStation’s NIST MS Library and PBM Benchtop MS libraries. Spectral matches and column retention times were compared with those of standard analytes.

2.6 Measurement of emission rates

Calibration curves were prepared to quantify MS detector response (peak area count) using the methodology developed by Akdeniz (2008). Akdeniz (2008) reported that there is no significant difference between dry and humid conditions (97% relative humidity) in quantification of dimethyl disulfide, dimethyl trisulfide, and pyrimidine. Therefore, calibration curves were prepared for only dry conditions. In this study, to simulate a full scale composting process, temperatures of the test units were changed on daily basis (figure 8). Thus, headspace VOCs were extracted at different temperatures. To be able to quantify MS detector response at different temperatures, calibration curves were prepared for 20, 30, 40, 50, and 60 °C. For this purpose after standard gases of marker compounds were generated and collected inside glass sampling bulbs as described in Akdeniz (2008), they were placed inside an oven (The Grieve Corporation, IL) with a temperature controller. Glass bulbs were kept inside the oven for 10 mins and headspaces were sampled for 1 h with 85 µm Carboxen/PDMS (polydimethylsiloxane) SPME fiber. Concentrations of the compounds were calculated using the calibration curve with the closest temperature to the sampling temperature. Emission rates of the marker compounds were calculated using the following equation (Xin, 2005):
\[ ER = Q \cdot C \cdot \frac{W_m}{V_m} \cdot \frac{T_{std}}{T_a} \cdot \frac{P_a}{P_{std}} \]  

(1)

Where ER is the emission rate (µg/min), Q is the air flow rate (L/min), C is the concentration of the analyte (ppmv), \( W_m \) is the molecular weight of the analyte (g/mol), \( V_m \) is the molar volume of the analyte at STP (L/mol), \( T_{std} \) is the standard temperature (273.15 K), \( T_a \) is the ambient temperature (K), \( P_{std} \) is the standard pressure (101.325 kPa), and \( P_a \) is the ambient pressure (kPa).

2.7 Statistical analysis

Experiments were run in triplicate (N=3). JMP v 6.0.2 package was used for statistical analysis. Data were subjected to one-way analysis of variance (ANOVA). Treatment means were compared using TUKEY’s honestly significant differences (HSD) test at 95 % confidence level (\( p \leq 0.05 \) and N=3).

3. Results and discussion

3.1 Air and moisture supply

Oxygen and carbon dioxide values of aerobic test units were found to be same with ambient air (0.03 % CO\(_2\), 20.94% O\(_2\)). The O\(_2\) values of anaerobic test units were measured as zero. The CO\(_2\) values of anaerobic test units were lower than ambient air ranging from 0.01 to 0.012%. It shows that there was no limitation in O\(_2\) supply to aerobic test units and no O\(_2\) contamination to anaerobic test units. Moisture contents of the corn silage, oat straw, alfalfa hay, and pig tissues were decreased to 29.1±1.01, 31.1±1.25, 32.0±0.98, and 32.2±1.21 %, respectively. It can be concluded that aerobic test units were humidified properly during the process and over drying of the compost materials due to excessive aeration was prevented.
3.2 Identification of marker VOCs

Forty three compounds including volatile fatty acids, esters, nitrogen-containing compounds, alcohols, phenols, ketones, sulfur-containing compounds and terpenes were identified from corn silage, oat straw, alfalfa hay and swine composts (table 6). Retention time and spectra of the compounds were matched with those of pure standards.

Among the forty three compounds, only three compounds were found to be evolved by decaying swine tissues but not produced by any of the plant materials. These marker compounds were dimethyl disulfide, dimethyl trisulfide and pyrimidine. They were produced under both aerobic and anaerobic conditions. The main reason they are not produced by decaying plant materials during composting can be explained by slower microbial activity and degradation rate of plant materials due to their cellulose and lignin contents.

Dimethyl disulfide and dimethyl trisulfide are believed to be produced from sulfur, which makes up about 0.15 % of a swine body. Sulfur is widely distributed throughout the body since proteins are present in every cell of a swine body and sulfur containing amino acids (e.g., methionine, cystine, and cysteine) are components of almost all proteins (0.6-0.8 % of a protein) (Shurson et al., 1998). Dimethyl disulfide and dimethyl trisulfide are produced by microbial decomposition of sulfur containing amino acids under aerobic and anaerobic conditions. The mechanism would likely entail the sequential steps of the breakdown of protein to form peptides and degradation of the peptides to form free sulfur-containing amino acids. Sulfur-containing amino acids are broken down to form methyl mercaptan, dimethyl disulfide and dimethyl trisulfide by oxidation and enzymatic activity (Kadota and Ishida, 1972; Shurson et al., 1998; Higgins et al, 2002; Tulio et al., 2003). In this study, methyl mercaptan was not detected from decaying swine tissues.

Pyrimidine is a heterocyclic aromatic organic compound similar to pyrazine, containing two nitrogen atoms at positions 1 and 3 of the six member ring. There is not any information about the Pyrazines and furans were found to be the major Maillard reaction
products (Jenq et al., 1994). Although there is not any solid evidence, pyrimididine could be produced by Maillard reactions that occur between reducing sugars and amino acids.

3.3 Quantification of marker VOCs

SPME temperatures were compared according to the amount of analytes extracted at 1 hour sampling time. The highest extraction efficiency was achieved at 20 °C (figure 9). As the temperature increased, the extraction efficiency decreased due to the exothermic nature of the adsorption process. The amount of analytes extracted at 20, 30, and 40 °C were found to be significantly different than each other and the ones extracted at 50 °C and 60 °C (figure 9). For this reason, calibration curves were prepared at 20, 30, 40, 50, and 60 °C (table 7). The points have relative standard deviations ranging from 0.22 to 6.15 % and correlation coefficients ranging from 94.7 to 99.7 %. The lowest and highest concentrations prepared for the calibration curves were 0.01 ppmv and 6.85 ppmv, respectively. Concentrations and then emission rates of the marker compounds were calculated using these calibration curves.

3.4 Emission rates of marker VOCs

Emission rates of the marker VOCs from aerobic and anaerobic test units were shown in figures 10 and 11. Marker compounds were not detected in the first week (2\textsuperscript{nd} day) of the process. In the second week (9\textsuperscript{th} day) of the process, marker compounds were found to be in the headspace of the test units. The highest emission rates were measured in the 3\textsuperscript{rd} week of the process. After the 3\textsuperscript{rd} week, emission rates were decreased gradually and after the 5\textsuperscript{th} week, marker compounds were not detected from the headspace of the test units. Although temperatures of the test units were increased after composting process is completed (week 9), emission rates of the marker compounds were not increased and marker compounds were not detected from the headspace. This indicates that the emission rates of the marker compounds did not reduce due to a decrease in temperature and volatility. The amount of analytes reduced as most of the available nutrient sources are used and microbial degradation process
is completed. These results are supported by respiration rate measurements. Respiration rates of the swine tissues were decreased from 8.72±0.85 to 3.25±0.12 mg CO₂-C/g VS*d. Thompson classifies compost materials with 3.25 mg CO₂-C/g VS*d respiration rates as stable composts.

Emission rates of the dimethyl disulfide and dimethyl trisulfide were approximately 12 times higher than emission rate of pyrimidine in both aerobic and anaerobic test units. It is hard to compare emission rates of aerobic and anaerobic test units since aerobic test units were sampled at dynamic conditions while sampling from anaerobic test units were completed at static conditions. The reason to keep anaerobic test units closed was to not disturb the anaerobic environment formed in the headspace.

High correlations (correlation coefficients ranging from 83.7 to 99.6%) were found between emission rates of dimethyl disulfide, dimethyl trisulfide, and pyrimidine (table 8). This shows that these organic compounds are produced simultaneously in the presence of microbial activity. Since same aeration rate was used during the entire process same correlation coefficients can be reported for concentrations of the compounds. Although there is a doubt that pyrimidine is generated via non-enzymatic reactions, concentration or emission rate of pyrimidine can be used to estimate completion of the carcass degradation as well as dimethyl disulfide and dimethyl trisulfide as these compounds were found to be produced simultaneously.

In further studies, correlations can be used to estimate emission rate of a marker compound when emission rate of another correlated compound is known.

4. CONCLUSIONS

Three compounds, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be produced by decaying swine tissues in a system simulating full scale swine mortality composting system. These compounds are found to be produced simultaneously and in the
first three weeks of the process. They are not detected after the fifth week of the process. The decrease in the emission rates is not caused by change in the volatility of the compounds. The main reason is the slower microbial activity due to limited nutrient sources and exposure to high temperatures. Concentration or emission rate of one of these three compounds can be used to estimate completion of the carcass degradation in biosecure swine mortality composting systems. In future studies, these compounds will be tested for a full scale swine mortality composting operation.

Acknowledgment

The authors gratefully acknowledge Canadian Food Inspection Agency for the financial support through a grant from the Canadian Research and Technology Initiative (CRTI Project # 04 0052 RD).

References


Table 6. Total VOCs identified specifically originating from swine carcass tissues

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(A: aerobic, An: anaerobic, CS: corn silage, OS: oat straw, AH: alfalfa hay, Pig: swine carcass tissue, (+) detected and (-) not detected in the headspace; retention times of all compounds are matched with pure standards; N=3)
Table 7. Calibration curves for SPME at five different sampling temperatures

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<td>R²= 9.83E-01</td>
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<td>y=1.66E+07x-2.64E+05</td>
<td>y=1.67E+07x+3.87E+06</td>
<td>y=1.39E+07x+3.70E+06</td>
</tr>
<tr>
<td></td>
<td>R²= 9.97E-01</td>
<td>R²= 9.84E-01</td>
<td>R²= 9.93E-01</td>
</tr>
</tbody>
</table>

(DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)

Table 8. Correlations between emission rates of DMDS, DMTS, and PR

<table>
<thead>
<tr>
<th>Aerobic test units</th>
<th>Anaerobic test units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(DMTS)= 8.91E-01(DMDS) + 1.47-04</td>
<td>(DMTS)= 1.43+00(DMDS) + 4.39E-05</td>
</tr>
<tr>
<td></td>
<td>R²= 9.86E-01</td>
</tr>
<tr>
<td>(PR)= 7.72E-02(DMDS) - 6.42E-06</td>
<td>(PR)= 1.62E-01(DMDS)-2.98E-05</td>
</tr>
<tr>
<td></td>
<td>R²= 8.76E-01</td>
</tr>
<tr>
<td>(PR)= 7.72E-02(DMTS) - 6.42E-06</td>
<td>(PR)= 1.15E-01(DMTS) + 2.29E-05</td>
</tr>
<tr>
<td></td>
<td>R²= 8.37E-01</td>
</tr>
</tbody>
</table>

(DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)
**Figure 6.** Schematic of the aerobic test units and dry/wet bulb temperature measurements (Only 3 of the 12 test units are shown)

**Figure 7.** Schematic of the anaerobic test units and O$_2$/CO$_2$ measurement (Only 3 of the 12 test units are shown)
Figure 8. Applied temperature profile to simulate a full scale warm season animal mortality composting process (Glanville et al., 2007)

Figure 9. Comparison of five different SPME temperatures at 1 h extraction time (Concentrations of DMDS 6.85 ppmv, DMTS 5.95 ppmv, and PR 6.25 ppmv; N=3)
Figure 10. Emission rates of DMDS, DMTS, and PR from aerobic swine tissue composting test units

(DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)
Figure 11. Emission rates of DMDS, DMTS, and PR from anaerobic swine tissue composting test units
(DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)
Appendix A. Calibration curves of CO$_2$ and O$_2$ sensors

**CO$_2$**

\[ y = 9.97 \times 10^{-1} x + 5.25 \times 10^{-2} \]

\[ R^2 = 1.00 \times 10^0 \]

**O$_2$**

\[ y = 1.69 \times 10^0 x + 1.04 \times 10^0 \]

\[ R^2 = 1.00 \times 10^0 \]
Appendix B. Calibration curves to quantify dimethyl disulfide (DMDS) at five different temperatures

20 ºC

\[ y = 2.72E+07x - 3.70E+06 \]

\[ R^2 = 9.74E-01 \]

30 ºC

\[ y = 2.18E+07x - 1.09E+06 \]

\[ R^2 = 9.90E-01 \]

40 ºC

\[ y = 1.78E+07x + 1.34E+05 \]

\[ R^2 = 9.93E-01 \]

50 ºC

\[ y = 1.66E+07x + 6.64E+05 \]

\[ R^2 = 9.96E-01 \]

60 ºC

\[ y = 1.66E+07x - 2.64E+05 \]

\[ R^2 = 9.97E-01 \]
Appendix C. Calibration curves to quantify dimethyl trisulfide (DMTS) at five different temperatures

\[ y = 1.88 \times 10^7 x + 5.58 \times 10^6 \]
\[ R^2 = 0.983 \]

\[ y = 1.47 \times 10^7 x + 5.00 \times 10^6 \]
\[ R^2 = 0.987 \]

\[ y = 1.43 \times 10^7 x + 4.31 \times 10^6 \]
\[ R^2 = 0.989 \]

\[ y = 1.40 \times 10^7 x + 4.27 \times 10^6 \]
\[ R^2 = 0.989 \]

\[ y = 1.39 \times 10^7 x + 3.70 \times 10^6 \]
\[ R^2 = 0.993 \]
Appendix D. Calibration curves to quantify pyrimidine (PR) at five different temperatures

- **20 °C**
  - $y = 2.34E+07x + 9.99E+06$
  - $R^2 = 9.47E-01$

- **30 °C**
  - $y = 2.13E+07x + 7.19E+06$
  - $R^2 = 9.75E-01$

- **40 °C**
  - $y = 1.95E+07x + 4.80E+06$
  - $R^2 = 9.68E-01$

- **50 °C**
  - $y = 1.67E+07x + 4.97E+06$
  - $R^2 = 9.82E-01$

- **60 °C**
  - $y = 1.67E+07x + 3.87E+06$
  - $R^2 = 9.84E-01$
**Appendix E.** Edlog (CR10) program code for CO₂, O₂ and temperature measurements

```plaintext
; [CR10]
*Table 1 Program
01: 10Execution Interval (seconds)
1: Batt Voltage (P10)
2: Temp (107) (P11)
; O₂ MEASUREMENTS
3: Volt (Diff) (P2)
; CO₂ MEASUREMENTS
4: Volt (Diff) (P2)
; ENABLE MULTIPLEXER
5: Do (P86)
; BEGIN MEASUREMENT LOOP
6: Beginning of Loop (P87)
; CLOCK PULS
7: Do (P86)
8: Excitation with Delay (P22)
; MEASURE 1 THERMOCOUPLE PER LOOP
9: Thermocouple Temp (DIFF) (P14)
; END MEASUREMENT LOOP
10: End (P95)
; DISABLE MULTIPLEXER
11: Do (P86)
; SAVE FINAL ARRAYS
12: If time is (P92)
13: Set Active Storage Area (P80)
14: Real Time (P77)
15: Average (P71)
16: Totalize (P72)
17: Totalize (P72)
18: Average (P71)

* Table 2 Program
02: 0.0000 Execution Interval (seconds)

*Table 3 Subroutines
End Program

(PC208W 3.1a-datalogger software)
### Appendix F. VOCs identified from mix compost of corn silage and pig tissues

<table>
<thead>
<tr>
<th>Compound name</th>
<th>CAS #</th>
<th>Aerobic</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volatile fatty acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>64-19-7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>79-09-4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3-Methyl butanoic acid</td>
<td>503-74-2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>109-52-4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>142-62-1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Esters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>79-20-9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>141-78-6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl propanoate</td>
<td>105-37-3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Propyl acetate</td>
<td>109-60-4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>105-54-4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Propyl butanoate</td>
<td>105-66-8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Isobutyl butanoate</td>
<td>539-90-2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Butyl butanoate</td>
<td>109-21-7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Butyl hexanoate</td>
<td>626-82-74</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>106-32-1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Nitrogen-containing compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrimidine</td>
<td>289-95-2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-piperidinone</td>
<td>675-20-7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1H-Indole</td>
<td>120-72-9</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>64-17-5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>67-63-0</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>78-92-2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>111-27-3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-Heptanol</td>
<td>111-70-6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>111-87-5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2-Heptanol</td>
<td>543-49-7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methoxy phenol</td>
<td>90-05-1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>108-95-2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4-Methyl phenol</td>
<td>106-44-5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4-Ethyl phenol</td>
<td>123-07-9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Butanone</td>
<td>78-93-3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>107-87-9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Octanone</td>
<td>111-13-7</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3-Octanone</td>
<td>106-68-3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>821-55-6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2-Decanone</td>
<td>886-33-9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sulfur-containing compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>624-92-0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dimethyl trisulfide</td>
<td>3658-80-8</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(Corn silage and pig tissues are mixed at the ratio of 15:1 (w/w); composted under aerobic and anaerobic conditions; temperature of a full scale composting process is simulated; N=3)
CHAPTER 5. IDENTIFICATION, EVALUATION AND QUANTIFICATION OF VOCS FROM FIELD SCALE BIOSECURE SWINE MORTALITY COMPOSTING OPERATIONS

A paper to be submitted to Environmental Science and Technology

Neslihan Akdeniz, Jacek A. Koziel, Hee-Kwon Ahn, Thomas D. Glanville, Benjamin P. Crawford, D. Raj Raman, Anthony L. Pometto III, Sam Beattie

ABSTRACT

Animal mortalities are composted by fully covering the nitrogen-rich carcasses with carbon-rich materials. In case of diseased swine mortality composting, special containment requirements are necessary to reduce the risk of spreading pathogens to the environment and subsequent disease transmission. These containment requirements and pathogen concerns make the monitoring and management of the process both more difficult and more crucial. The fundamental goal of this field scale composting study was to determine the potential of using VOCs to non-invasively monitor the state and completion of full scale swine mortality composting operations. The objectives of the study were to determine if specific compounds can be correlated with different phases of swine mortality composting process and to determine the effects of compost operating parameters on the chemical make-up of gaseous emissions. To this end, solid phase microextraction (SPME) coupled with gas chromatography (GC)-mass spectrometry (MS) was used to collect gas samples from diseased swine mortality composts. A total of 55 compounds were identified. Of these, three compounds, dimethyl disulfide, dimethyl trisulfide, and pyrimidine, were assessed as the marker compounds of swine mortality composting process. It was showed that VOC production decreases in the later stages of the process and was closely related to the
degradation rates of swine carcasses. The highest concentrations of the VOCs were detected from swine carcasses with the highest respiration rates and lowest degradation rates.

**Keywords.** Compost, GC-MS, SPME, swine mortality, VOCs.

1. **Introduction**

1.1 **Composting basics**

Composting is the aerobic or oxygen-requiring degradation process which takes place under controlled conditions (Rynk, 1992; Haug, 1993; Keener et al., 1993). Active composting generates considerable heat, water vapor, and large quantities of carbon dioxide and/or volatile organic compounds. Composting also reduces the volume and mass of the raw substrate while transforming them into a valuable soil conditioner (Rynk, 1992). During composting, many factors need to be controlled including moisture content, oxygen availability, carbon and nitrogen balance, and heat retention (Glanville, 2001). These factors affect odorous compound emissions and stability of the compost (Walker, 1993). A proper C/N ratio will result in a composting process that produces little odor and offers an environment where beneficial microorganisms can flourish (Keener et al., 2000). Poorly aerated and high moisture compost causes offensive odor release and decreases compost quality. Well aerated compost will improve the quality of the compost, but may lead to rapid drying of the compost before it has fully stabilized. Compost envelope materials are used to provide desired ratio of C and N, essential nutrients, biological diversity, adequate but not excessive moisture and aeration. The envelope materials that are often used include legume and grass hay, leaves, farm and garden plant residues, vegetable and fruit processing residue, chopped corn silage, soybean stems, straw and wood shaving bedding and animal manure (Biernbaum and Fogiel, 2004).
1.2 Diseased animal mortality composting

Animal mortalities are composted by fully covering the nitrogen-rich carcasses with carbon rich materials. Mortalities are converted into humic acids, bacterial biomass, and compost (Harper and Estienne, 2003). Specific criteria must be met for animal mortality composting to assure human pathogenic bacteria are not present in the compost. A sufficient number of turns, with specific temperatures (131- 170ºF), in a certain period of time (3 days for static aerated pile and 15 days for windrow) are required (Biernbaum and Fogiel, 2004). However, in case of diseased animal mortality composting, special requirements will be necessary to reduce the risks of pathogen survival and disease transmission. One of the most important requirements is to not turn piles during decomposition to minimize the risk of releasing pathogens into the wind (Glanville 2006). To further reduce the risks of pathogen release, the Canadian Food Inspection Agency used plastic bio-security barriers to compost poultry during 2004 avian influenza outbreak (Spencer et al., 2004).

1.3 Stabilization of composts

Stabilization of composts affects the response of plants to compost applications, potential for microbial activity, odor generation, and pathogen regrowth (Zucconi et al., 1985; Miller 1993). Also, the degree of stability achieved within a certain time can be used to evaluate performance of the process (Stentiford, 1993). Stability is generally defined as an indicator of microbial activity and it can be determined by O₂ uptake rate, CO₂ production rate or heat release (Chen and Inbar, 1993; Wu et al., 2000). Respirometric techniques are widely used as stability indicators (Sadaka et al., 2006). The basis of these methods is that immature compost has higher O₂ consumption and CO₂ production rates due to high levels of microbial activity (Gomez et al., 2005). Thompson (2002) classified compost stability based on respiration rates as very stable (1 mg CO₂-C/ g VS. d), stable (2-4 mg CO₂-C/ g VS. d),
moderately unstable (5-7 mg CO₂-C/ g VS. d), unstable raw compost (8-9 mg CO₂-C/ g VS. d), raw compost (10-11 mg CO₂-C/ g VS. d), and raw feedstock (>11 mg CO₂-C/ g VS. d).

1.4 Monitoring diseased animal mortality composting

Four parameters including temperature, moisture content, oxygen/ carbon dioxide, and VOC production can be monitored to assess composting process. Temperature can be easily monitored during composting. There are four thermal phases. These phases are mesophilic starting phase, thermophilic phase, cooling phase and maturation phase (Peigne and Girardin, 2004). If temperature of a pile is not increasing, nutrient supply, moisture level or aeration may not be sufficient. Other reason may be over aeration of the pile which causes removal of the produced heat. Thus, a decline in temperature does not guarantee that the composting process is completed. The moisture content of a compost material during active phase should be at a level that some water can be squeezed out by hand (45-65%) (Haug 1993). However there is not any easy and affordable way to collect representative samples from large compost piles. Oxygen levels should be above 5% if compost is aerated properly. Oxygen and CO₂ levels can be measured with a probe to test aeration status of a compost pile (Biernbaum and Fogiel, 2004). However, O₂ and CO₂ levels do not indicate completion of a composting process since.

Measurement of VOCs evolved from decaying mortalities is another alternative way to test aeration and status of a composting process (Kim et al., 2005). It is known that a wide variety of VOCs are produced during the process. These include volatile fatty acids (VFAs), ammonia, and other nitrogen compounds, inorganic, and organic sulfur compounds, ketones, aldehydes, alcohols, and terpenes (Golueke et al., 1954; Chanysak et al., 1982; Fisher et al., 1986; Koe, and Ng, 1987; Derikx et al., 1990; Miller, 1993; Williams, and Miller, 1993; Day et al., 1998; Krzymien et al. 1999; Kim et al., 2005).

Solid phase microextraction (SPME) coupled with GC-MS has been shown as a convenient method to detect VOC production in various matrices (Koziel et al., 1999; Kim et
al 2005; Koziel et al., 2006; Akdeniz et al., 2007a). Koziel et al. (1999) and Koziel (2006) used SPME for a wide scope of air monitoring including livestock odors. Kim et al. (2005) characterized headspace odorants from commercial composts of 14 different producers using SPME and GC-MS. Akdeniz et al. (2007a) identified VOCs evolved during biological decomposition of plant materials utilizing SPME and GC-MS. VOC emissions from swine mortality composting processes have not been identified or measured with any sampling technique. In this study, VOCs generated during degradation of swine mortality composts were identified and monitored on a weekly basis from two replicated field trials carried out using 6 different envelope materials.

Objectives of the study were (a) to develop a comprehensive chemical library of VOCs emitted from swine mortality composts surrounded by plastic bio-security barriers, (b) to determine if specific compounds can be correlated with different phases of the composting process and be used to determine completion of composting, (c) to determine the effects of compost operating parameters on the chemical make-up of gases.

2. METHODOLOGY

2.1 Composting test units

In this study, the emergency treatment used during the 2004 outbreak of avian influenza in British Columbia (Spencer et al., 2004; Stepushyn, 2004) were taken as a model and applied to swine mortalities. The experiments were conducted in May-October 2007 at Livestock Environment Building and Research Center of Iowa State University, Ames, Iowa. Test units were constructed on 2 m × 2 m platforms with 1.2 m high sidewalls. Inside of the test units were covered with a synthetic rubber liner to capture and retain leachate. Test units were loaded with approximately 250 kg swine carcasses. The bottom 30 cm of the test units were filled with an envelope material and swine carcasses were placed on this envelope material. The same envelope material (60 cm) was used between and over the carcasses.
Outside of the test units were insulated with 5 cm thick Styrofoam (plastic barrier). Aeration of the test units was achieved by inserting three passive aeration tubes beneath swine carcasses. (Ahn et al., 2007; Glanville et al., 2007). The purpose of the plastic barrier was to minimize the risk of spreading pathogens to the surrounding. Vaccine strains of Avian encephalomyelitis (AE) and *Newcastle disease virus* (NDV) were placed inside the test units by another research group of the project to test pathogenic virus survival. Virus survival data is not included in this paper and will be reported separately.

Six envelope materials including corn silage, oat straw, corn stalks, wood shavings, soybean straw, and alfalfa hay were tested. Except corn silage and wood shavings, all envelope materials were ground into 5 cm length to reduce their size, improve liquid and VOC absorbency and heat retention. Moisture contents of the envelope materials were not changed. Corn silage, oat straw and corn stalks test units were run in triplicate (N=3) from May 29\textsuperscript{th} to July 29\textsuperscript{th}, and wood shavings, soybean straw and alfalfa hay test units were run from August 20\textsuperscript{th} to October 20\textsuperscript{th}. Swine carcasses were composted for eight weeks since all the experiments were supposed to terminate during the warm season of Iowa (May 29\textsuperscript{th}-October 20\textsuperscript{th}).

Multi port gas and temperature sampling probe constructed of 3 cm diameter PVC (polyvinyl chloride) piping placed in the center of the test units to make measurements at bottom (envelope material), middle (swine carcass), and top (envelope material) layers (depths) of the test units. PTFE (polytetrafluoroethylene) air sampling tubings (0.62 cm I.D., E&S Technologies, Chelmsford, MA) and thermocouples (Omega Engineering, Stamford, CT) were tightly placed inside the sampling probes. An Apogee O\textsubscript{2} sensor (Logan, UT) was used to measure O\textsubscript{2} level.
2.2 Headspace SPME

Air samples were drawn from the test units using SKC pumps (224-PCXR4, PA). Air flow rate was 1.0 L/min and sampling time was chosen as 5 min (5 hydraulic residence times) to let the system to reach equilibrium. Air samples were passed through 250 mL glass sampling bulbs (Supelco, Bellofonte, PA). After 5 min sampling time, PTFE stopcocks of glass bulbs were closed and air samples were stored in the bulbs. Then, air samples were carried to Atmospheric Air Quality Laboratory where they were sampled using an 85 µm Car/PDMS SPME fiber (Supelco, Bellefonte, PA) and 1 hr extraction time. This fiber coating and extraction time was chosen based on previous studies (Akdeniz, 2008). New SPME fibers were first conditioned according to the manufacturer directions. In addition, SPME fibers were inserted into the injection port of GC for 5 min to thermally desorb impurities on the fiber immediately before sampling. After this 5 min cleaning, all SPME fibers were analyzed as blanks for a possible carry-over and other impurities to minimize interferences.

2.3 Sample analysis

All gas analyses were completed using integrated multi dimensional GC-MS system consisting of a 6890N GC and 5973 MS (Agilent Inc., Wilmington, DE) system. Ultrahigh pure (99.9995 %) helium (Praxair, Danbury, CT) was used as the carrier gas at constant pressure. The injector and SPME fiber desorption temperature was 260 ºC. The initial temperatures of the GC oven were 40 ºC with 3 min holding time, followed by a ramp of 7 ºC/min until reaching 220 ºC, where it was held for 11.29 min to complete the 40 min run. The analytes were separated on two capillary columns that were connected in series: a 12 m × 0.53 mm i.d. non-polar pre-column, and a 25 m × 0.53 mm i.d. polar analytical column (SGE, Austin, TX). The heart-cut valve between the pre-column, and analytical column was opened between 0.05-35 min, and backflush of the pre-column was activated between 36-40 min to prepare the system for the following run. The MS mass/charge (m/z) ratio was set
between 33 and 150. For the first eight min scanning rate was 5.89 scan/s. After the first eight min, the m/z ratio was open between 34 and 280 m/z with 5.64 scan/s scanning rate. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 ºC, respectively. The electron multiplier voltage was between 1000 and 1388 eV. Autotuning of MS was performed at least once a week.

2.4 Data analysis

Chromatography data acquisition software consisted of MSD ChemStation (Agilent) and BenchTop/PBM™ V. 3.2.4 (Palisade Corporation, Ithaca, NY) was used to analyze data. Separated compounds were identified using mass spectral matches with ChemStation’s NIST MS Library and PBM Benchtop MS libraries. Spectral matches and column retention times were compared with those of standard analytes. HPLC-grade standards of compounds were purchased from Sigma-Aldrich (Milwaukee, WI).

MS detector responses (peak area count) were converted to concentrations (ppmv) using the calibration curves reported in Akdeniz (2008) for dry conditions. Akdeniz (2008) prepared calibration curves for dry and humid conditions. Since no significant difference was reported between dry and humid, dry condition calibration curves were used in this study.

2.5 Respiration rate measurements

Swine carcasses were composted for eight weeks. After eight week composting, plastic barriers of the test units were opened and it was observed that there was still remaining animal tissue. Respiration rates of the remaining animal tissues were measured. Samples were collected from each pig composted in the experiments. Twenty gram of homogenous soft tissue samples was prepared. Titration method was used to measure the evolved CO₂ during respiration. The method is described in Sadaka et al., 2006. For respiration rate calculations, moisture content and volatile solids of the compost samples were analyzed using standard methods. Moisture content was determined by drying samples
at 105 °C for 24 h. Volatile solid contents of dried samples were measured by combusting at 550 °C for 5 hrs (Ahn et al., 2005).

2.6 Statistical analysis

Experiments were conducted in triplicate (3 test units for each envelope material). Statistical tests were conducted using JMP v 6.0.2 package. Data were subjected to one-way analysis of variance (ANOVA). Treatment means were compared using Tukey’s honestly significant differences (HSD) test at the significance level p<0.05.

3. Results and discussion

3.1 Evaluation of sampling location

Results from sampling probes placed in the center of the test units to collect air samples from bottom, middle and top layers (depths) of the test units are presented in figure 12. It is noted that amounts of marker compounds extracted during eight week composting time was added and average of the three test units were presented. Relative standard deviation of the data was ranging from 3 to 18%. Dimethyl disulfide and pyrimidine amount in corn silage, wood shavings, soybean straw, and alfalfa hay test units, was found to be significantly different in middle layer compared to bottom and top layers. In the rest of the test units (oat straw and corn stalks test units), no significant difference was observed between middle and bottom layers, but remarkable differences were detected compared to the top layer. Highest amounts of dimethyl trisulfide were measured from the middle and bottom layers of the test units. It is believed that marker compounds were produced during degradation of swine carcasses in the contaminated middle and bottom layers. The low amounts of compounds detected in the top layer can be explained by absorption of the compounds by overlying envelope materials. Since the highest amounts of marker VOCs were measured from the middle layer, this layer was decided to be the most representative
layer of the test units and results associated with this layer were presented in the following sections.

### 3.2 Identification and evaluation of VOCs

Fifty five compounds were identified from middle location (depth) of warm season swine mortality composting operations (table 9). Retention times and spectra of 35 compounds were matched with those of pure standards and these compounds are verified. Compounds detected from all test units, regardless of the envelope material used, were dimethyl disulfide, dimethyl trisulfide, and pyrimidine. These compounds were considered as marker compounds of the swine mortality degradation due to two reasons. The first reason was these compounds were found to be produced during degradation of swine tissues in laboratory scale composting experiments. Akdeniz (2008) reported that dimethyl disulfide, dimethyl trisulfide and pyrimidine were not produced during degradation of corn silage, oat straw, and alfalfa hay but produced from decaying swine tissues. The second reason was these marker compounds were produced regardless of the plant (envelope) material used in the field trials. This evidence also suggests that these compounds were evolved from decaying swine carcasses. Dimethyl disulfide, dimethyl trisulfide and pyrimidine were used to monitor completion of the swine tissue degradation and composting process. Detection of these compounds from all 18 field test units indicates that these compounds can be produced and detected under different composting conditions (e.g., different envelope material, moisture content, temperature profile). In this sense, these three compounds are believed to be reliable markers of the swine carcass degradation process and can be used to evaluate completion of a swine mortality composting process.

Volatile fatty acids (VFAs) and mercaptans are known to be produced during anaerobic degradation (Haug, 1993). In this study, several VFAs (acetic, propanoic, 3-methyl butanoic, pentanoic and hexanoic acids) and mercaptans (methanethiol and ethanethiol) were
detected from corn silage, oat straw and corn stalks test units. However, they were not
detected from wood shavings, soybean straw and alfalfa hay test units. Oxygen levels of the
test units can be potentially used to explain this difference. Oxygen levels of all test units are
shown in figure 13. Relative standard deviation of oxygen data was ranging from 0.19-45.1%
. Oxygen levels of all test units were high and ranging from 13 to 20.9%. There was no
evidence of anaerobic macro-environment formation. However, higher initial moisture
contents of corn silage, oat straw and corn stalks (56.2-64.8%) than wood shavings, soybean
straw and alfalfa hay (11.2-17.7%) may have caused anaerobic micro-environment formation.
Anaerobic microenvironment formation can explain anaerobic VOC production in corn
silage, oat straw and corn stalks test units. It can be concluded that VFAs and mercaptans can
be used to evaluate aeration status of a composting system.

3.3 Evaluation of carcass degradation

Concentrations of the marker compounds measured from middle layer of the test units
are shown in figures 14 through 19. In corn silage, oat straw and corn stalks test units, the
highest concentrations were observed for the second and third weeks of the process (figures
14, 15, and 16). After the third week, concentrations of the marker compounds decreased
gradually. In wood shavings, soybean straw and alfalfa hay test units, the highest
concentrations were measured for the first week and concentrations were decreased gradually
after the first week. A gradual decrease in the concentrations of the marker compounds
indicates that carcass degradation and marker VOC production slowed down. Akdeniz (2008)
used a laboratory scale set-up and showed that dimethyl disulfide, dimethyl trisulfide, and
pyrimidine were not detected from the headspace of swine tissue comports after the fifth
week of the process. In replicated field trials, although concentrations of the marker
compounds were decreased after eight week composting, they still continued to be detected
from the test units. Concentrations of the compounds in the last week of the process are
shown in figure 20 with a lower scale compared to figures 14 to 19. Dimethyl disulfide was detected from all test units in the 8th week of the trial. Its concentration decreased from a range of 0.29-4.34 ppmv (during weeks 1 and 2) to 0.006-0.160 ppmv. Dimethyl trisulfide was detected in the 8th week of the trial from wood shavings, soybean straw, and alfalfa hay test units at concentrations of 0.43, 0.017, and 0.019 ppmv, respectively. Pyrimidine was only detected from wood shavings and alfalfa hay test units at concentration of 0.013 ppmv. It was concluded that composting process was not stabilized in any of the test units and needed to continue until concentrations of these cannot be detected from the middle layers of the test units. Since DMDS concentrations were lowest in corn stalks and oat straw test units, the highest degradation was found in these test units. Wood shavings and alfalfa hay test units were evaluated as the test units with the lowest degradation rate as all the three marker compounds were detected from these test units (figure 20). Based on the concentrations of the marker compounds in week 8 (figure 20), the carcass degradation in the test units was ranked from the highest to the lowest as: corn stalks ≈ oat straw > corn silage > soybean straw > alfalfa hay > wood shavings. This finding was supported by respiration rate measurements (table 10). Samples collected from corn silage, oat straw and corn stalks test units were classified as moderately unstable compost and samples collected from wood shavings, soybean straw and alfalfa hay test units were classified as unstable raw compost. The highest respiration rates (lowest degradation) were detected for wood shavings and alfalfa test units. It can be concluded that concentrations of the marker compounds are good indicators of carcass degradation and stabilization of the swine mortality composting process. These marker compounds can be used to evaluate completion of the swine mortality composting process.

The incomplete carcass degradation can be explained by substantial loss of water from the test units, resulting in desiccation of carcasses and cessation of breakdown. Since test units were wrapped with plastic barriers, no additional water was added to the test units.
during the process. Corn silage, oat straw and corn stalks were initially moistened by rain (56.2-64.8%) but there was strong evidence of excessive moisture loss due to over aeration. This problem was made worse in wood shavings, soybean straw, and alfalfa hay test units by the lower initial moisture contents (11.2 to 17.7%) as well as by over aeration. The very low initial moisture contents of wood shavings, soybean straw, and alfalfa hay caused lower degradation of swine carcasses in these test units. Moreover, many terpenes (e.g., camphene, limonene, beta pinene, beta phellandrene) were detected from pine wood shaving and alfalfa hay test units. These compounds cause the specific odor of pine and alfalfa plants. These monoterpenes have been found to have antimicrobial effect on some pathogenic bacteria. Demirci et al. (2007) tested antimicrobial effect of \textit{Charephyllum libanoticum}'s essential oil which is rich in monoterpenes including camphene, limonene, $\beta$-pinene and $\beta$-phellandrene. Results showed that common Gram (+) and Gram (-) human pathogenic bacteria including methicillin-resistant \textit{S. aureus} and the yeast \textit{C. albicans} survived exposure to the essential oil, \textit{P. aeruginosa} and \textit{S. epidermidis} were reduced. The oil was defined as weak to moderate in antibacterial activities. This weak to moderate antimicrobial effect of terpenes may partially explain lower microbial activity, lower temperatures and higher respiration rates of remaining carcass tissues in wood shavings and alfalfa hay test units (figures 17, 19, 20 and table 10).

Temperature data collected from middle depth of the center sampling probe and ambient air temperature are presented in figures 14 through 19. Average of the three test units was reported. Relative standard deviations of the temperature data range from 0.86 to 20.4%. The highest temperatures were measured during the 1\textsuperscript{st} and 2\textsuperscript{nd} weeks of the process. After the 2\textsuperscript{nd} week, temperatures of the test units started to decrease. It is known that a decline in temperature does not necessarily show completion of the process (Biernbaum and Fogiel, 2004). In this study, it is also shown that there is no relation between the temperatures of the test units and the degradation (respiration) rate of swine carcasses. In the last week of
the process, the lowest temperatures (around 35 ºC) were observed for oat straw, soybean straw and alfalfa hay test units (figures 15, 18, and 19). Temperatures of corn silage, corn stalks, and wood shavings test units were around 55, 45, and 45 ºC, respectively (figures 14, 16, and 17). Although higher final temperatures were recorded for corn silage, corn stalks and wood shavings test units compared to oat straw, soybean straw and alfalfa hay test units, the most complete carcass degradation was observed for corn silage and corn stalks test units. It was concluded that temperature data does not necessarily indicate the final extent of degradation of swine carcasses. A better estimate of carcass degradation can be made by measuring dimethyl disulfide, dimethyl trisulfide and pyrimidine concentrations in the last week of the process.

4. CONCLUSIONS

A comprehensive chemical library of VOCs was developed for swine mortality composts surrounded by plastic barriers. Dimethyl disulfide, dimethyl trisulfide and pyrimidine were shown to be marker compounds of the swine mortality composting process. These compounds were produced from all test units although different plant (envelope) materials at different moisture levels were used in each test unit. This indicates that these compounds can be produced under various conditions (e.g., different plant material, moisture content, porosity, temperature). In this study, swine mortalities were not composted until they were completely degraded. However, it is showed that VOC production decreases in the later stages of the process and are consistent with the final extent of degradation of the swine carcasses. No close relation was observed between the temperature data and degradation rates of carcasses. A better estimate of carcass degradation is made by measuring dimethyl disulfide, dimethyl trisulfide and pyrimidine concentrations in the last week of the process. In future studies, dimethyl disulfide, dimethyl trisulfide and pyrimidine can be monitored for swine or other livestock mortality composting operations where all the carcass degradation is
completed. Also, volatile fatty acids, methanethiol, and ethanethiol can be further investigated as indicators of insufficient aeration in animal mortality composting operations.

**Acknowledgment**

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**References**


Thompson, **2002**. Test methods for the examination of composting and compost (TMECC) on CD, June 2002. The Composting Council Research and Education Foundation.


Table 9. VOCs identified from middle location (depth) of warm season swine mortality compost

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(CSi: corn silage, OS: oat straw, CSt: corn stalks, WS: wood shavings, SS: soybean straw, AH: alfalfa hay; (+) presence, and (-) absence of the compounds in the air sample)
Table 9 continues…

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<td>24406-05-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Calamene</td>
<td>483-77-2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>α-Calacorene</td>
<td>21391-99-1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Azulene</td>
<td>275-51-4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(CSi: corn silage, OSt: oat straw, CSt: corn stalks, WSh: wood shavings, SSSt: soybean straw, AHa: alfalfa hay; (+) presence, and (-) absence of the compounds in the air sample; * retention time matched with standard analytes; N=3).

Table 10. Respiration rates of the remaining carcass tissues

<table>
<thead>
<tr>
<th>Test units</th>
<th>MC (%)</th>
<th>mg CO₂-C / g VS⁻¹ d⁻¹</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>59.62±3.25</td>
<td>6.40±0.42D</td>
<td>moderately unstable compost</td>
</tr>
<tr>
<td>Oat straw</td>
<td>48.59±1.25</td>
<td>5.52±0.3E</td>
<td>moderately unstable compost</td>
</tr>
<tr>
<td>Corn stalks</td>
<td>49.52±2.8</td>
<td>5.21±0.82E</td>
<td>moderately unstable compost</td>
</tr>
<tr>
<td>Wood shavings</td>
<td>53.26±3.2</td>
<td>9.25±0.85A</td>
<td>unstable raw compost</td>
</tr>
<tr>
<td>Soybean straw</td>
<td>37.57±4.9</td>
<td>7.85±0.65C</td>
<td>unstable raw compost</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>52.80±4.7</td>
<td>8.56±0.38B</td>
<td>unstable raw compost</td>
</tr>
</tbody>
</table>

(Different letters are significantly different, α= 0.05; N=3)
Figure 12. Comparison of bottom, middle and top layers (depths) of the test units (DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)
Figure 13. Oxygen concentrations of the middle layer
(Average of three test units; N=3)
**Figure 14.** Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for corn silage test units

(Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during May 29th-July 29th)
Figure 15. Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for oat straw test units

(Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during May 29th-July 29th)
Figure 16. Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for corn stalks test units

(Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during May 29th-July 29th)
Figure 17. Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for wood shavings test units.
(Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during August 20\(^{th}\)-October 20\(^{th}\))
Figure 18. Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for soybean straw test units

(Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during August 20\(^{th}\)-October 20\(^{th}\))
Figure 19. Average concentrations of the marker compounds, test unit temperature, and ambient air temperature for alfalfa hay test units (Concentrations and temperature values from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3; composted during August 20<sup>th</sup>-October 20<sup>th</sup>)
Figure 20. DMDS, DMTS, PR concentrations in week eight

(Concentrations from middle layer; DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine; N=3)
Appendix A. Photos of full scale swine mortality composting test units and air sample collection
Appendix B. Photos of test units and air sample collection

Figure 1. Multi port VOC, O₂, CO₂ and temperature sampling probe (a) and sampling locations inside a test unit (b)
Figure 2. Inside view of test units and multi port gas-temperature sampling probe (a), (b), (c) and plastic sheet wraps (d)
Figure 3. Overview of test units, sampling tubings, glass sampling bulbs, SPME at Atmospheric Air Quality Laboratory and GC-MS analysis
### Appendix C. Degradation rates of the swine carcasses in different test units

<table>
<thead>
<tr>
<th>Test units</th>
<th>MC (%)</th>
<th>Carcass decomposition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>59.62±3.25</td>
<td>64.4±0.1</td>
</tr>
<tr>
<td>Oat straw</td>
<td>48.59±1.25</td>
<td>71.8±0.6</td>
</tr>
<tr>
<td>Corn stalks</td>
<td>49.52±2.8</td>
<td>74.8±0.9</td>
</tr>
<tr>
<td>Wood shavings</td>
<td>53.26±3.2</td>
<td>63.6±5.1</td>
</tr>
<tr>
<td>Soybean straw</td>
<td>37.57±4.9</td>
<td>71.0±1.9</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>52.80±4.7</td>
<td>64.3±3.3</td>
</tr>
</tbody>
</table>

1. Carcass decomposition rate (%) = \{((Initial carcass weight-Final carcass weight) / (Initial carcass weight-Bone weight in initial carcass))\} * 100

2. Bone content about 12% of body weight
CHAPTER 6. SAMPLING METHOD OF VOCS FOR BIOSECURE COMPOSTING OPERATIONS

A short communication paper to be submitted to Bioresource Technology

Neslihan Akdeniz, Jacek A. Koziel, Hee-Kwon Ahn, Thomas D. Glanville, Benjamin, P. Crawford, D. Raj Raman, Anthony L. Pometto III, Sam Beattie

Abstract

Monitoring volatile organic compounds (VOCs) as biosecure markers of carcass degradation was reported to be a promising method to test progress and completion of the process. Three compounds, dimethyl disulfide, dimethyl trisulfide, pyrimidine, were found to be marker compounds of swine carcass degradation. In this study, different air sampling locations were compared for a full scale swine mortality composting system. The objectives of the study were (a) to test if passive aeration tubes (inlet and outlet) were practical sampling locations of marker VOCs and (b) to monitor VOC concentrations from passive aeration tubes in order to decide air flow direction of the test units. It was shown that marker VOC concentrations were significantly different in middle location (depth) and upper-aeration duct compared to other locations. In case of an emergency swine disposal upper-aeration duct could be used to collect air samples. However, upper aeration duct was not always a reliable sampling location as the air flow direction could change during the process. In the study, higher concentrations of the VOCs were measured from the upper aeration duct and this duct was claimed to be an outlet. Sampling VOCs could be used to evaluate air flow direction of passively aerated systems. Further studies are needed to show consistency of the findings.

Keywords. Air flow, Biosecurity, Compost, SPME, Swine, VOC
1. Introduction

Iowa has led the US in swine production and pig inventory for one hundred twenty years (Honeyman and Duffy, 2006). In 2005, Iowa produced 28.4% (8 billion pounds) of all the swine in U.S.A (Iowa Agricultural Statistics Bulletin). This intensive production of swine in one area inevitably results in production of high amount of piggery waste including animal carcasses. Animal carcasses, like all animal waste, contain useful nutrients which can be recycled into agricultural land. However, this recycling must be done in an environmentally sound, economically feasible, and socially acceptable manner especially when carcasses are capable of spreading diseases in soil, plants, animals, and humans (Imbeah, 1997, Kalbasi et al., 2005).

In AI outbreak in British Columbia (Canada) in 2004, 1.25 million infected birds were disposed by burial, burning, and composting. At the beginning of the outbreak, carcasses were transported to other locations and they were buried and burnt in these locations. In the middle of the outbreak, the disposal strategy switched to on-farm composting. The first stage of the composting was conducted in the poultry houses. After 5 days, the windrows were re-formed on a layer of heavy black plastic over wood shavings. Piles were passively aerated by black agricultural drainage pipe aligned across the plastic. The windrows were covered with vapor barriers, wood shavings, and finally black plastic (Spencer et al., 2004). Glanville et al. (2007) and Ahn et al. (2007) investigated application of the same composting system to diseased swine carcasses in case of an outbreak. In biosecure composting applications, carcasses are not allowed to be exposed to the environment. They are fully covered with plant materials and sometimes with plastic sheets. Akdeniz (2008) reported that monitoring VOCs as biosecure markers of carcass degradation is a promising method to test progress and completion of the process. Akdeniz (2008) indicated that three compounds, dimethyl disulfide, dimethyl trisulfide, pyrimidine, are marker compounds of swine carcass degradation. In this study, different air sampling
locations within full scale swine mortality composting test units are compared. The objectives of the study were (a) to test if passive aeration tubes (inlet and outlet) are practical sampling locations of marker VOCs and (b) to monitor VOC concentrations from passive aeration tubes in order to decide air flow direction of the test units.

2. Methods

2.1 Biosecure compost test units and VOC sampling locations

In this study, the innovative composting system used during the 2004 outbreak of avian influenza in British Columbia (Spencer et al., 2004) was applied to compost swine mortalities. The experiments were conducted in April-June 2008 at Livestock Environment Building and Research Center of Iowa State University, Ames, Iowa. Three envelope materials were used including wood shavings, soybean straw, and alfalfa hay. The details of the system are given in Ahn et al. (2007), Glanville et al. (2007), Akdeniz (2008).

Air samples were drawn from five different locations of test units in the 3rd week of the process. The first three weeks are known to be the most active phase of the process (Haug, 1993). Vertical PVC (polyvinyl chloride) piping placed in the center of the test units were used to collect VOC samples from bottom (envelope material), middle (swine carcasses), and top (envelope material) layers (depths) of the test units (figure 21). ¼ inch ID PTFE (polytetrafluoroethylene) tubes were tightly placed inside PVC pipes at bottom, middle and top depths. In addition, VOC samples were collected from upper and lower aeration ducts by placing PTFE tubings inside the ducts (figure 21).

2.2 Sampling and analysis of VOCs

Air samples were drawn using SKC pumps (224-PCXR4, PA) at 1.0 L/min air flow rate. Air samples were passed through 250 mL glass sampling bulbs (Supelco, Bellofonte, PA) during 5 minutes (5 hydraulic residence time). After 5 minute sampling, PTFE stopcocks
were closed and air samples were captured inside the bulbs. 85 µm SPME fiber and 1 h extraction time was used to extract VOCs in air samples. This fiber coating and extraction time was chosen based on previous published data (Akdeniz, 2008). After extraction, SPME fibers were kept in the refrigerator until inserting into the injection port of the GC. To test if there is any sample loss during transferring glass sampling bulbs to the laboratory, results of SPME in the field (just after capturing air samples) and SPME in the laboratory (after transferring glass bulbs to the laboratory) were compared.

Glass sampling bulbs were used (a) to decrease sample collection time in the field, (b) to avoid damage on fiber in rough field conditions, and (c) to be able to do SPME in the laboratory at same conditions. Sample collection method using glass sampling bulbs was compared with dynamic sample collection using a SPME fiber in the field. For this purpose, samples were collected by inserting a SPME fiber inside a stainless steel bulkhead union, which was connected to the PTFE tubing from middle layer of the test unit in one end and sealed with a thermogreen septum in the other end. Air passed through tubings for 5 minutes before starting to sample and SPME were performed for 1 h. After sampling SPME fibers were immediately carried to Atmospheric Air Quality Laboratory at Iowa State University for the analysis and kept in the refrigerator until analysis.

Samples were run using a 6890N GC and 5975 MS (Agilent Inc., DE). Pure helium (99.995 %) was used as the carrier gas at constant 1.7 m/s flow rate. The injector temperature was 240 °C. The initial temperatures of the GC oven were 40 °C with 3 min holding time. It followed by a ramp of 8 °C/min until reaching 220 °C, where it was held for 10 min. A 60 m polar capillary column (BP21, 0.32 mm ID* 0.25 µm film thickness, SGE, TX) was used to separate compounds. The MS mass/charge (m/z) ratio was set between 29 and 150 for the first 8 minutes. After the first 8 minutes, MS detection was between 34 and 280 m/z. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 °C,
respectively. Chromatography data acquisition software, MSD ChemStation (Agilent) and BenchTop/PBM™ V. 3.2.4 (Palisade Corporation, Ithaca, NY), were used to analyze data.

2.3 Statistical Analysis

Three test units were prepared for each envelope material and samples were collected from each test unit (N=3). JMP v 6.0.2 package was used for statistical analysis. Data were subjected to one-way analysis of variance (ANOVA). Treatment means were compared using TUKEY’s honestly significant differences (HSD) test at 95 % confidence level.

3. Results and discussion

The amounts of analytes extracted using a SPME fiber in the field (immediately after capturing air samples inside glass bulbs) and in the laboratory (after transferring sampling bulbs to the laboratory) are compared. The difference in MS area counts is found to be ranging from 0.1 to 1.3%. It is believed this difference is not significant and caused by the temperature change during transferring sampling bulbs to the laboratory. Relative standard deviation of the data is found to be ranging from 1.8 to 11.3%. Samples were drawn from replicated field trials and the difference between replicates is most likely to be caused due to the differences between test units. These low relative standard deviations show that sample loss during waiting in the refrigerator is minimal.

The amount of analytes extracted was compared for two different sample collection methods. No significant difference was found between SPME in the field using bulkhead unions and SPME in the laboratory using glass sampling bulbs (figure 22). Although no significant difference was found, glass sampling bulbs are recommended to collect air samples. It is easier to handle with glass sampling bulbs compared to fragile SPME fibers in rough field conditions. Also, it will provide comparable data when the samples are collected at different ambient temperatures. The disadvantages could be carry over of the analytes if proper cleaning is not applied and cleaning might be time consuming.
Concentrations of the marker compounds sampled from different locations can be ranked from the highest concentration to the lowest as follows: middle ≈ upper aeration duct > top > bottom = lower aeration duct (figure 23). The highest concentrations of dimethyl disulfide, dimethyl trisulfide, and pyrimidine were measured from middle layer and upper aeration duct of the test units. In all the test units, these locations were found to be significantly different than the other locations. The second highest concentrations of the marker compounds were measured from top layer and the lowest concentrations from bottom and lower aeration duct. It is hard to explain the difference in concentrations between top layer and upper-aeration duct. Although there is no solid evidence, it might be caused by a short circuiting of air flow in the top layer of the test units.

It has been shown that the most reliable locations for VOC sampling are middle and upper-aeration duct. In case of an emergency composting, placing PVC pipes to collect air samples would not be a practical option. Thus, the upper aeration duct is recommended to collect VOC samples to test progress and completion of animal degradation without opening plastic bio-security barriers. The problem associated with sampling from aeration ducts is alteration in air flow direction during the process depending on the porosity, temperature of the test units, and maybe wind direction. In this study, lower aeration duct is found to be an inlet due to low concentrations of the VOCs in this location. Upper-aeration duct is indicated as an outlet as the highest concentrations of VOCs were measured from this location. It is showed that VOC concentrations can be used to decide air flow direction of a compost test unit. However, further studies are needed to show consistency of this finding. After deciding air flow direction, air samples can be collected from the outlet aeration tubing.

In this type of composting systems, a downward air flow direction is not desirable. An upward air flow allows the volatile compounds emitted from carcasses to be filtered by top layer plant materials. An easy way of testing air flow direction by applying SPME would be helpful to have more control on passively aerated systems. Air flow direction might be
changed from downward to upward by employing aeration ducts at different heights, locations, and directions.

4. Conclusions

Glass sampling bulbs can be used to collect air samples from composting systems. Using glass sampling bulbs to capture air samples and applying SPME in laboratory conditions would be a practical option to analyze VOCs from composting operations. In the study, middle location where the carcasses are located and upper-aeration duct are recommended to collect air samples. The concentrations of the marker compounds were found to be significantly different than the other locations. In case of an emergency disposal of carcasses, upper aeration ducts can be preferred to collect air samples. However, upper aeration duct was not always a reliable sampling location as the air flow direction can change during the process. In the study, higher concentrations of the VOCs were measured from the upper aeration duct and this duct is claimed to be an outlet. Sampling location should be decided after air flow direction is figured out. Sampling VOCs has a potential to be a simple way of deciding air flow direction of passively aerated systems. Further studies are needed to show practical application of this finding.

Acknowledgment

The authors gratefully acknowledge Canadian Food Inspection Agency for the financial support through a grant from the Canadian Research and Technology Initiative (CRTI Project # 04 0052 RD).

References


Figure 21. Schematic of the swine compost test units and sampling locations
Figure 22. Comparison of VOC sample collection methods (N=3)
Figure 23. Comparison of different air sample collecting locations (N=3)
CHAPTER 7. GENERAL CONCLUSIONS

1. General conclusions

The following conclusions can be drawn from this research:

• Solid phase microextraction is a powerful sample collection technique that can be used to analyze volatile organic compounds from headspace of laboratory and full scale swine mortality composting units. It overcomes the difficulties associated with VOC sampling at low concentrations.

• Mass spectrometry is a useful detector to identify compounds from complex matrices such as mortality composting environments.

• Eighty five µm CAR/PDMS is shown to extract the highest amount of analytes at one hour sampling time. One hour sampling time is the longest extraction time that does not cause replacement of the analytes.

• The developed method to quantitatively analyze dimethyl disulfide, dimethyl trisulfide, pyrimidine, acetic acid, propanoic acid, 3-methyl butanoic acid, pentanoic acid and hexanoic acid gives accurate results. The percentage of the error is calculated to be ranging from 1.47 to 20.96 %, which is believed to be in an acceptable range.

• It is possible to detect analytes at low concentrations. Method detection limits are ranging from 0.01 to 580 ppbv.

• Relative humidity of the sampling environment does not affect the efficiency of dimethyl disulfide, dimethyl trisulfide, pyrimidine and hexanoic acid sampling. However, lower concentrations of acetic acid, propanoic acid, 3-methyl butanoic acid, and pentanoic acid are detected at 97% humid conditions compared to 0 % humid conditions. A range of concentrations can be reported for 0 and 97% relative humidity conditions, if the relative humidity of the sampling environment is not known.
• Dimethyl disulfide, dimethyl trisulfide, and pyrimidine are found to be produced during degradation of swine carcass tissues but not produced from decaying plant (envelope) materials. These compounds are marker compounds of swine carcass degradation process.

• Marker compounds are produced in the first three weeks of the swine carcass process. After the first three weeks, their concentrations and emission rates are decreased gradually. Laboratory studies show that marker compounds cannot be detected when the respiration rates of carcasses decrease to a level of 3.25 mg CO$_2$-C/g VS*d.

• Field studies showed that when carcass degradation is incomplete, detection of marker compounds is still possible in the eighth week of the process. After eight week composting time, the highest concentrations of marker compounds are detected for the carcass samples with the highest respiration rates (least stabilization). No relation is observed between temperature data and degradation rates of carcasses. A better estimate of carcass degradation is made by measuring concentrations of the marker compounds.

• Dimethyl disulfide, dimethyl trisulfide, and pyrimidine are produced from all compost units under various conditions regardless of the plant material, moisture content, porosity and temperature. These compounds are reliable marker compounds that can be followed to test completion of a swine mortality composting process when the carcasses are fully covered by plant materials and plastic sheets due to biosecurity reasons.

• Volatile fatty acids, methanethiol and ethanethiol are detected from composting units with relatively high moisture contents (56.2-64.8%). These compounds can be further investigated as indicators of insufficient aeration in animal mortality composting operations.
• Using glass sampling bulbs to collect air samples and applying SPME in laboratory conditions can be a practical option to analyze VOCs from composting operations.

• In case of an emergency disposal of carcasses, upper aeration duct can be proffered to collect air samples when upper aeration duct is an outlet.

• Sampling VOCs has a potential to be a simple way of monitoring air flow direction of passively aerated systems. Higher concentrations of VOCs are detected in the outlet of the aeration ducts. Further studies are needed to show practical application of this finding.

**2. Recommendations for future studies**

Suggestions for future studies are presented in the following:

• Usage of marker VOCs can be tested for swine carcass composting operations where the carcass degradation is completed.

• Marker VOCs can be tested for poultry and livestock mortality composting operations and different composting systems (e.g., windrow, rotating vessel).

• A portable device can be developed for field sampling of dimethyl disulfide, dimethyl trisulfide or pyrimidine.

• Air flow rate of full scale composting units can be measured using an anemometer and these air flow rates can be compared with theoretical air flow rates. Theoretical air flow rates can be calculated by employing theoretical emission rates and measured concentrations of CO$_2$, O$_2$, H$_2$O, and NH$_3$.

• Usage of VOCs to test inlet and outlet of passively aerated systems can be further investigated. The necessary sampling frequency can be searched for matrices with different properties (e.g., porosity, moisture content, temperature).
• The findings of the study can be modeled by including VOC concentrations, temperature, respiration rate, oxygen and moisture contents.

• A possible usage of SPME coupled with GC-MS can be investigated to find marker compounds of other biological systems.
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