

# **FIELD SCALE EVALUATION OF VOLATILE ORGANIC COMPOUND PRODUCTION INSIDE BIOSECURE SWINE MORTALITY COMPOSTS**

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**ABSTRACT**

Emergency mortality composting associated with a disease outbreak has special requirements to reduce the risks of pathogen survival and disease transmission. The most important requirements are to cover mortalities with biosecure barriers and avoid turning compost piles until the pathogens are inactivated. Temperature is the most commonly used parameter for assessing success of a biosecure composting process, but a decline in compost core temperature does not necessarily signify completion of the degradation process. In this study, gas concentrations of volatile organic compounds (VOCs) produced inside biosecure swine mortality composting units filled with six different cover/plant materials were monitored to test the state and completion of the process. Among the 55 compounds identified, dimethyl disulfide, dimethyl trisulfide, and pyrimidine were found to be marker compounds of the process. Temperature at the end of eight week was not found as an indicator of swine carcass degradation. However, gas concentrations of the marker compounds at the end of eight weeks were found to be related to carcass degradation. The highest gas concentrations of the marker compounds were measured for the test units with the lowest degradation (highest respiration rates). Dimethyl disulfide was found to be the most robust marker compound as it was detected from all composting units in the eighth week of the trial. Concentration of dimethyl disulfide decreased from a range of 290 to 4340 ppmv to 6 to 160 ppbv. Dimethyl trisulfide concentrations decreased to a range of below detection limit to 430 ppbv while pyrimidine concentrations decreased to a range of below detection limit to 13 ppbv.

**Keywords.** Mortality compost, dimethyl disulfide, gas chromatography-mass spectrometry, respiration rate, solid phase microextraction, volatile organic compound

## 1. Introduction

Proper disposal of mortalities is required to protect public and animal health (Kalbasi et al., 2005; Xu et al., 2008). Before the occurrence of bovine spongiform encephalopathy (BSE) in North America, mortalities were primarily disposed by rendering. After BSE, a variety of alternatives are being investigated for the disposal of mortalities due to risks associated with transporting large amounts of pathogen-contaminated carcasses over long distances (Stanford et al., 2007; Wilkinson, 2007; Benson et al., 2008, Guan et al., 2008; Glanville et al., 2009; Guan et al., 2009; Stanford et al., 2009; Xu et al., 2009). Composting has been successfully employed for disposing mortalities in a few outbreaks in North America, including 2002 avian influenza (H7N2) outbreak in the central Shenandoah Valley (Virginia) (Bendfeldt et al., 2005a and b), 2004 outbreaks on the Delmarva Peninsula of Maryland and Delaware (Malone et al., 2004), and in British Columbia (Canada) (Spencer et al., 2005a and b).

Emergency mortality composting associated with a disease outbreak has special requirements to reduce the risks of pathogen survival and disease transmission. One of the most important requirements of composting diseased mortalities is to avoid turning compost piles during decomposition to minimize the risk of aerosol transmission of pathogens (Ahn et al., 2008a; Ahn et al., 2008b). To further reduce the risk of pathogen release, plastic sheets may be used to cover compost piles. During the highly pathogenic outbreak in British Columbia (2004), the poultry composting windrows were built from brown building paper (isolation), wood shavings and covered with black plastic sheets (Wilkinson, 2007). One of the greatest challenges to such biosecure systems was the lack of reliable tools to safely monitor the process.

To date, temperature is the most commonly used parameter for assessing success of a composting process (Ahn et al., 2009). If the temperature of a compost unit fails to increase in the initial stages of the process, nutrient supply, moisture level or aeration may not be sufficient (Haug, 1993) and low carcass decomposition and potential survival of pathogens is expected. Other reasons for low core compost temperatures may be over-aeration of the composting units and low heat retention properties of the plant (cover) materials (Glanville et al., 2007). Thus, measuring temperature does not necessarily help to evaluate completion of a composting process. A recent approach to assess performance of a biosecure composting process is to measure volatile organic compound (VOC) concentrations inside the biosecure composting units. It is known that a wide variety of gases including odorous VOCs and greenhouse gases are produced during composting (Rosenfeld et al., 2007; Xu et al., 2007). However, we are not aware of any study that focuses on monitoring VOC production inside field-scale biosecure composting units.

In this pioneering study, we identified VOCs generated during degradation of swine mortalities in biosecure composts and monitored VOCs on a weekly basis from two sequential field trials carried out using six different plant (cover) materials. This study focuses on VOCs that can be determined by SPME and GC-MS to find a biomarker compounds of the composting process. Objectives of the study were (a) to develop a comprehensive chemical library of VOCs originating from field scale swine mortality composts surrounded by plastic bio-security barriers; (b) to quantify specific compounds that can be correlated with different phases of the composting process and used to determine completion of the process; (c) to determine the effects of compost operating parameters on the chemical make-up of VOCs.

## 2. Materials and Methods

*Composting Units.* In this study, the biosecure composting system used during the 2004 outbreak of avian influenza in British Columbia (Wilkinson, 2007) was adapted to swine mortalities. The experiments were conducted from May 2007 to October 2007 at Livestock Environment Building and Research Center of Iowa State University, Ames, Iowa. Composting units were constructed on 2 m×2 m platforms with 1.2 m high sidewalls (Ahn et al., 2007; Glanville et al., 2007). The insides of the composting units were covered with a synthetic rubber liner to capture and retain leachate. Composting units were loaded with approximately 250 kg swine carcasses (four or five carcasses per unit). The bottom 30 cm of the composting units were filled with cover material and swine carcasses were placed on this cover material. The same cover material (60 cm) was used between and over the carcasses. The ratio of plant material to swine carcass was 15 (plant): 1 (animal) (w/w). The moisture contents, volatile solids contents and respiration rates of the plant materials were given in Akdeniz et al. (2010). The outsides of the composting units were insulated with 5 cm thick Styrofoam plastic barrier. Aeration of the composting units was achieved by inserting three passive aeration tubes beneath the swine carcasses. The details of the composting units were reported in Ahn et al. (2007) and Glanville et al. (2007). The purpose of the plastic barrier placed on top of each unit was to minimize the risk of spreading pathogens to the surrounding environment during an emergency mortality composting operation.

Six plant (cover) materials including corn silage, oat straw, corn stalks, wood shavings, soybean straw, and alfalfa hay were used to cover swine carcasses. Except for corn silage and wood shavings, all plant materials were ground into 5 cm length to reduce

size and leachate and improve VOC absorbency and heat retention (Ahn et al., 2007; Glanville et al., 2007). Initial moisture contents, volatile solids, and respiration rates of the plant (cover) materials are shown in Table 1. Eighteen test units were prepared (6 cover materials\*3 replicates=18 test units). Corn silage, oat straw and corn stalks composting units were run from May 29 to July 29, and wood shavings, soybean straw and alfalfa hay composting units were run from August 20 to October 20. Multiport gas and temperature sampling probes constructed of 3 cm diameter PVC (polyvinyl chloride) piping were placed in the center of each unit. Three PTFE (polytetrafluoroethylene) air sampling tubing (0.62 cm I.D., E&S Technologies, Chelmsford, MA) and three thermocouples (Omega Engineering, Stamford, CT) were tightly placed inside the sampling probes at bottom, middle and top depths to make measurements from these layers (depths) of the units. An Apogee O<sub>2</sub> sensor (Logan, UT) was used to measure the O<sub>2</sub> level.

*Headspace Gas Sampling with Solid Phase Microextraction.* Air samples were drawn from the composting units on weekly basis using SKC pumps (model 224-PCXR4, Eighty Four, PA). Air flow rate was 1.0 L/min. Air samples were collected with 250 mL glass sampling bulbs (Supelco, Bellefonte, PA) after pumping air for 5 min (20 hydraulic residence times). After 5 min, PTFE stopcocks of glass bulbs were closed and air samples were captured inside the bulbs. Then the glass sampling bulbs were carried to Atmospheric Air Quality Laboratory where they were sampled using an 85 µm Carboxen/PDMS (Polydimethylsiloxane) SPME (solid phase microextraction) fiber (Supelco, Bellefonte, PA) in a 1 h sampling time. New solid SPME fibers were first conditioned according to the manufacturer's directions. In addition, SPME fibers were

inserted into the injection port of GC for 5 min to thermally desorb impurities on the fiber 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. The details of the SPME-based gas sampling and sample preparation were described in Akdeniz et al. (2009).

*Gas Sample Analysis.* All gas analyses were completed using an integrated multi-dimensional GC-MS (gas chromatography-mass spectrometry) system consisting of a 6890N GC and 5973 MS (Agilent Inc., Wilmington, DE) system. Ultrahigh pure (99.995 %) helium (Praxair, Danbury, CT) was used as the carrier gas at constant pressure. The injector and SPME fiber desorption temperature was 260 °C. The initial temperatures of the GC oven were 40 °C with 3 min holding time, followed by a ramp of 7 °C/min until reaching 220 °C, 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 and 35 min, and backflush of the pre-column was activated between 36 and 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 °C, respectively. The electron multiplier voltage was between 1000 and 1388 eV. MS was auto-tuned on weekly basis.

*Data Analysis.* Chromatography data acquisition software consisting 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. GC-grade standards of compounds were purchased from Sigma-Aldrich (Milwaukee, WI).

Gas concentrations were calculated using the calibration curves prepared by Akdeniz et al (2009). Calibration curves were reported for both dry (~0% relative humidity) and humid (~97% relative humidity) conditions. Since no significant difference was reported between dry and humid conditions, dry condition calibration curves were used in this study.

*Respiration Rate Measurements.* Swine carcasses were composted for eight weeks. After eight weeks of composting, the plastic barriers of the composting units were opened and partially decomposed animal tissues were collected. Respiration rates of the partially decomposed animal tissues were measured using 20 g of homogenous soft tissue from each carcass. Titration method was used to measure the CO<sub>2</sub> production during respiration of partially decomposed carcass tissues (Sadaka et al., 2006). For respiration rate calculations, moisture content and volatile solids of the tissues were analyzed using standard methods. Moisture content was determined by drying samples at 105 °C for 24 h. Volatile solid contents of samples were measured by combusting dried samples at 550 °C for 5 hrs (APHA 2540E).



*Statistical Analysis.* Experiments were run in triplicate. ANOVA tests were conducted using JPM software version 6.0.2 from SAS (SAS Institute Inc, Cary, NC). Data had a normal distribution. Concentrations of gases collected from bottom, middle, and top layers were compared using Tukey's honestly significant differences (HSD) at the significance level  $P < 0.05$ . Respiration rates of the remaining carcasses and dimethyl disulfide concentrations at the end of week 8 were also compared using Tukey test at the same significance level. Average and relative standard deviation (RSD) of the concentrations were reported. Relative standard deviation is reported in percentage and calculated as  $(\text{standard deviation}/\text{average}) \times 100$ . Average and RSD values were calculated for three independent composting units.

## **Results and Discussion**

### *Identification and Evaluation of VOCs Generated by Decaying Plant and Animal Tissues.*

Fifty-five compounds were identified in gas samples collected from warm season swine mortality composting units. A list of compounds is reported in Table 2. Thirty-five compounds were verified by matching capillary GC column retention times and MS spectra with those of pure standards. Compounds detected from all composting units, regardless of the plant material used, were dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and pyrimidine. These compounds were considered as marker compounds of the swine mortality degradation process for two reasons. The first reason was that these compounds were also found to be produced during degradation of swine tissues in laboratory scale composting experiments. It was reported that DMDS, DMTS and pyrimidine were produced from decaying swine tissues but not produced from decaying corn silage, oat straw and alfalfa hay (Akdeniz et al., 2010). The second reason

was that these marker compounds were produced regardless of the plant material used to cover swine carcasses. Detection of the marker compounds from all composting units in the present study indicated that these marker compounds can be produced and detected under different composting conditions (e.g., moisture content, temperature, bulk density). For these reasons, these three compounds were found to be reliable markers of the swine carcass degradation process and they can be used to evaluate completion of a biosecure swine mortality composting process.

Other VOCs that were detected in this study were volatile fatty acids (VFAs) and mercaptans. Several VFAs (acetic, propanoic, 3-methyl butanoic, pentanoic and hexanoic acids) and mercaptans (methanethiol and ethanethiol) were detected in gas samples collected from corn silage, oat straw and corn stalks composting units but not detected in gas samples collected from wood shavings, soybean straw and alfalfa hay composting units. It is important to discuss the role of other VFAs and mercaptans since they are known to be produced during anaerobic degradation (Haug, 1993). It was reported that VFAs were produced from decaying corn silage, oat straw and alfalfa hay under anaerobic conditions (Akdeniz et al., 2010). Measured oxygen levels inside the composting units could be potentially used to explain the difference in VFA contents of the composting units (Fig. 1). Oxygen levels of all composting units were high and ranged from 13.4 to 21.10%. Relative standard deviation of oxygen concentrations ranged from 0.19 to 45.1 %. RSD values were high as some natural factors (e.g., wind direction and sun light) affecting replicates could not be controlled. There was no evidence of anaerobic macro-environment formation inside compost. However, higher initial moisture contents of corn silage, oat straw and corn stalks (56.2 to 64.8%)

compared to wood shavings, soybean straw and alfalfa hay (11.2 to 17.7%) may have caused anaerobic microenvironment formation (Table 1). Anaerobic microenvironment formation can explain anaerobic VOC production in corn silage, oat straw and corn stalks composting units. Thus, the VFAs and mercaptans production can be an indicator of the aeration status of a composting system.

*Evaluation of Representativeness of Gas Sampling Locations.* Concentrations of the marker compounds sampled from the bottom, middle, and top layers (depths) of the test units were presented in Fig. 2. Measured concentrations of the marker compounds were added for all samples collected within each trial and the average of the three test units was reported. Relative standard deviations of the measured concentrations ranged from 1% to 18%. The concentrations of DMDS and pyrimidine sampled from corn silage, wood shavings, soybean straw, and alfalfa hay composting units were found to be significantly different in the middle (carcass) layer compared to bottom and top (plant) layers. No significant difference was observed between marker compound concentrations measured in the middle and bottom layers of oat straw and corn stalks test units. However, measured marker VOC concentrations in these layers were found to be different than those in the top layer. The highest concentrations of DMTS were measured from the middle and bottom layers of the all composting units. It is most likely that marker compounds were produced during degradation of swine carcasses in the middle layer and the bottom layer was contaminated with leachate from the middle layer. The relatively low concentrations of the marker compounds detected in the top layer can be explained by absorption of the compounds by overlying plant materials and/or microbial utilization/biofiltration. Since the highest amounts of marker VOCs were measured from

the middle layer (carcass layer), this layer was chosen as the most relevant and representative location to sample VOCs. Results associated with this layer were presented in the following sections.

*Evaluation of Carcass Degradation.* Gas concentrations of the marker compounds measured over the duration of the eight week study from the middle layer of the composting units are shown in Figs. 3 and 4. The highest gas concentrations of the marker compounds from corn silage, oat straw and corn stalks test units were observed for the second and third weeks of the process (Fig. 3). After the third week, gas concentrations of the marker compounds decreased gradually. For wood shavings, soybean straw, and alfalfa hay test units, the highest gas concentrations were measured for the first week and decreased gradually after the first week (Fig. 4). A gradual decrease in the gas concentrations of the marker compounds indicated a reduced rate of carcass degradation and marker VOC production (Kim et al., 2005; Akdeniz et al., 2010).

These results are consistent with those from the laboratory scale study which was planned to simulate this field scale study. In the laboratory scale study, it was shown that DMDS, DMTS, and pyrimidine were not detected in gas samples collected from the headspace of swine tissue composts after the sixth week of the experiment (Akdeniz et al., 2010). In this study, gas concentrations of the marker compounds followed a similar trend as in the laboratory-scale trials. Dimethyl disulfide was detected from all composting units in the eighth week of the trial. Its concentration decreased from a range of 290 to 4340 ppmv (during weeks 1 and 2) to 6 to 160 ppbv. Dimethyl trisulfide was detected in the eighth week of the trial from wood shavings, soybean straw and alfalfa hay composting units at concentrations of 430, 17 and 19 ppbv, respectively. Pyrimidine

was only detected from wood shavings and alfalfa hay composting units at a concentration of 13 ppbv in the last week of the process. The concentrations of the marker compounds at the end of eight weeks were shown in Table 3. Although logarithmic scale was used these concentrations could not be clearly shown in Figures 3 and 4.

Based on the concentrations of the marker compounds in week 8, it was concluded that composting process was not stabilized in any of the composting units. The composting process should not have been terminated until the concentrations of the marker compounds decreased under method detection limits. Method detection limits of DMDS, DMTS, and pyrimidine were reported as 1, 5.7 and 0.01 ppbv, respectively (Akdeniz et al., 2009). Since DMDS concentrations were lowest in corn stalks and oat straw composting units, the highest degradation was expected for these test units. Wood shavings and alfalfa hay composting units were confirmed as the composting units with the lowest degradation rates, as even in the last week of the process all three marker compounds were detected in gas samples collected from these composting units.

Based on the concentrations of the marker compounds in week 8 (Table 3), carcass degradation in the composting units is ranked from the highest to the lowest as: corn stalks  $\approx$  oat straw > corn silage > soybean straw > alfalfa hay > wood shavings. DMDS was found to be the most robust marker compound as it was detected from all test units at the end of eight week. No significant difference was found between DMDS concentrations of corn stalks and oat straw composting units at the end of eight weeks. DMDS concentrations were found to be significantly different in corn silage, soybean straw, alfalfa hay and wood shavings composting units (Table 3). These findings were

supported by respiration rate measurements conducted after the termination of each trial (Table 4). Collection of representative samples for determination of respiration rates could not be conducted during the trial for biosecurity reasons. Samples collected from remaining swine tissues in corn silage, oat straw and corn stalks composting units were classified as ‘moderately unstable’ compost and samples collected from wood shavings, soybean straw and alfalfa hay composting units were classified as ‘unstable raw’ compost (Sadaka et al., 2006). The highest respiration rates (lowest degradation) and the highest concentrations of marker compounds were measured for alfalfa hay and wood shavings composting units. The lowest respiration rates (and therefore highest degradation) and lowest gas concentrations of marker compounds were reported for corn stalks and oat straw composting units (Tables 2 and 3, Fig. 3 and 4).

The incomplete carcass degradation can be explained by substantial losses of water from the composting units (Glanville et al., 2007), resulting in desiccation of carcasses, lower microbial activity, and cessation of breakdown. Since the test units were wrapped with plastic barriers, no additional water was added during the process. Corn silage, oat straw and corn stalks were initially moistened by rain (56.2 to 64.8%) but during the process excessive moisture was lost due to over-aeration (Glanville et al., 2007). This problem of incomplete carcass degradation was made worse in wood shavings, soybean straw and alfalfa hay composting units with lower initial moisture contents (11.2 to 17.7%) and over-aeration caused due to their higher porosity. The low initial moisture contents of wood shavings, soybean straw and alfalfa hay caused lower degradation of swine carcasses in these composting units. Moreover, many terpenes (e.g., camphene, limonene,  $\beta$ -pinene,  $\beta$ -phellandrene) were detected in gas samples collected

from pine wood shaving and alfalfa hay test units (Table 2). These compounds cause the specific odor of pine wood and alfalfa but they have been found to have an antimicrobial effect on some pathogenic bacteria (Demirci et al., 2007). 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 composting units (Table 4, Fig. 4). At the beginning of the study, it was difficult to predict the potential impact of terpenes on the composting process as there was no published data about it.

Middle layer and ambient temperatures were presented in Fig. 3 and 4. The average of the three composting units was reported. Relative standard deviations of the temperature data ranged from 0.86 to 20.4%. The highest temperatures were measured during the first and second weeks of the process. After the second week, temperatures of the composting units started to decrease. In the last week of the process, the lowest temperatures (around 35 °C) were observed for oat straw, soybean straw and alfalfa hay composting units. Temperatures of corn silage, corn stalks and wood shavings test units at week 8 were around 55, 45, and 45 °C, respectively. Lower temperatures at the end of the process (week 8) did not necessarily indicate higher degradation rates and stabilization of the carcasses. For example, higher final temperatures were measured for corn silage, corn stalks and wood shavings test units compared to oat straw, soybean straw and alfalfa hay test units. However, the most complete carcass degradation was observed for corn silage and corn stalks test units. This finding suggests that temperature at eight weeks cannot be a reliable tool to monitor the completion of tissue degradation inside biosecure composts. It can be concluded that a better estimate of carcass

degradation can be made by measuring gas concentrations of DMS, DMTS and pyrimidine in the last week of the process.

### **Conclusions**

A comprehensive chemical library of VOCs emitted from biosecure swine mortality composting processes was developed. Among the 55 VOCs identified in gas samples collected from compost, dimethyl disulfide, dimethyl trisulfide and pyrimidine were found to be marker compounds of the process. Six plant (cover) materials at different moisture levels were tested and these three VOCs were consistently produced in all replicated composting units.

The highest concentrations of the marker compounds were detected from the middle layer (depth) of the composting units, which indicates that marker compounds were produced during degradation of the carcasses in this layer and bottom and top layers were contaminated later. Middle layer was the most relevant and representative location of the test units to collect VOC samples.

The highest gas concentrations of the VOC marker compounds were measured in the first weeks after which, their gas concentrations decreased gradually. A gradual decrease in the gas concentrations indicated that carcass degradation and marker VOC production slowed down. These results are consistent with those from the laboratory scale study. Since concentrations of marker compounds were still above the method detection limit in the last week of the process, it was concluded that composting process was not stabilized in any of the test units. Dimethyl disulfide was found to be the most robust marker compound as it was detected from all composting units in the eighth week



of the trial. These findings were supported by respiration rate measurements. The highest gas concentrations of the three marker compounds were detected in the gas samples collected for the test units with the highest respiration rates (lowest degradation).

In future studies, more relevant (easier to access in field conditions) sampling locations (e.g., passive aeration tubes) could be investigated. Also, portable instruments to measure concentrations of marker compounds could be developed.

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### Figure Captions

**Fig. 1.** Oxygen concentrations for the middle layer of the test units (average of the three replicated units, n=3).

**Fig. 2.** Measured gas concentrations of marker compounds in the bottom, middle and top layers of the test units. Gas concentrations for eight weeks were added and average of the three replicated units was reported. DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine. Different letters within each compound mark significant differences ( $\alpha=0.05$ ).

**Fig. 3.** Average gas concentrations of the marker compounds during eight weeks and temperatures of the test units and ambient air. Three replicated corn silage, corn stalks, oat straw test units which were composted during May 29-July 29. DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine. For clarity purposes relative standard deviations of the data were only reported in the text. Concentrations at the end of eight weeks could not be clearly shown on this figure but were reported in Table 3.

**Fig. 4.** Average gas concentrations of the marker compounds during eight weeks and temperatures of the test units and ambient air. Three replicated wood shavings, soybean straw and alfalfa hay test units which were composted during August 20-October 20. DMDS: dimethyl disulfide, DMTS: dimethyl trisulfide, PR: pyrimidine. For clarity purposes relative standard deviations of the data are only reported in the text. Concentrations at the end of eight weeks could not be clearly shown on this figure but were reported in Table 3.

Table 1. Initial moisture contents, volatile solids and respiration rates of the plant (cover) materials

Composting unit name	Moisture content (%)	Volatile solids (% dry basis)	Respiration rate (mgCO <sub>2</sub> -C/gVS <sup>-1</sup> d <sup>-1</sup> )	Classification
Corn Silage	56.2±0.95	95.01±2.05	4.45±0.35	moderately unstable
Oat Straw	58.6±1.11	96.00±1.23	4.32±0.36	moderately unstable
Corn Stalks	64.8±1.1	95.00±1.12	4.55±0.21	moderately unstable
Wood shavings	11.2±0.85	98.6±2.12	4.11±0.12	moderately unstable
Soybean straw	17.7±0.74	93.5±1.95	4.22±0.13	moderately unstable
Alfalfa hay	17.6±0.86	95.80±1.21	5.25±0.09	moderately unstable



**Table 2.** Summary of volatile organic compounds detected in gas samples collected from the middle layer of the test units (n=3). (+) detected and (-) not detected in the headspace.

(\*) GC column retention time and MS spectra were confirmed by using standards.

Compound name	CAS number	Test unit					
		Corn silage	Oat straw	Corn stalks	Wood shavings	Soybean straw	Alfalfa hay
Volatile fatty acids							
Acetic acid*	64-19-7	+	+	+	-	-	-
Propanoic acid*	79-09-4	+	+	+	-	-	-
Isovaleric acid*	503-74-2	+	+	+	-	-	-
Valeric acid*	109-52-4	+	+	+	-	-	-
Hexanoic acid*	142-62-1	+	+	+	-	-	-
Esters							
Methyl acetate*	79-20-9	+	-	-	+	-	+
Ethyl butanoate*	105-54-4	+	-	-	-	-	-
Butyl butanoate*	109-21-7	+	-	-	-	-	-
Butyl hexanoate*	626-82-4	+	-	-	-	-	-
Nitrogen-containing compounds							
Pyrimidine*	289-95-2	+	+	+	+	+	+
Pyrazine*	290-37-9	+	+	+	-	+	-
Pyridine*	110-86-1	+	+	+	-	-	-
2-Methyl pyrazine*	109-08-0	+	+	+	-	-	-
2,6-Dimethyl pyrazine	108-50-9	-	-	-	-	+	-
Pyrrole	109-97-7	-	-	-	-	+	-
Alcohols							
1-Octanol*	111-87-5	-	-	-	+	-	+
A-Fenchol	1632-73-1	-	-	-	+	-	+
1-Decanol*	112-30-1	-	-	-	+	-	-
Ketones							
2-Butanone*	78-93-3	+	+	+	-	+	-
3-Methyl 2-butanone*	563-80-4	+	+	+	-	-	-
2-Heptanone*	110-43-0	+	+	+	+	-	-
2-Octanone*	111-13-7	+	+	+	-	-	-
2-Nonanone*	821-55-6	+	+	+	+	-	-
2-Decanone*	693-54-9	-	-	-	+	-	-
Aldehydes							
Butanal	123-72-8	+	+	-	-	-	-
Isobutanal*	78-84-2	+	+	+	-	-	-
3-Methyl butanal*	590-86-3	+	+	+	-	-	-
Pentanal	110-62-3	+	+	+	-	-	-
Hexanal*	66-25-1	+	+	+	-	-	-
Myrtenal	23727-16-4	-	-	-	+	-	-
Safranal	116-26-7	-	-	-	+	-	-
Phellandral	21391-98-0	-	-	-	+	-	-
Sulfur-containing compounds							
Methanethiol*	74-93-1	+	+	+	-	-	-
Dimethyl sulfide*	75-18-3	+	+	+	-	-	-
Ethanethiol	75-08-1	+	+	+	-	-	-
Dimethyl disulfide*	624-92-0	+	+	+	+	+	+
Dimethyl trisulfide	3658-80-8	+	+	+	+	+	+

**Table 2 continues...**

Compound name	CAS number	Test unit					
		Corn silage	Oat straw	Corn stalks	Wood shavings	Soybean straw	Alfalfa hay
Terpenes							
Camphene*	79-92-5	-	-	-	+	-	+
B-Pinene	127-91-3	-	-	-	+	-	+
β-Myrcene*	123-35-3	-	-	-	+	-	-
Delta 3-carene*	13466-78-9	-	-	-	+	-	+
α-Terpinene*	99-86-5	-	-	-	+	-	+
Limonene*	138-86-3	-	-	-	+	-	-
β-phellandrene	555-10-2	-	-	-	+	-	+
α-Terpinolene*	586-62-9	-	-	-	+	-	+
Fenchone	1195-79-5	-	-	-	+	-	-
Camphor*	76-22-2	-	-	-	+	-	-
α-Longipinene	5989-08-2	-	-	-	+	-	-
Copaene	3856-25-5	-	-	-	+	-	-
Isolongifolene	1135-66-6	-	-	-	+	-	+
α-Muurolene	24406-05-1	-	-	-	+	-	+
Calamenene	483-77-2	-	-	-	+	-	+
α-Calacorene	21391-99-1	-	-	-	+	-	+
Azulene	275-51-4	-	-	-	+	-	-

**Table 3.** Average concentrations (ppbv) and  $\pm$  standard deviations of marker compounds at the end of eight week composting. Some values could not be clearly shown on Figure 3 and 4 but presented separately on this table.

Composting unit name	DMDS	DMTS	Pyrimidine
Corn Silage	19 $\pm$ 0.5 <sup>D*</sup>	Below MDL**	Below MDL*
Oat Straw	16 $\pm$ 0.5 <sup>E</sup>	Below MDL*	Below MDL*
Corn Stalks	16 $\pm$ 0.5 <sup>E</sup>	Below MDL*	Below MDL*
Wood shavings	160 $\pm$ 2.4 <sup>A</sup>	430 $\pm$ 3.2	13 $\pm$ 0
Soybean straw	79 $\pm$ 2.5 <sup>C</sup>	17 $\pm$ 0.4	Below MDL*
Alfalfa hay	129 $\pm$ 3.0 <sup>B</sup>	19 $\pm$ 0.5	13 $\pm$ 0.5

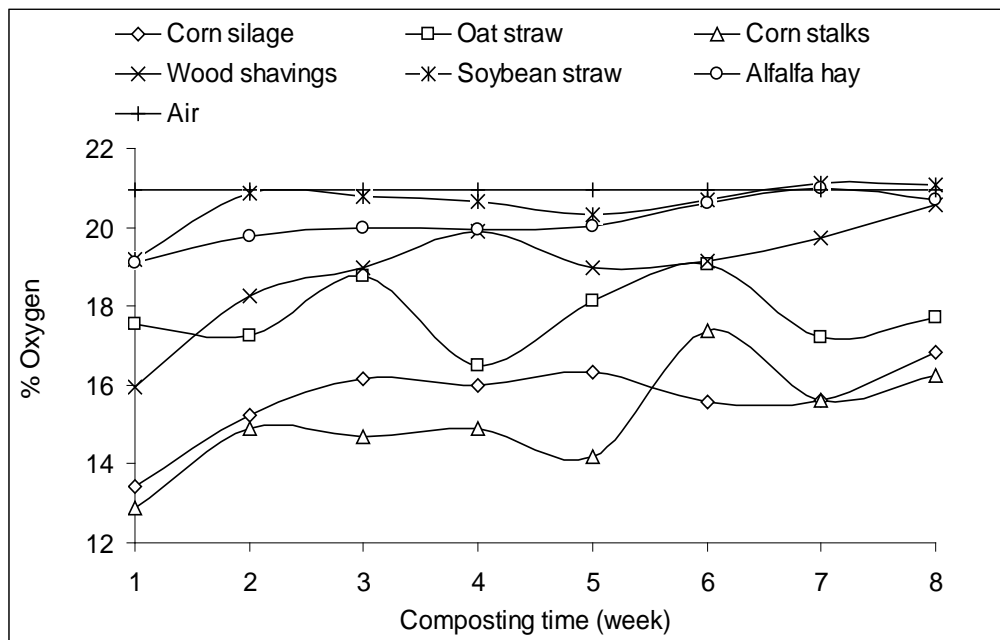
\*DMDS means with different superscripts are significantly different ( $P<0.05$ ).

\*\*Method detection limits (MDL) of dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and pyrimidine were 1, 5.7, and 0.01 ppbv, respectively.

**Table 4.** Respiration rates of the partially decomposed carcass tissues collected at the end of the eight week trials (6 cover materials\*3 replicated test units\*3 replicates per test unit= 54 tests).

Composting unit name	Moisture content (%)	Respiration rate (mgCO <sub>2</sub> -C/gVS <sup>-1</sup> d <sup>-1</sup> )	Classification
Corn Silage	59.62±3.25	6.40±0.42 <sup>D*</sup>	moderately unstable compost
Oat Straw	48.59±1.25	5.52±0.3 <sup>E</sup>	moderately unstable compost
Corn Stalks	49.52±2.8	5.21±0.82 <sup>E</sup>	moderately unstable compost
Wood shavings	53.26±3.2	9.25±0.85 <sup>A</sup>	unstable raw compost
Soybean straw	37.57±4.9	7.85±0.65 <sup>C</sup>	unstable raw compost
Alfalfa hay	52.80±4.7	8.56±0.38 <sup>B</sup>	unstable raw compost

\*Respiration rates with different superscripts are significantly different ( $P < 0.05$ ).



**Fig. 1.**

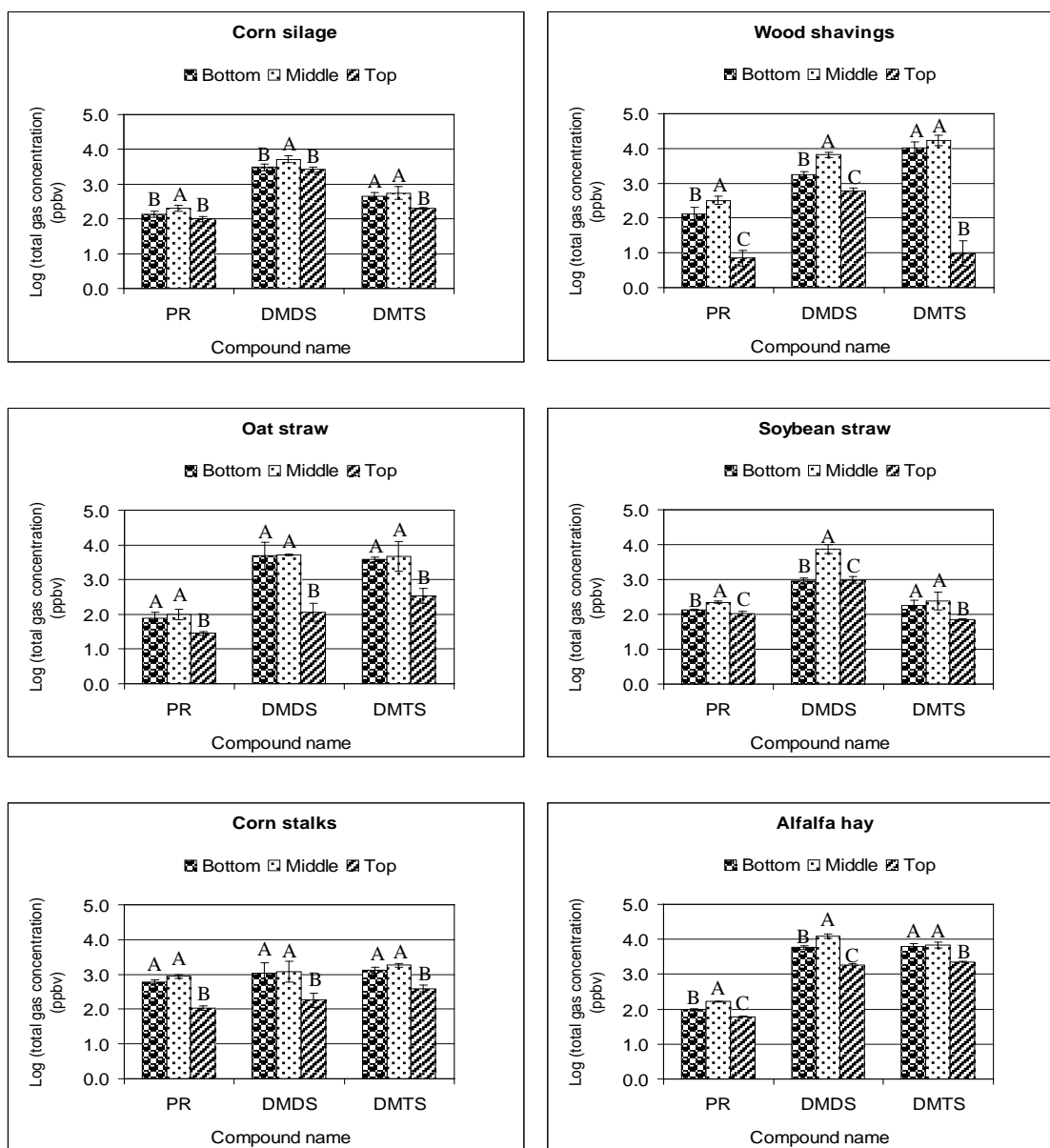


Fig. 2.

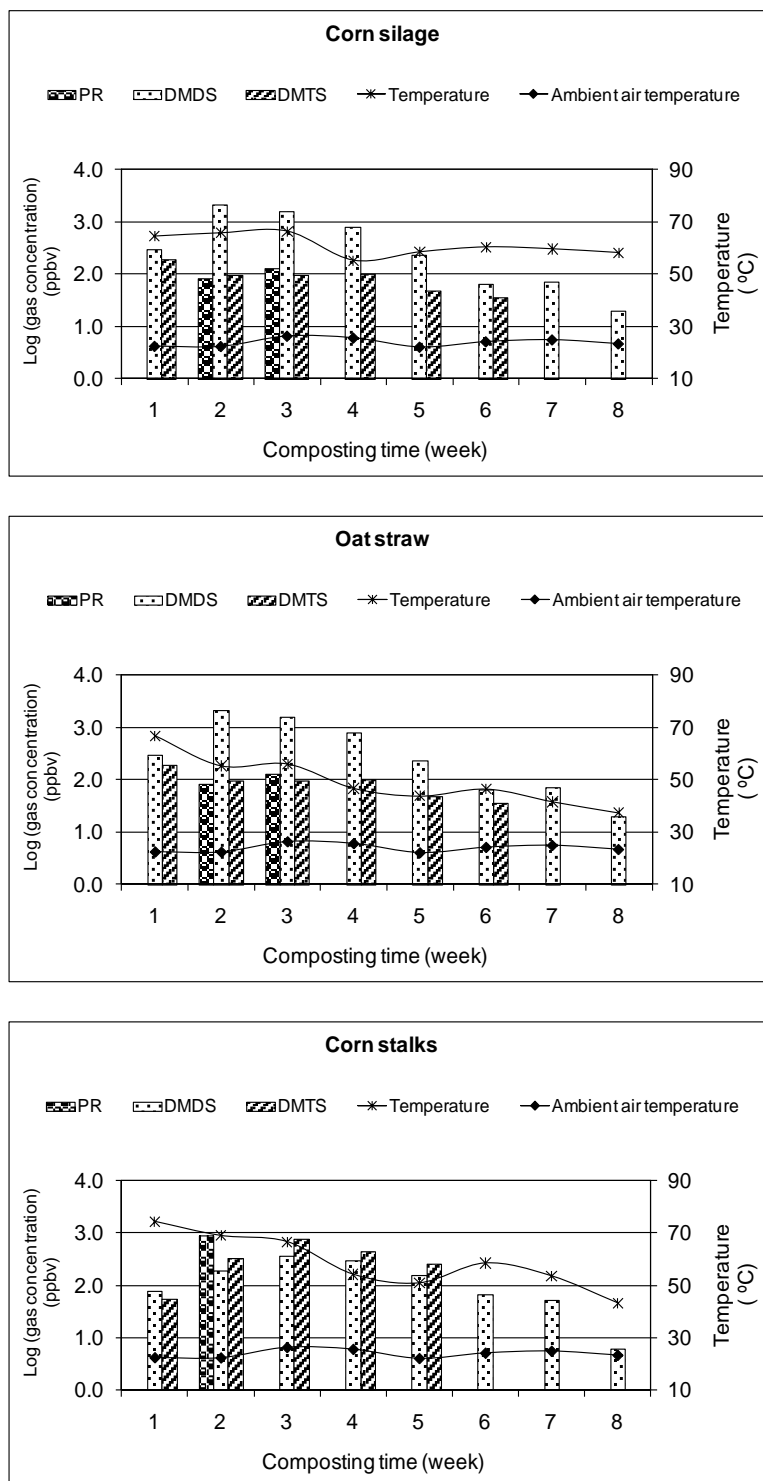


Fig. 3.

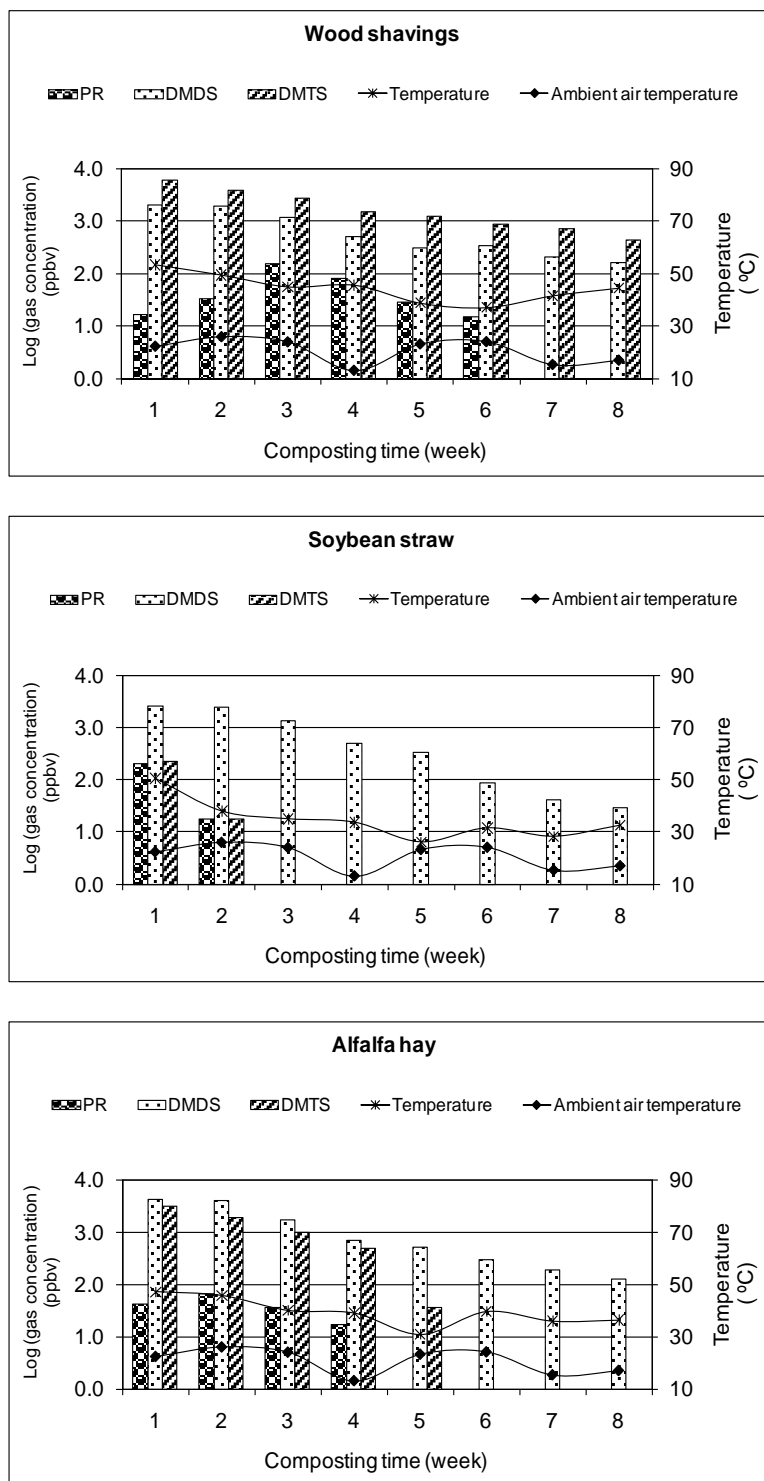


Fig. 4.