

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

4
5 Neslihan Akdeniz^{a,†}, Jacek A. Koziel^{a,b,*}, Hee-Kwon Ahn^{a,‡}, Thomas D. Glanville^a,
6 Benjamin P. Crawford^a, D. Raj Raman^a

7
8 ^aDepartment of Agricultural and Biosystems Engineering, Iowa State University,
9 Ames, IA 50011

10 ^bDepartment of Civil, Construction and Environmental Engineering, Iowa State
11 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

14 **Abstract**

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

34

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

36

37 **1. Introduction**

38 The safe disposal of carcasses during emergency animal disease (EAD) outbreaks is
39 a significant issue since diseases can be easily spread to humans and other livestock
40 (Wilkinson, 2007). Improper disposal of carcasses, such as illegal dumping, has become a
41 problem due to cost of mortality disposal and limited number of licensed landfills,
42 incineration and rendering services (Bonhotal et al., 2002). Biosecurity agencies in USA,
43 Canada, Australia, and New Zealand have recognized the potential benefits of composting
44 for emergency management of livestock mortalities (Wilkinson et al., 2007). Composting
45 of diseased mortalities was first developed for the poultry industry (Spencer et al., 2004;
46 Bendfeldt et al., 2006) with more recent studies being conducted to adapt mortality
47 composting for swine and cattle emergency disposal (Glanville et al., 2006a, 2006b;
48 Glanville et al., 2007; Ahn et al., 2007)

49 In case emergency disposal is necessary, a passively-aerated plastic wrapped
50 mortality composting system has been designed to reduce the potential of spreading live
51 viruses to the environment (Spencer et al., 2004; Ahn et al., 2007; Glanville et al., 2007). In
52 this type of biosecure composting application, carcasses are fully covered with envelope
53 (typically silage, straw, or hay) materials and the entire matrix is wrapped with plastic
54 sheets. It is not safe to turn, mix, or expose the diseased carcasses to the environment until
55 carcass degradation is completed, temperatures have been reached and maintained to
56 inactivate pathogens, and compost is considered stable (Wilkinson, 2007). Since visual
57 inspection of the carcasses is not possible, a new method is needed to assess progress and
58 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.,

81 2008), landfills (Davoli et al., 2003; Kim et al., 2005a), commercial composts (Kim et al.,
82 2005b) and decaying plant and animal materials (Akdeniz et al., 2007a, 2007b).

83 There are many variables affecting analytical method development and
84 quantification with SPME. One of the factors affecting air sampling with a SPME fiber is
85 air temperature. Although there are more compounds in the vapor phase when the
86 temperature increases, amounts of volatiles adsorbed can decrease (Jia et al., 1998;
87 Demyttenaere et al., 2003). This can be explained by the fact that adsorption is an
88 exothermic and competitive process (Pawliszyn, 1997). In addition, adsorption induces heat
89 release, which can increase the temperature of the adsorbent and decrease the amounts of
90 analyte adsorbed (Nongonierma et al., 2006).

91 In this study, a laboratory scale composting system, which simulated a field-scale
92 biosecure composting systems was designed, built and tested to specifically monitor VOC
93 emissions. Several operating variables were controlled, (i.e., composting time, temperature,
94 and aeration flow rate resulting) in aerobic or anaerobic conditions. The objectives of the
95 study were (a) to identify and compare VOCs evolved from decaying swine tissues and
96 plant materials in order to find marker compounds of decaying swine tissues, (b) to develop
97 air sampling and analyses methods for different air temperatures to accurately quantify
98 marker VOC emissions, and (c) to evaluate a potential usage of VOC emissions to test
99 completion of carcass degradation.

100 **2. Methods**

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

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

111 *2.1. Aerobic and anaerobic test units*

112 Two oilless pumps (Combined Fluid Products, Lake Zurich, IL) were used to aerate
113 aerobic test units. Air was pumped through hydrocarbon traps (Supelco, Bellefonte, PA) to
114 prevent any VOC contamination from the ambient air. Curved PTFE tubing with equal
115 number of holes were used to aerate test units (Fig. 1a). Air flow rate was set at 100 ± 1.66
116 mL/min by using stainless steel 3-way needle valves (Swagelok, Omaha, NE). As Rynk et
117 al. (1992) suggested airflow rates of 707.7 to 2,831 L/min per dry tonne of compost were
118 required for aerobic composting, we chose an air flow rate of 1.42 mL/min per dry gram
119 (1,420 L/min per dry ton-average of 707.7 and 2,831 L/min per dry ton) and kept constant
120 in all test units. Air flow rate of each test unit was set at 100 ± 2 mL/min and checked
121 weekly from the outlet port of the test units (Fig. 1a). One hour before headspace VOC
122 sampling, aeration was stopped and aerobic test units were kept closed to let the VOCs
123 reach equilibrium in the headspace.

124 Humidified air was pumped through aerobic test units. Relative humidity at the
125 outlet of the test units was calculated by measuring dry and wet bulb temperatures. Outlet
126 of the test units were connected to small glass jars and dry/wet bulb temperatures were
127 measured inside these jars using thermocouples (Omega Engineering, Stamford, CT).
128 Wetted gauze was used to measure wet bulb temperatures (Fig. 1a). A datalogger (Model
129 CR10X, Campbell Scientific, Logan, UT) was utilized to record temperature.

130 Anaerobic conditions were maintained by tightly closing the test units. Pure N₂
131 (99.995 % pure; Chemistry Store, Iowa State University) was passed through the test units
132 for two minutes once a week to make O₂ and CO₂ measurements. Mass flow rate of N₂ was
133 set at 1 L/min and checked by a mass flow controller (DryCal, Butler, NJ). Oxygen and
134 CO₂ concentration measurements were completed immediately after VOC sampling. The
135 O₂ trap (Alltech Associates, Chicago, IL) was used on N₂ delivery line to prevent any O₂
136 contamination and compromising anaerobic conditions.

137 *2.2. Sample preparation and composting conditions*

138 Plant materials (corn silage, oat straw, and alfalfa hay) were collected from central
139 Iowa. Oat straw and alfalfa hay were chopped to approximately 10 cm lengths to prevent
140 formation of an excessively porous structure. A whole swine carcass (6.3 kg, one month
141 old), including bones, internal organs, and skin, was shredded using an industrial grinder in
142 order to get a representative 70 g sample of the entire carcass. The initial moisture contents
143 of the corn silage, oat straw, alfalfa hay, and swine tissues were 43.6, 37.7, 44.2, and
144 65.0 %, respectively. Moisture contents of the plant materials were adjusted to 65 % by

145 spraying deionized water and incubated at 30 °C 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 °C 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 °C and then decreased to 22 °C 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 °C, 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 CO₂ and O₂ measurements*

174 The headspace CO₂ and O₂ concentrations were measured on weekly basis from the
175 outlet of the test units using a CO₂ (Vaisala, Louisville, CO) and O₂ (Apogee, Logan, UT)
176 probe. The CO₂ and O₂ 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 O₂ contamination. Carbon dioxide and O₂ probes
179 were calibrated weekly using clean ambient air and gas mixtures (5% CO₂-15 % O₂ and
180 15 % CO₂-5 % O₂) 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 °C. The initial temperatures of the GC oven were 40 °C with 3 min

189 holding time, followed by a ramp of 8 °C/min until reaching 220 °C, where it was held for
190 10 min. A polar capillary column (BP-21, 60 m × 0.32 mm ID × 0.25 µm film thickness,
191 SGE, Austin, TX) was used to separate VOCs. The MS mass/charge (m/z) ratio was set
192 between 29 and 150 for the first 8 min. After that, MS detection was between 34 and 280
193 m/z. The transfer line, quadrupole, and MS source temperatures were 240, 150, and 230 °C,
194 respectively. Chromatography data acquisition software including MSD ChemStation
195 (Agilent, New Castle, DE) and BenchTop/PBM™ V. 3.2.4 (Palisade Corporation, Ithaca,
196 NY) were used to analyze data. Separated compounds were identified using mass spectral
197 matches with ChemStation's NIST MS Library and PBM Benchtop MS libraries. Spectral
198 matches and column retention times were compared with those of standard VOCs (Sigma-
199 Aldrich, Milwaukee, WI).

200 *2.5 Emission rate measurements*

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

211 described in Akdeniz et al. (2009) and then they were subsequently placed inside an oven
212 (The Grieve Corporation, Round Lake, IL) with a temperature controller. Glass bulbs were
213 kept inside the oven for 10 min and headspaces were sampled for 1 h with 85 µm
214 CAR/PDMS SPME fiber. Concentrations of the compounds were calculated using the
215 calibration curve with the closest temperature to the sampling temperature. Emission rates
216 of the marker compounds were calculated based on the following equation (Xin, 2005):

217

$$218 \quad ER = Q * C * \frac{W_m}{V_m} * \frac{T_{std}}{T_a} * \frac{P_a}{P_{std}}$$

219

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

225 *2.6 Respiration rate measurements*

226 Compost materials were incubated for nine weeks and after nine weeks 20 g of
227 samples were collected from each unit and respiration rates of the remaining compost
228 materials were measured. A titration method was used to measure the CO₂ production
229 during respiration of remaining carcass tissues (Sadaka et al., 2006). For respiration rate
230 calculations, moisture content was determined by drying samples at 105 °C for 24 h.
231 Volatile solid contents of samples were measured by combusting dried samples at 550 °C
232 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 CO₂ concentrations in aerobic test units were found to be same as
243 those in ambient air (0.03 % CO₂, 20.94 % O₂). The O₂ concentrations in anaerobic test
244 units were always measured as zero. The CO₂ 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 O₂ supply to aerobic test units and no O₂ 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.,

254 2007) depending on the initial moisture content and the progress of the process. It is known
255 that lowest moisture content at which bacterial activity takes place is from 12 to 15%
256 (Shammas and Wang, 2007). In this study the final moisture contents were above 15% and
257 it can be concluded that aerobic test units were humidified properly and over-drying of the
258 compost materials due to excessive aeration was prevented.

259 *3.2 Identification of marker VOCs*

260 Forty three compounds including volatile fatty acids, esters, nitrogen-containing
261 compounds, alcohols, phenols, ketones, sulfur-containing compounds and terpenes were
262 identified in the headspace of corn silage, oat straw, alfalfa hay and swine composts (Table
263 1). Retention time and spectra of the compounds were matched with those of pure standards.

264 Among the forty three compounds, only three compounds were found to be evolved
265 exclusively by decaying swine tissues, and were not produced by any of the plant materials
266 tested. These marker compounds were dimethyl disulfide, dimethyl trisulfide and
267 pyrimidine, which were produced under both aerobic and anaerobic conditions. Plant
268 materials may be degraded slowly since their cellulose and lignin contents are high
269 (Richard, 1996). In the current study, marker compounds were not produced or produced
270 under method detection limits by plant materials. Method detection limits of dimethyl
271 disulfide, dimethyl trisulfide, and pyrimidine were 1.10, 5.50 and 0.011 ppbv, respectively
272 (Akdeniz et al., 2009).

273 In addition to swine composts, the marker compounds were also detected from
274 additional test units (both aerobic and anaerobic) where plant and animal materials were
275 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 °C (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 °C were found to be significantly higher than those extracted at 50 °C and 60 °C (Fig.
300 3). For this reason, calibration curves were prepared at 20, 30, 40, 50, and 60 °C (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)

320 of the process, marker compounds were found to be in the headspace of the test units. The
321 highest emission rates were measured in the 3rd week of the process. In this week, under
322 aerobic conditions, the emission rates were 1.24×10^{-2} , 1.43×10^{-2} , and 6.80×10^{-4} $\mu\text{g}/\text{min}$ for
323 dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. At anaerobic
324 conditions the emission rates were 3.93×10^{-5} , 5.53×10^{-5} , and 5.93×10^{-6} $\mu\text{g}/\text{min}$ for dimethyl
325 disulfide, dimethyl trisulfide, and pyrimidine, respectively. After the 3rd week, emission
326 rates significantly decreased. After the 6th week, marker compounds were not detected
327 from the headspace of the test units. In week 6, concentrations of the marker compounds in
328 the headspace were lower than the MDLs and the available sources of marker emissions
329 were most likely depleted. Under aerobic conditions, the emission rates were estimated to
330 be below 0.51×10^{-4} , 3.43×10^{-4} , and 4.35×10^{-7} $\mu\text{g}/\text{min}/(\text{final g VS of swine tissue})$ for
331 dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively. At anaerobic
332 conditions the emission rates were 0.37×10^{-7} , 2.51×10^{-7} and 3.19×10^{-10} $\mu\text{g}/\text{min}/(\text{final g VS}$
333 of swine tissue) for dimethyl disulfide, dimethyl trisulfide, and pyrimidine, respectively.
334 These emission rates were calculated based on MDLs, final g VS of swine tissues and
335 compost temperature in week 6. Emission rates were reported per final g VS (VS of
336 compost materials after 9 weeks) to facilitate comparisons between the results of this lab-
337 scale study and larger scale composting studies.

338 Temperatures of the test units were purposefully raised at the end of the composting
339 process (week 8) (Fig. 2). This increase in temperature did not result in increased emission
340 rates of the marker compounds and marker compounds were not detected in the headspace
341 (Figs. 4a and b). This indicated that the emission rates of the marker compounds did not
342 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 CO₂-C/g VS/d (Table 3). Thompson (2002)
346 classified compost materials with respiration rates below 5 mg CO₂-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

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

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

378 **4. Conclusions**

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

387 emission rates were detected during the third week of the composting process. Marker
388 compounds were not detected after the 6th week of the process. The decrease in the
389 emission rates of marker compounds after week 6 was not caused by change in the
390 temperature and lower volatility of the compounds. The main reason was likely slower
391 microbial activity due to limited nutrient sources and earlier exposure to high temperatures.
392 Respiration rates of the swine tissues were measured at the end of the trial (week 9).
393 Volatile fatty acids were only detected in the headspace of decaying plant materials under
394 anaerobic conditions. Significant correlations (correlation coefficients ranging from 83.7 to
395 99.6 %) were found between emission rates of dimethyl disulfide, dimethyl trisulfide, and
396 pyrimidine. Consequently, the emission rate of one of these three marker compounds could
397 be used to estimate completion of the carcass degradation in biosecure swine mortality
398 composting systems. Therefore, future studies to monitor completion of biosecure field-
399 scale swine mortality compost systems using the VOCs characterized in this study are
400 warranted.

401 **Acknowledgments**

402 The authors gratefully acknowledge Anthony Pometto III and Sam Beattie for their
403 significant contribution. This project is supported by Canadian Food Inspection Agency
404 through a grant from the Canadian Research and Technology Initiative (CRTI Project # 04
405 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

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

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

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

459 Jia, M., Zhang, Q.H., Min, D.B., 1998. Optimization of solid phase microextraction
460 analysis for headspace flavor compounds of orange juice. J. Agr. Food Chem., 46(7),
461 2744-2747.

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

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

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

469 Koziel, J.A., Novak, I., 2002. Sampling and sample preparation strategies based on solid
470 phase microextraction for analysis of indoor air. Trends in Analytical Chemistry.
471 21(12), 840-850.

472 Koziel, J.A., Jia, M., Khaled, A., Noah, J., Pawliszyn, J., 1999. Field air analysis with
473 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 Crop 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 Shamas, N.K., Wang, L.K. 2007. Biosolids composting, in Wang, L.K., Shamas, 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

496 http://www.manure.umn.edu/research/sulfide_emmissions.html (access date
497 05/16/2008).

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

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

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

506 Wilkinson, K., 2007. The biosecurity of on-farm mortality composting. J. Appl. Microbiol.,
507 102, 609-618.

508 Xin, H., 2005. Instruments for measuring concentrations and emission rates of gases and
509 particulates from animal feeding operations. Iowa State University Extension, online
510 available at <http://www.extension.iastate.edu/Publications/PM1990.pdf> (access date
511 05/13/2008).

512

513 **Figure captions**

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

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

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

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