1-2010

Laboratory scale evaluation of volatile organic compound emissions as indication of swine carcass degradation inside biosecure composting units

Neslihan Akdeniz
Iowa State University

Jacek A. Koziel
Iowa State University, koziel@iastate.edu

Hee-Kwon Ahn
Iowa State University

Thomas D. Glanville
Iowa State University

Benjamin P. Crawford
Iowa State University

Laboratory scale evaluation of volatile organic compound emissions as indication of swine carcass degradation inside biosecure composting units

Abstract
Biosecure livestock mortality composting systems have been used to dispose of diseased livestock mortalities. In those types of system, visual inspection of carcass degradation is not possible and monitoring VOCs (volatile organic compounds) released by carcasses is a new approach to assess progress of the composting process. In this study, field-scale livestock mortality composting systems were simulated and a laboratory scale composting system with aerobic and anaerobic test units was designed to collect VOC samples from the headspace of decaying plant materials (70 g dry weight) and swine tissues (70 g dry weight) at controlled operating temperatures. Headspace samples were collected with SPME (solid phase microextraction) and analyzed by a GC–MS (gas chromatography–mass spectrometry) system. Among the 43 VOCs identified, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be marker compounds of the mortality composting process. These compounds were only found to be produced by decaying swine tissues but not produced by decaying plant materials. The highest marker VOC emissions were measured during the first three weeks, and VOCs were not detected after the 6th week of the process, which indicates degradation processes were completed and compost materials microbially stabilized (no additional VOC production). Results of respiration tests also showed that compost materials were stabilized. Results of this study can be useful for field-scale composting operations but more studies are needed to show the effects of size and aeration rate of the composting units.

Keywords
Dimethyl disulfide, Emissions, Mortality compost, SPME, VOCs

Comments
This is a manuscript of an article published as Akdeniz, Neslihan, Jacek A. Koziel, Hee-Kwon Ahn, Thomas D. Glanville, Benjamin P. Crawford, and D. Raj Raman. "Laboratory scale evaluation of volatile organic compound emissions as indication of swine carcass degradation inside biosecure composting units." *Bioresource Technology* 101, no. 1 (2010): 71-78. DOI: 10.1016/j.biortech.2009.07.076. Posted with permission.

Authors
Neslihan Akdeniz, Jacek A. Koziel, Hee-Kwon Ahn, Thomas D. Glanville, Benjamin P. Crawford, and D. Raj Raman

Laboratory scale evaluation of volatile organic compound emissions as indication of swine carcass degradation inside biosecure composting units

Neslihan Akdeniz[a,†], Jacek A. Koziel[a,b,*], Hee-Kwon Ahn[a,‡], Thomas D. Glanville[a], Benjamin P. Crawford[a], D. Raj Raman[a]

[a]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011

[b]Department of Civil, Construction and Environmental Engineering, Iowa State University, Ames, IA 50011

[*] Corresponding author [telephone (515) 294-4206; fax (515) 294-4250; e-mail koziel@iastate.edu]

[†] Present address: Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN 55108

[‡] Present address: Beltsville Agricultural Research Center, United States Department of Agriculture, Beltsville, MD 20705

Abstract

Biosecure livestock mortality composting systems have been used to dispose of diseased livestock mortalities. In those types of system, visual inspection of carcass degradation is not possible and monitoring VOCs (volatile organic compounds) released by carcasses is a new approach to assess progress of the composting process. In this study, field-scale livestock mortality composting systems were simulated and a laboratory scale composting system with aerobic and anaerobic test units was designed to collect VOC samples from the headspace of decaying plant materials (70 grams dry weight) and swine tissues (70 grams dry weight) at controlled operating temperatures. Headspace samples were collected with SPME (solid phase microextraction) and analyzed by a GC-MS (gas chromatography – mass spectrometry) system. Among the 43 VOCs identified, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be marker compounds of the mortality composting process. These compounds were only found to be produced by decaying swine tissues but not produced by decaying plant materials. The highest marker VOC emissions were measured during the first three weeks, and VOCs were not detected after the 6^{th} week of the process, which indicates degradation processes were completed and compost materials microbially stabilized (no additional VOC production). Results of respiration tests also showed that compost materials were stabilized. Results of this study can be useful for field-scale composting operations but more studies are needed to show the effects of size and aeration rate of the composting units.

Keywords: Dimethyl disulfide; Emissions; Mortality compost; SPME; VOCs

1. Introduction

The safe disposal of carcasses during emergency animal disease (EAD) outbreaks is a significant issue since diseases can be easily spread to humans and other livestock (Wilkinson, 2007). Improper disposal of carcasses, such as illegal dumping, has become a problem due to cost of mortality disposal and 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 for emergency management of livestock mortalities (Wilkinson et al., 2007). Composting of diseased mortalities was first developed for the poultry industry (Spencer et al., 2004; Bendfeldt et al., 2006) with more recent studies being conducted to adapt mortality composting for swine and cattle emergency disposal (Glanville et al., 2006a, 2006b; Glanville et al., 2007; Ahn et al., 2007)

In case emergency disposal is necessary, a passively-aerated plastic wrapped mortality composting system has been designed to reduce the potential of spreading live viruses to the environment (Spencer et al., 2004; Ahn et al., 2007; Glanville et al., 2007). In this type of biosecure composting application, carcasses are fully covered with envelope (typically silage, straw, or hay) materials and the entire matrix is wrapped with plastic sheets. It is not safe to turn, mix, or expose the diseased carcasses to the environment until carcass degradation is completed, temperatures have been reached and maintained to inactivate pathogens, and compost is considered stable (Wilkinson, 2007). Since visual inspection of the carcasses is not possible, a new method is needed to assess progress and completion of the degradation process without compromising biosecurity. Temperature is

59 the most commonly used parameter for assessing performance of a composting process.
60 However, monitoring temperature profiles does not necessarily help to evaluate progress
61 and completion of the process. Reasons for low core compost temperatures may be over-
62 aeration of the composting units and low heat retention properties of the envelope materials
63 (Ahn et al., 2007; Glanville et al., 2007). Under these conditions, temperature may not
64 increase but the degradation process can still continue. A new approach is to monitor VOCs
65 (volatile organic compounds) released by decaying mortalities inside biosecure compost
66 units (Akdeniz et al., 2009).
67 Volatile organic compounds emitted from composting of various wastes include a
68 wide group of organic compounds, including sulfur-containing compounds, nitrogen-
69 containing compounds, alcohols, phenols, ketones, esters, volatile fatty acids, and terpenes
70 (Pagans, et al., 2006). Typically, VOCs are found in low concentrations (pg/L to μg/L) in
71 the headspace of composting units. Sample preparation for VOCs in headspace requires
72 pre-concentration (Dewulf and Van Langenhove, 2002; Clemitshaw et al., 2004).
73 Ultraviolet differential absorption spectroscopy (UV-DOAS), Fourier transform infrared
74 spectroscopy (FT-IR), and colorimetric sensors are the techniques that allow measurement
75 of VOCs in headspace without sample preparation but the number of analytes that can be
76 detected is limited (Lin et al., 2004). Gas phase SPME (solid phase microextraction)
77 coupled with GC-MS (gas chromatography-mass spectrometry) based analyses is a more
78 convenient (does not require sample preparation) method for measurement of VOCs in air
79 and headspace (Koziel et al., 1999; Koziel and Novak, 2002). Solid phase microextraction
80 has been used to sample VOCs from biofilter-treated effluent air of swine barns (Chen et al.,

2008), landfills (Davoli et al., 2003; Kim et al., 2005a), commercial composts (Kim et al., 2005b) and decaying plant and animal materials (Akdeniz et al., 2007a, 2007b).

There are many variables affecting analytical method development and quantification with SPME. One of the factors affecting air sampling with a SPME fiber is air temperature. Although there are more compounds in the vapor phase when the temperature increases, amounts of volatiles adsorbed can decrease (Jia et al., 1998; Demyttenaere et al., 2003). This can be explained by the fact that adsorption is an exothermic and competitive process (Pawliszyn, 1997). In addition, 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, which simulated a field-scale biosecure composting systems was designed, built and tested to specifically monitor VOC emissions. Several operating variables were controlled, (i.e., composting time, temperature, and aeration flow rate resulting) in aerobic or anaerobic conditions. The objectives of the study were (a) to identify and compare VOCs evolved from decaying swine tissues and plant materials in order to find marker compounds of decaying swine tissues, (b) to develop air sampling and analyses methods for different air temperatures to accurately quantify marker VOC emissions, and (c) to evaluate a potential usage of VOC emissions to test completion of carcass degradation.

2. Methods

Compost test units were designed, prepared, and run in triplicate to identify and compare VOCs evolved from decaying plant and animal materials. These tests included

separate plant and animal material compost units and layered (plant-animal material) compost units (n=3 for each treatment). The separate units consisted of corn silage, oat straw, and alfalfa hay, and swine tissues (70 dry grams) composted separately at aerobic and anaerobic conditions (total of 24 units). The layered compost tissues consisted of combinations of corn silage and swine tissues (70 dry grams total), composted under aerobic and anaerobic conditions (total of 6 units). Corn silage was chosen for this combination because it emitted a higher number of VOCs among all plant materials tested (Akdeniz et al., 2007a).

2.1. Aerobic and anaerobic test units

Two oilless pumps (Combined Fluid Products, Lake Zurich, IL) were used to aerate aerobic test units. Air was pumped through hydrocarbon traps (Supelco, Bellefonte, PA) to prevent any VOC contamination from the ambient air. Curved PTFE tubing with equal number of holes were used to aerate test units (Fig. 1a). Air flow rate was set at 100±1.66 mL/min by using stainless steel 3-way needle valves (Swagelok, Omaha, NE). As Rynk et al. (1992) suggested airflow rates of 707.7 to 2,831 L/min per dry tonne of compost were required for aerobic composting, we chose an air flow rate of 1.42 mL/min per dry gram (1,420 L/min per dry ton-average of 707.7 and 2,831 L/min per dry ton) and kept constant in all test units. Air flow rate of each test unit was set at 100±2 mL/min and checked weekly from the outlet port of the test units (Fig. 1a). One hour before headspace VOC sampling, aeration was stopped and aerobic test units were kept closed 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, Stamford, CT). Wetted gauze was used to measure wet bulb temperatures (Fig. 1a). A datalogger (Model CR10X, Campbell Scientific, Logan, UT) was utilized to record temperature.

Anaerobic conditions were maintained by tightly closing the test units. Pure N_2 (99.995 % pure; Chemistry Store, Iowa State University) was passed through the test units for two minutes once a week to make O_2 and CO_2 measurements. Mass flow rate of N_2 was set at 1 L/min and checked by a mass flow controller (DryCal, Butler, NJ). Oxygen and CO_2 concentration measurements were completed immediately after VOC sampling. The O_2 trap (Alltech Associates, Chicago, IL) was used on N_2 delivery line to prevent any O_2 contamination and compromising anaerobic conditions.

2.2. Sample preparation and composting conditions

Plant materials (corn silage, oat straw, and alfalfa hay) were collected from central Iowa. Oat straw and alfalfa hay were chopped to approximately 10 cm lengths to prevent formation of an excessively porous structure. A whole swine carcass (6.3 kg, one month old), including bones, internal organs, and skin, was shredded using an industrial grinder in order to get a representative 70 g sample of the entire carcass. The initial moisture contents of the corn silage, oat straw, alfalfa hay, and swine tissues were 43.6, 37.7, 44.2, and 65.0 %, respectively. Moisture contents of the plant materials were adjusted to 65 % by

145 spraying deionized water and incubated at 30 ºC for 24 h (Ahn et al., 2005). Moisture loss
146 during incubation was calculated from weight change and added (sprayed) back.
147 Each separate test unit consisted of approximately 70 dry grams of plant and animal
148 materials placed inside 0.946 L glass jars (Mason, Wal-Mart, Ames, IA) (Figs. 1a and b).
149 Layered plant-animal material units simulated biosecure field-scale mortality composting
150 conditions where plant and animal materials are layered and decayed together (Ahn et al.,
151 2007; Glanville et al., 2007). In these test units, plant and animal materials were layered at
152 the ratio of 1 (animal):15 (plant) (w/w) (70 dry grams total). This ratio was chosen based on
153 previous field-scale biosecure composting experiments (Ahn et al., 2007; Glanville et al.,
154 2007).
155 Glass jars that were used to compost plant and animal materials (Figs. 1a and b)
156 were first washed with deionized water and heated at 110 ºC overnight to drive off volatile
157 impurities. Polytetrafluoroethylene (PTFE) liners with 0.8 mm thickness (USP, Lima, OH)
158 were placed on the lids (inner side) to separate headspace of the jars from tin lids and to
159 minimize interfering compounds from tin lids and seals. Sampling ports for SPME insertion
160 and VOC collection were made by drilling 5 mm holes in the middle of the lids.
161 Thermogreen half-hole septa (Supelco, Bellefonte, PA) were tightly placed in these holes.
162 Air handling system was assembled from clean 6.35 mm O.D. Teflon (PTFE) tubing (E&S
163 Technologies, Chelmsford, MA) and stainless steel fittings (Swagelok, Omaha, NE) to
164 prevent any VOC contamination. All the test units were placed in water baths (Fisher
165 Scientific, Chicago, IL). Temperatures of the water baths were adjusted every day manually
166 (Fig. 2). Temperatures were increased up to 67 ºC and then decreased to 22 ºC during the
167 first 8 weeks. This temperature profile was based on the average core temperature of a

168 warm season field-scale biosecure swine mortality composting process (Ahn et al., 2007;
169 Glanville et al., 2007). After temperature decreased to 22 ºC, it was increased again (Fig. 2)
170 to check the potential for VOC emissions caused solely by temperature increase after the
171 composting process is completed. This was done to determine if the biological activity had
172 fully ceased during the composting period and compost materials were fully stabilized.

173 *2.3 CO_2 and O_2 measurements*

174 The headspace CO_2 and O_2 concentrations were measured on weekly basis from the
175 outlet of the test units using a CO_2 (Vaisala, Louisville, CO) and O_2 (Apogee, Logan, UT)
176 probe. The CO_2 and O_2 concentrations in the aerobic test units were recorded to test
177 aeration status of the test units. Oxygen concentrations of anaerobic test units were
178 measured in order to check for possible O_2 contamination. Carbon dioxide and O_2 probes
179 were calibrated weekly using clean ambient air and gas mixtures (5% CO_2-15 % O_2 and
180 15 % CO_2-5 % O_2) prepared by the Chemistry Store, Iowa State University, Ames, IA.

181 *2.4 Headspace SPME and GC-MS analysis*

182 An 85 μm Carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber and 1 h
183 extraction time was used to collect VOCs from the headspace of the test units. This SPME
184 fiber and extraction time was chosen based on air sampling and analysis method
185 development and optimization described elsewhere (Akdeniz et al., 2009). Samples were
186 run using a 6890N GC and 5975 MS (Agilent Inc., DE). Pure helium (99.995 %) was used
187 as the carrier gas at constant flow (1.7 m/s). The injector and SPME fiber desorption
188 temperature was 240 ºC. The initial temperatures of the GC oven were 40 ºC with 3 min

holding time, followed by a ramp of 8 ºC/min until reaching 220 ºC, where it was held for 10 min. A polar capillary column (BP-21, 60 m × 0.32 mm ID × 0.25 μm film thickness, SGE, Austin, TX) was used to separate VOCs. The MS mass/charge (m/z) ratio was set between 29 and 150 for the first 8 min. After that, MS detection was between 34 and 280 m/z. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 ºC, respectively. Chromatography data acquisition software including MSD ChemStation (Agilent, New Castle, DE) 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 VOCs (Sigma-Aldrich, Milwaukee, WI).

2.5 Emission rate measurements

Calibration curves for air sampling and analysis were prepared using the methodology developed by Akdeniz et al. (2009). These authors reported that there was no significant difference between dry (0% relative humidity) and humid conditions (97% relative humidity) in extraction efficiency for dimethyl disulfide, dimethyl trisulfide, and pyrimidine. Therefore, calibration curves were prepared for only dry conditions. In this study, to simulate a field-scale composting process, temperatures of the test units were changed on daily basis (Fig. 2) and headspace VOCs were extracted at different temperatures. Thus, calibration curves were prepared at 20, 30, 40, 50, and 60 ºC, respectively, to quantify MS detector response at different temperatures. First, standard gases of marker compounds were generated and collected inside glass sampling bulbs as

described in Akdeniz et al. (2009) and then they were subsequently placed inside an oven (The Grieve Corporation, Round Lake, IL) with a temperature controller. Glass bulbs were kept inside the oven for 10 min and headspaces were sampled for 1 h with 85 μm CAR/PDMS 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 based on the following equation (Xin, 2005):

$$ER = Q * C * \frac{W_m}{V_m} * \frac{T_{std}}{T_a} * \frac{P_a}{P_{std}}$$

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).

2.6 Respiration rate measurements

Compost materials were incubated for nine weeks and after nine weeks 20 g of samples were collected from each unit and respiration rates of the remaining compost materials were measured. A titration method was used to measure the CO_2 production during respiration of remaining carcass tissues (Sadaka et al., 2006). For respiration rate calculations, moisture content was determined by drying samples at 105 ºC for 24 h. Volatile solid contents of samples were measured by combusting dried samples at 550 ºC for 5 hrs (APHA, 1989). Sadaka et al. (2006) adjusted moisture contents of compost

233 samples to 50% (w.b) but in this study moisture contents of the samples were increased to
234 65% since it was the initial moisture content of the swine tissues.

235 *2.6 Statistical analysis*

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

240 ## 3. Results and discussion

241 *3.1 Aeration and moisture supply*

242 Oxygen and CO_2 concentrations in aerobic test units were found to be same as
243 those in ambient air (0.03 % CO_2, 20.94 % O_2). The O_2 concentrations in anaerobic test
244 units were always measured as zero. The CO_2 concentrations in anaerobic test units were
245 lower than ambient air and ranged from 0.01 to 0.012 %. The results demonstrate that there
246 was no limitation in O_2 supply to aerobic test units and no O_2 contamination to anaerobic
247 test units. Moisture contents of the corn silage, oat straw, alfalfa hay, and swine tissues
248 decreased to 29.1±1.01, 31.1±1.25, 32.0±0.98, and 32.2±1.21 %, respectively, at the end of
249 the study (Table 3). The optimum initial moisture content of the compost materials is in the
250 range of 40 to 60% (Haug, 1993). In this study, the initial moisture contents of the plant
251 materials were increased to 65% (to approximate moisture content of the swine tissues) and
252 during the process moisture contents of the plant materials and swine tissues decreased to
253 approximately 30%. Final moisture contents can vary from 21% to 77% (Glanville, et al.,

2007) depending on the initial moisture content and the progress of the process. It is known that lowest moisture content at which bacterial activity takes place is from 12 to 15% (Shammas and Wang, 2007). In this study the final moisture contents were above 15% and it can be concluded that aerobic test units were humidified properly 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 in the headspace of corn silage, oat straw, alfalfa hay and swine composts (Table 1). 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 exclusively by decaying swine tissues, and were not produced by any of the plant materials tested. These marker compounds were dimethyl disulfide, dimethyl trisulfide and pyrimidine, which were produced under both aerobic and anaerobic conditions. Plant materials may be degraded slowly since their cellulose and lignin contents are high (Richard, 1996). In the current study, marker compounds were not produced or produced under method detection limits by plant materials. Method detection limits of dimethyl disulfide, dimethyl trisulfide, and pyrimidine were 1.10, 5.50 and 0.011 ppbv, respectively (Akdeniz et al., 2009).

In addition to swine composts, the marker compounds were also detected from additional test units (both aerobic and anaerobic) where plant and animal materials were layered and composted together at the ratio of 1 (animal):15 (plant) (w/w). In this study,

276 only corn silage, which contained the most complex matrix of VOC emissions (Akdeniz et
277 al., 2007a), was used to test the production of marker compounds when plant materials
278 were layered with swine tissues.

279 The presence of sulfur-containing VOCs as marker compounds of swine tissue
280 decay is reasonable. Sulfur makes up about 0.15 % of a swine tissue mass (Shurson et al.,
281 1998) and it is widely distributed throughout the body since sulfur-containing amino acids
282 (e.g., methionine, cystine, and cysteine) are components of almost all proteins (0.6 to 0.8 %
283 of a protein) (Shurson et al., 1998). Dimethyl disulfide and dimethyl trisulfide are produced
284 by microbial decomposition of sulfur-containing amino acids under aerobic and anaerobic
285 conditions. This process likely entails the sequential steps of the breakdown of protein to
286 form peptides and degradation of the peptides to form free sulfur-containing amino acids. It
287 has been shown that sulfur-containing amino acids are broken down to form methyl
288 mercaptan, dimethyl disulfide and dimethyl trisulfide by oxidation and enzymatic activity
289 (Kadota and Ishida, 1972; Shurson et al., 1998; Higgins et al, 2002; Tulio et al., 2003). In
290 this study, methyl mercaptan was not detected from decaying swine tissues. Pyrimidine is a
291 heterocyclic aromatic organic compound similar to pyrazine, containing two N atoms at
292 positions 1 and 3 of the six-member ring. No information has been found about possible
293 processes responsible for the production of pyrimidine.

294 *3.3 Quantification of marker VOCs*

295 Extraction temperatures for SPME were compared according to the amount of
296 analytes extracted at 1 hr sampling time. The highest extraction efficiency was achieved at
297 20 ºC (Fig. 3). As the temperature increased, the extraction efficiency decreased due to the

298 exothermic nature of the adsorption process. The amount of analytes extracted at 20, 30,
299 and 40 ºC were found to be significantly higher than those extracted at 50 ºC and 60 ºC (Fig.
300 3). For this reason, calibration curves were prepared at 20, 30, 40, 50, and 60 ºC (Table 2).
301 The replicates exhibited relative standard deviations ranging from 0.22 to 6.15 % and
302 correlation coefficients ranging from 94.7 to 99.7 %. The lowest and highest
303 concentrations prepared for the calibration curves were 0.01 ppmv and 6.85 ppmv,
304 respectively. Concentrations and emission rates of the marker compounds were estimated
305 using these calibration curves.

306 *3.4 Concentrations and emission rates of marker VOCs*

307 Measured concentrations of the marker compounds ranged from below method
308 detection limits (MDLs) to 36.86 ppbv for dimethyl disulfide, 31.66 ppbv for dimethyl
309 trisulfide, and 2.40 ppbv for pyrimidine under aerobic conditions. Under anaerobic
310 conditions, measured concentrations ranged from below MDLs to 59.04 ppbv for dimethyl
311 disulfide, 62.01 ppbv for dimethyl trisulfide, and 10.46 ppbv for pyrimidine. The relative
312 standard deviation (RSD) range of measured concentrations from triplicate units was 2.37
313 to 10.0 %. The MDLs for these marker compounds in gas phase were reported as 1.10, 5.50
314 and 0.011 ppbv for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively
315 (Akdeniz et al., 2009).

316 Estimates of emission rates of the marker VOCs from aerobic and anaerobic test
317 units are shown in Figs. 4a and b. In these figures, emission rates were reported for the
318 whole mass of the compost material at the time of measurement. Marker compounds were
319 not detected in the 1st week (sampled on the 2nd day) of the process. In the 2nd week (9th 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 3rd week of the process. In this week, under aerobic conditions, the emission rates were 1.24×10^{-2}, 1.43×10^{-2}, and 6.80×10^{-4} µg/min for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. At anaerobic conditions the emission rates were 3.93×10^{-5}, 5.53×10^{-5}, and 5.93×10^{-6} µg/min for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. After the 3rd week, emission rates significantly decreased. After the 6th week, marker compounds were not detected from the headspace of the test units. In week 6, concentrations of the marker compounds in the headspace were lower than the MDLs and the available sources of marker emissions were most likely depleted. Under aerobic conditions, the emission rates were estimated to be below 0.51×10^{-4}, 3.43×10^{-4}, and 4.35×10^{-7} µg/min/(final g VS of swine tissue) for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. At anaerobic conditions the emission rates were 0.37×10^{-7}, 2.51×10^{-7} and 3.19×10^{-10} µg/min/(final g VS of swine tissue) for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. These emission rates were calculated based on MDLs, final g VS of swine tissues and compost temperature in week 6. Emission rates were reported per final g VS (VS of compost materials after 9 weeks) to facilitate comparisons between the results of this lab-scale study and larger scale composting studies.

Temperatures of the test units were purposefully raised at the end of the composting process (week 8) (Fig. 2). This increase in temperature did not result in increased emission rates of the marker compounds and marker compounds were not detected in the headspace (Figs. 4a and b). This indicated that the emission rates of the marker compounds did not decrease due to a lower temperature and volatility. It is most likely that available nutrient

343 sources were depleted and microbial degradation process was completed. These results
344 were supported by the respiration rate measurements. Respiration rates of the swine tissues
345 decreased from 8.82±0.36 to 3.25± 0.12 mg CO_2-C/g VS/d (Table 3). Thompson (2002)
346 classified compost materials with respiration rates below 5 mg CO_2-C/g VS/d as stable
347 composts.

348 Emission rates of marker VOCs were much higher under aerobic conditions
349 compared to anaerobic conditions. During the period where emission rates were apparently
350 highest (Figs. 4a and b), emission rate of dimethyl disulfide under aerobic conditions was
351 12.39×10^{-3} µg/min compared to 3.92×10^{-5} µg/min under anaerobic conditions. This
352 represents an approximately 200-fold difference. The higher emission rates of dimethyl
353 disulfide and dimethyl trisulfide under aerobic conditions might be explained considering
354 the production mechanism. Possibly oxidation (in aerobic conditions) of sulfur-containing
355 amino acids to form dimethyl disulfide and dimethyl trisulfide might be faster and preferred
356 pathway than enzymatic degradation (in anaerobic conditions) since most degradation
357 processes occur more rapidly under aerobic conditions (Haug, 1993).

358 An important observation was that emissions of volatile fatty acids (VFAs) were
359 detected from only anaerobic test units of plant materials (Table 1). These results were
360 expected since VFAs are known to be produced during anaerobic degradation (Haug, 1993).
361 In further field-scale biosecure composting studies, a potential use of VFAs to evaluate
362 aeration status of compost materials could be evaluated.

363 Emission rates of dimethyl disulfide and dimethyl trisulfide were approximately 12
364 times higher than the emission rates of pyrimidine. This ratio was consistent in both aerobic

and anaerobic test units. Significant correlations (correlation coefficients ranging from 83.7 to 99.6 %) were found between emission rates of dimethyl disulfide, dimethyl trisulfide, and pyrimidine (Table 4), indicating that these organic compounds were likely produced simultaneously by microbial activity. Based on these results, emission rates of the three marker compounds can also be estimated if emission rate of one of the marker compounds is known (Table 4).

The results of this study serve as a 'stepping stone' to field-scale research on the degradation progress of biosecure swine mortalities. In this study, aeration rate, temperature profile, composting time, carbon to nitrogen ratio, moisture content of the compost materials were carefully chosen to simulate a field-scale biosecure composting process. The results of this study will be supported by subsequent field-scale biosecure swine mortality composting studies since there could be additional variables affecting larger-scale degradation processes.

4. Conclusions

Volatile organic compounds emitted from decaying swine and plant materials were characterized in a laboratory-scale system simulating biosecure field-scale swine mortality composting operations. The novel approach involving SPME coupled with collection of VOCs from a complex matrix of decaying plant and animal tissues, and analysis of VOCs on a GC-MS was useful in identifying marker compounds that could be used as indicators of the process status and its completion. Three marker compounds, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be produced only by decaying swine tissues. Marker compounds were found to be produced simultaneously and the highest

emission rates were detected during the third week of the composting process. Marker compounds were not detected after the 6th week of the process. The decrease in the emission rates of marker compounds after week 6 was not caused by change in the temperature and lower volatility of the compounds. The main reason was likely slower microbial activity due to limited nutrient sources and earlier exposure to high temperatures. Respiration rates of the swine tissues were measured at the end of the trial (week 9). Volatile fatty acids were only detected in the headspace of decaying plant materials under anaerobic conditions. Significant correlations (correlation coefficients ranging from 83.7 to 99.6 %) were found between emission rates of dimethyl disulfide, dimethyl trisulfide, and pyrimidine. Consequently, the emission rate of one of these three marker compounds could be used to estimate completion of the carcass degradation in biosecure swine mortality composting systems. Therefore, future studies to monitor completion of biosecure field-scale swine mortality compost systems using the VOCs characterized in this study are warranted.

Acknowledgments

The authors gratefully acknowledge Anthony Pometto III and Sam Beattie for their significant contribution. This project is supported by Canadian Food Inspection Agency through a grant from the Canadian Research and Technology Initiative (CRTI Project # 04 0052 RD).

406 ## References

407 Ahn, H.K., Richard, T.L., Glanville, T.D., Harmon, J.D., Reynolds, D.L., 2005. Estimation
408 of optimum moisture levels for biodegradation of compost bulking materials. ASABE
409 Annual International Meeting, ASABE paper No 054089, Tampa, Florida.

410 Ahn, H.K., Glanville, T.D., Crawford, B.P., Koziel, J.A., Akdeniz, N., 2007. Evaluation of
411 the biodegradability of animal carcasses in passively aerated bio-secure composting
412 system. ASABE Annual International Meeting, ASABE paper No 074037,
413 Minneapolis, Minnesota.

414 APHA 2540E, Fixed and Volatile Solids Ignited at 550°C, "Standard Methods for the
415 Examination of Water and Waste water", 17th Edition, 1989, American Public Health
416 Association (APHA), 1740 Broadway, New York, USA.

417 Akdeniz, N., Koziel, J.A., Ahn, H.K., Crawford B., Glanville, T.D., 2007a. Qualitative
418 characterization of volatile compound emissions during biological decomposition of
419 plant materials using SPME-GC-MS. ASABE Annual International Meeting,
420 ASABE paper No 074041, Minneapolis, Minnesota.

421 Akdeniz, N., Koziel, J.A., Ahn, H.K., Crawford B., Glanville, T.D., 2007b. Stability
422 evaluation of simulated plant and animal composts utilizing respiration rates and
423 VOC emissions. ASABE Annual International Meeting, ASABE paper No 074155,
424 Minneapolis, Minnesota.

425 Akdeniz, N., Koziel, J.A., Ahn, H.K., Glanville, T.D., Crawford, B.P., Raman, R. D. 2009.
426 Air sampling and analysis for VOCs related to field-scale mortality composting
427 operations. J. Agric. Food Chem., 57, 5658-5664.

428 Bonhotal, J., Telega, L., Petzen, J., 2002. Natural rendering: composting livestock mortality
429 and butcher waste. Cornell University Cooperative Extension, Ithaca, New York.
430 Clemitshaw, K.C., 2004. A review of instrumentation and measurement techniques for
431 ground-based and airborne field studies of gas-phase tropospheric chemistry. Critical
432 Rev. Environ. Sci. Tech., 34(1), 1-108.
433 Chen, L., Hoff, S.J., Koziel, J.A., Cai, L., Sun, G. 2008. Performance evaluation of a
434 woodchip-based biofilter using solid-phase microextraction and gas chromatography-
435 mass spectrometry-olfactometry. Bioresour. Tech., 99, 7767-7780.
436 Davoli, E., Gangai, M.L., Morselli, L., Tonelli, D., 2003. Characterization of odorants
437 emissions from landfills by SPME and GC/MS. Chemosphere, 51, 357-368.
438 Demyttenaere, J.C.R., Sanchez Martinez, J.I., Verhe, R., Sandra, P., de Kimpe, N., 2003.
439 Analysis of volatiles of malt whisky by solid phase microextraction and stir bar
440 sorptive extraction. J. Chromatogr. A, 985 (1-2), 221-232.
441 Dewulf, J., Van Langenhove, H., 2002. Analysis of volatile organic compounds using gas
442 chromatography. Trends Anal. Chem., 21, 637-646.
443 Glanville, T.D., Richard, T.L., Harmon, J.D., Reynolds, D.L., Ahn, H.K., Akinc, S., 2006a.
444 Composting livestock mortalities. Biocycle, 47, 42-46.
445 Glanville, T.D., Ahn, H.K., Richard, T.L., Harmon, J.D., Reynolds, D.L., Akinc, S., 2006b.
446 Environmental impacts of emergency livestock mortality composting- leachate
447 release and soil contamination. ASABE Annual International Meeting, ASABE paper
448 No 054089, Portland, Oregon.
449 Glanville, T.D., Ahn, H.K., Koziel, J.A., Akdeniz, N., Crawford, B.P., 2007. Performance
450 evaluation of a passively aerated plastic wrapped composting system designed for

emergency disposal of swine mortalities. ASABE Annual International Meeting, ASABE paper No 074038, Minneapolis, Minnesota.

Haug, R.T., 1993. The Practical Handbook of Compost Engineering. Ann Arbor, Mich: Lewis Publication.

Higgins, M.J., Yarosz, D.P., Chen, Y.C., Murthy, S.N., Mass, N.A., Cooney, J.R., 2002. Mechanisms of volatile sulfur compound and odor production in digested biosolids. Residuals and Biosolids, online available at http://www.mabiosolids.org/docs/25843.doc (access date 05/19/2008).

Jia, M., Zhang, Q.H., Min, D.B., 1998. Optimization of solid phase microextraction analysis for headspace flavor compounds of orange juice. J. Agr. Food Chem., 46(7), 2744-2747.

Kadota, H., Ishida Y., 1972. Production of volatile sulfur compounds by microorganisms. Annu. Rev., 1591, 127-138.

Kim, K.H., Choic, Y.J., Jeon, E.C., Sunwoo, Y., 2005a. Characterization of malodorous sulfur compounds in landfill gas. Atmos. Environ., 39, 1103-1112.

Kim, H., McConnell, L.L. and Millner, P., 2005b. Comparison of odorous volatile compounds from fourteen different commercial composts using solid-phase microextraction. Trans. ASAE, 48(1), 315-320.

Koziel, J.A., Novak, I., 2002. Sampling and sample preparation strategies based on solid phase microextraction for analysis of indoor air. Trends in Analytical Chemistry. 21(12), 840-850.

Koziel, J.A., Jia, M., Khaled, A., Noah, J., Pawliszyn, J., 1999. Field air analysis with SPME device. Anal. Chim. Acta. 400(1-3), 153-162.

474 Nongonierma, A., Cayot, P., Quere, J.L.L., Springett, M., Voilley, A., 2006. Mechanism of
475 extraction of aroma compounds from foods using adsorbents: effect of various
476 parameters. Food Rev. Int., 22, 51-94.
477 Pagans, E., Font, X., Sanchez, A., 2006. Emission of volatile organic compounds from
478 composting of different solid wastes: abatement by biofiltration. J. Hazard. Mater.,
479 131, 179-186.
480 Pawliszyn, J., 1997. SPME method development, in Pawliszyn J. (Ed.), Solid Phase
481 Microextraction- Theory and Practice. Wiley-VCH, New York, pp.97-140.
482 Richard, T. 1996. The effect of lignin on biodegradability. Cornell Waste Management
483 Institute, Department of Corp and Soil Sciences. Online available at
484 http://www.css.cornell.edu/compost/calc/lignin.html (access date 05/25/2009).
485 Rynk, R., van de Kamp, M., Willson, G.B., Singley, M.E., Richard, T.L., Kolega, J.J.,
486 Gouin, F. R., Jr. Laliberty, L., Kay, D., Murphy, D.W., Hoitink, H.A.J., Brinton, W.F.,
487 1992. On-Farm Composting Handbook. Northeast Regional Agric. Eng. Serv., pp. 24-
488 42.
489 Sadaka, S.S.; Richard, T.L.; Loecke, T.D.; Liebman, M. 2006. Determination of compost
490 respiration rates using pressure sensors. Compost Sci. Uti., 14, 124-131.
491 Shammas, N.K., Wang, L.K. 2007. Biosolids composting, in Wang, L.K., Shammas, N.K.,
492 Hung, Y.T (Eds), Handbook of Environmental Engineering: Biosolids Treatment
493 Process. Volume 6, Humana Press. pp. 645-685.
494 Shurson, J., Whitney, M., Nicolai, R., 1998. Nutritional manipulation of swine diets to
495 reduce hydrogen sulfide emissions. Available at

http://www.manure.umn.edu/research/sulfide_emmisions.html (access date 05/16/2008).

Spencer, J.L., Rennie, B., Guan, J., 2004. Emphasis on biosecurity for composting Poultry and Manure during an outbreak of highly pathogenic *Avian influenza* in British Columbia. Canadian Animal Health Net Bull., 9, 21-23.

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

Tulio, A.Z., Yamanaka, H., Ueda, Y., Imahori, Y., Chachin, K., 2003. Reversible and irreversible emission of methanethiol and dimethyl disulfide from anaerobically stored broccoli. J. Agri. Food Chem., 51, 6774-6781.

Wilkinson, K., 2007. The biosecurity of on-farm mortality composting. J. Appl. Microbiol., 102, 609-618.

Xin, H., 2005. Instruments for measuring concentrations and emission rates of gases and particulates from animal feeding operations. Iowa State University Extension, online available at http://www.extension.iastate.edu/Publications/PM1990.pdf (access date 05/13/2008).

Figure captions

Fig. 1. Schematic of the test units and dry/wet bulb temperature measurements (only 3 of the 12 test units are shown): (a) aerobic, (b) anaerobic

Fig. 2. Temperature profile used to simulate field-scale warm season animal mortality composting process based on previous work (Ahn et al., 2007; Glanville et al., 2007).

Fig. 3. Effect of SPME extraction temperatures at 1 h air sampling time (concentrations of dimethyl disulfide=6.85 ppmv, dimethyl trisulfide=5.95 ppmv, and pyrimidine=6.25 ppmv; three measurements for each point of the calibration curves).

Fig. 4. Measured emission rates of dimethyl disulfide, dimethyl trisulfide, and pyrimidine from swine tissue composting test units (from triplicate model compost units): (a) aerobic, (b) anaerobic