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Lab-scale evaluation of aerated burial concept for treatment and emergency disposal of infectious animal carcasses

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Abstract

Nearly 55,000 outbreaks of animal disease were reported to the World Animal Health Information Database between 2005 and 2016. To suppress the spread of disease, large numbers of animal mortalities often must be disposed of quickly and are frequently buried on the farm where they were raised. While this method of emergency disposal is fast and relatively inexpensive, it also can have undesirable and lasting impacts (slow decay, concerns about groundwater contamination, pathogens re-emergence, and odor). Following the 2010 foot-and-mouth disease outbreak, the Republic of Korea's National Institute of Animal Science funded research on selected burial alternatives or modifications believed to have potential to reduce undesirable impacts of burial. One such modification involves the injection of air into the liquid degradation products from the 60–70% water from decomposing carcasses in lined burial trenches. Prior to prototype development in the field, a laboratory-scale study of aerated decomposition (AeD) of poultry carcasses was conducted to quantify improvements in time of carcass decomposition, reduction of potential groundwater pollutants in the liquid products of decomposition (since trench liners may ultimately leak), and reduction of odorous VOCs emitted during decomposition. Headspace gases also were monitored to determine the potential for using gaseous biomarkers in the aerated burial trench exhaust stream to monitor completion of the decomposition. Results of the lab-scale experiments show that the mass of chicken carcasses was reduced by 95.0 ± 0.9% within 3 months at mesophilic temperatures (vs. negligible reduction via mesophilic anaerobic digestion typical of trench burial) with concomitant reduction of biochemical oxygen demand (BOD; 99%), volatile suspended solids (VSS; 99%), total suspended solids (TSS; 99%), and total ammonia nitrogen (TAN; 98%) in the liquid digestate. At week #7 BOD and TSS in digestate met the U.S. EPA standards for treated wastewater discharge to surface water. *Salmonella* and *Staphylococcus* were inactivated by the AeD process after week #1 and #3, respectively. Five gaseous biomarkers: pyrimidine; *p*-cresol; phenol; dimethyl disulfide; and dimethyl trisulfide; were identified and correlated with digestate quality. Phenol was the best predictor of TAN (R = 0.96), BOD (R = 0.92), and dissolved oxygen (DO) (R = −0.91). Phenol was also the best predictor populations of *Salmonella* (R = 0.95) and aerobes (R = 0.88). *P*-cresol was the best predictor for anaerobes (R = 0.88). The off-gas from AeD will require biofiltration or other odor control measures for a much shorter time than anaerobic decomposition. The lab-scale studies indicate that AeD burial has the potential to make burial a faster, safer, and more environmentally friendly method for emergency disposal and treatment of infectious animal carcasses and that this method should be further developed via prototype-scale field studies.

Keywords

Emergency disposal, Infectious animal carcasses, Aerated burial, Salmonella, MRSA, Odor mitigation

Disciplines

Animal Sciences | Bioresource and Agricultural Engineering | Environmental Health | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Lab-scale evaluation of aerated burial concept for treatment and emergency disposal of infectious animal carcasses

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Abstract

Nearly 55,000 outbreaks of animal disease were reported to the World Animal Health Information Database between 2005 and 2016. To suppress the spread of disease, large numbers of animal mortalities often must be disposed of quickly and are frequently buried on the farm where they were raised. While this method of emergency disposal is fast and relatively inexpensive, it also can have undesirable and lasting impacts (slow decay, concerns about groundwater contamination, pathogens re-emergence, and odor). Following the 2010 foot-and-mouth disease outbreak, the Republic of Korea's National Institute of Animal Science funded research on selected burial alternatives or modifications believed to have potential to reduce undesirable impacts of burial. One such modification involves the injection of air into the liquid degradation products from the 60-70% water from decomposing carcasses in lined burial trenches. Prior to prototype development in the field, a laboratory-scale study of aerated decomposition (AeD) of poultry carcasses was conducted to quantify improvements in time of

carcass decomposition, reduction of potential groundwater pollutants in the liquid products of decomposition (since trench liners may ultimately leak), and reduction of odorous VOCs emitted during decomposition. Headspace gases also were monitored to determine the potential for using gaseous biomarkers in the aerated burial trench exhaust stream to monitor completion of the decomposition. Results of the lab-scale experiments show that the mass of chicken carcasses was reduced by 95.0 ± 0.9% within 3 months at mesophilic temperatures (vs. negligible reduction via mesophilic anaerobic digestion typical of trench burial) with concomitant reduction of biochemical oxygen demand BOD (99%), volatile suspended solids VSS (99%), total suspended solids TSS (99%), and total ammonia nitrogen TAN (98%) in the liquid digestate. At week #7 BOD and TSS in digestate met the U.S. EPA standards for treated wastewater discharge to surface water. *Salmonella* and *Staphylococcus* were inactivated by the AeD process after week #1 and #3, respectively. Five gaseous biomarkers: pyrimidine; *p*-cresol; phenol; dimethyl disulfide; and dimethyl trisulfide; were identified and correlated with digestate quality. Phenol was the best predictor of total ammoniacal nitrogen, TAN (R = 0.96), BOD (R = 0.92), and dissolved oxygen (DO) (R = -0.91). Phenol was also the best predictor populations of *Salmonella* (R = 0.95) and aerobes (R = 0.88). *P*-cresol was the best predictor for anaerobes (R = 0.88). The off-gas from AeD will require biofiltration or other odor control measures for a much shorter time than anaerobic decomposition. The lab-scale studies indicate that AeD burial has the potential to make burial a faster, safer, and more environmentally friendly method for emergency disposal and treatment of infectious animal carcasses and that this method should be further developed via prototype-scale field studies.

Keywords: Emergency disposal; infectious animal carcasses; aerated burial; *Salmonella; MRSA;* odor mitigation

1. Introduction

1.1. Outbreaks of diseases and emergency animal mortality management – a global concern

Animal disease outbreaks and associated emergency mortality management issues are a global concern. The World Organization for Animal Health (OIE) Database (WAHIS Interface, 2017) shows that nearly 55,000 cases involving 116 diseases were reported during the period 2007-2016. Epidemiologically significant events between 2005 and 2016 are summarized in Fig. S1, Supplementary Material. The disease type, timing, location, and the number of cases were highly variable from year to year, but the overall trend of total cases is slowly rising, emphasizing the need for preparedness and effective emergency disposal methods. Nearly 70% of these cases were reported in Europe, followed by 15% in Asia, 11% in Africa, 5% in Americas, and 1% in Oceania. The Republic of Korea alone, reported as many as 941 cases constituting ~2% of world's total. In 2010 approximately 3.5 million animals were culled and buried at ~4,580 burial sites during a Foot-and-Mouth (FMD) outbreak in the Republic of Korea (Park et al., 2013). Managing emergency carcass disposal requires special care and urgency compared with routine daily non-disease-related disposal of relatively small numbers of carcasses via burial, incineration, composting, rendering, lactic acid fermentation, alkaline hydrolysis, or anaerobic digestion (AnD) (NADC, 2004).

1.2 Pros and cons of burial for animal mortalities disposal

Burial is the most common disposal method worldwide and is likely to remain that way (NABC, 2004). Regulatory & governmental agencies and the general public understand it and generally approve (NABC, 2004). Burial requires no specialized equipment. High-capacity excavators are available nearly everywhere. The risk of spreading disease via air is reduced because carcasses are not transported (assuming burial on the farm), and pathogens are quickly sequestered beneath a soil filter. However, because burials are often completed in haste, they can cause: chemical and microbial contamination in the groundwater around burial pits due to the poor site selection and improper construction (Kim and Kim, 2012); odor complaints (Kasper et al., 2012); declining property values; and disruption of long-term land use plans (Brglez and Hahn, 2008).

Burial is not simple if the necessary steps are taken to avoid the problems above. These steps are time-consuming (evaluating local geologic/hydrologic hazards, and/or trucking contaminated carcasses long distances to specially constructed landfills) yet they must be accomplished quickly when thousands of carcasses need to be managed. When done in haste, offensive odor release, slow carcass decomposition (angering farmers because land used for burial remains out of production much longer than anticipated), and widespread public concern over possible leakage of the burial sites and contamination of groundwater, can occur.

1.3. Need for improved in-trench burial approaches

While emergency disposal by burial is fast and relatively inexpensive, it also can have undesirable and lasting impacts. An example is the 2010 outbreak of foot-and-mouth disease (FMD) in the Republic of Korea in which nearly 3.5 million cattle and swine were buried on 4580 farms. While this solved the immediate bio-security concerns, it spawned a variety of

unanticipated problems. Carcass decay within anoxic burial trenches was slower than anticipated, angering farmers who were barred by the government from reusing valuable burial sites until carcasses were fully decomposed. In some instances buried carcasses were subsequently exhumed and disposed of via more rapid on-site composting. Surrounding communities also were impacted. Foul odors were emitted from some burial sites, and hasty burial, without regard for local geology, raised public fear of groundwater contamination.

Following the 2010 FMD outbreak, the National Institute of Animal Science (NIAS) of the Korean Rural Development Administration embarked on a comprehensive scientific evaluation of improved methods for emergency management of diseased carcasses. One method of interest was to use aeration in conjunction with in-trench burial. In this proposed method, carcasses would be buried in trenches lined with plastic or other impermeable sheeting and equipped with aeration tubing (perforated PVC or similar small diameter pipe, embedded in gravel to reduce impact during trench loading and to prevent plugging of perforations), creating a temporary underground aerated treatment vessel. This could possibly (*i*) speed carcass decay (thus, reducing the time land is taken out of production), (*ii*) reduce odor emissions (by oxidizing odorous volatile organic compounds (VOCs) into less offensive compounds), (*iii*) inactivate pathogens, and (*iv*) produce a treated liquid digestate that is less likely to pollute groundwater, in the event that the plastic trench liner fails over time. This method is analogous to the plastic-wrapped swine mortality composting procedure tested by Glanville et al. (2016) that was designed to reduce emissions of odor, leachate, and pathogens while creating a gas flow regime that could be monitored (for biomarker VOCs) to track process progress. Because on-farm burial is one of most commonly-used disposal methods used worldwide, the proposed aerated burial-AeD hybrid

concept could become a useful animal disposal alternative for adoption by animal health professionals, emergency responders, and policymakers.

1.4. Aerobic digestion as a part of 'aerated burial hybrid' emergency disposal technology

Aerobic carcass degradation carried out in small above-ground vessels was reported as a novel technology that provides an effective option for storing and pretreating of animal (sheep) carcasses prior to final disposal in the UK (Williams et al., 2009). It was shown that high numbers of bacteria *Salmonella enterica* (serotypes Senftenberg and Poona), *Enterococcus faecalis*, *Campylobacter jejuni* and *coli*, and *Escherichia coli* O157) in sheep carcasses were inactivated in the digestate within 3 months of AeD (Gwyther et al., 2012). Combining aeration with burial raises questions that have not been researched. These include: (*i*) acceleration of whole carcass decomposition during treatment; (*ii*) adherence of the treated digestate to U.S. EPA effluent guidelines (in case in-trench liner ruptures, the digestate needs to be pumped out, or the site needs to be decommissioned); (*iii*) minimally-invasive and biosecure means for process monitoring; and (*iv*) ability to inactivate pathogens.

1.5. Gaseous biomarkers of process completion

VOCs can be used as biomarkers of process completion in potentially infectious environments. Previous research has shown that selected VOCs such as pyrimidine, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) can be detected and quantified in a complex matrix of gases produced by decaying swine mortalities in a biosecure composting process (Akdeniz et al., 2009; 2010a; 2010b). The presence of these gasses was successfully used to biosecurely monitor process completion without the need to dig into the compost pile. It is

hypothesized that the same VOCs could be used to monitor degradation of poultry carcasses, but this has yet to be verified.

1.6. Study objectives

The primary objective of this research was to conduct a preliminary laboratory-scale study of the effectiveness of laboratory-scale treatment and biosecure disposal of whole infectious carcasses in the context of in-trench burial-AeD hybrid approach. The key questions to answer were:

(1) *Carcass decomposition*: What % of carcass decomposition is observed in 13 weeks of AeD? Is this faster than anaerobic carcass digestion typical within conventional burial sites?

(2) *Digestate quality*: Does aerated carcass decomposition significantly reduce pollutants (BOD, TSS, VSS, TAN) in burial trench leachate that threaten the quality of shallow groundwater or hydrologically connected streams? Does the aerated carcass digestate meet U.S. EPA effluent guidelines (for TSS and BOD)?

(3) *Gaseous process biomarkers*: Which of the VOCs in the effluent aeration stream are indicators of completion of carcass degradation? Are they the same biomarkers of process completion identified in earlier swine carcass composting research? Does aeration reduce odor impact potential?

(4) *Improved process monitoring*: How do the VOC data correlate with traditional digestate biological, chemical and physical treatment parameters (TSS, VSS, BOD, DO, TAN and pH)?

(5) *Managing the risk of pathogen re-emergence*: Does the AeD process reliably inactivate common pathogens such as Salmonella and Staphylococcus or will post-digestion disinfection be needed?

If answers to the above show AeD has potential to improve traditional burial practices, follow-up field studies are envisioned to identify and address implementation and maintenance challenges.

2. Materials and Methods

Digestion of whole poultry carcasses was selected for this work due to (1) relevancy to continued large outbreaks of infectious diseases affecting poultry, (2) animal size suited for lab-scale study, and (3) availability of poultry carcasses from other ongoing university studies.

2.1. Preliminary trials using anaerobic digestion for carcass disposal

Important lessons learned during a 22-week preliminary trial of AnD of poultry carcass led to the AeD study reported here. The AnD experiments (summarized in accompanying data article) simulated the environment found within conventional burial trenches, sometimes with a topical application of quicklime as it is required in the Republic of Korea. Three 6 L reactors were used for the (*i*) whole (with quicklime added), (*ii*) quarter (1/4 portion of whole chicken) and (*iii*) coarse cut (~2. 5 cm wide cuts of whole chicken) carcass digestion with added (1.2 – 1.5 L) water at 35 °C. There was no visible breakdown of carcass material, i.e., at the end of 22-week study carcass (or carcass quarters and coarse cuts) still resembled the initial material. The assessment of digestate quality showed that neither pH, ammonia, nor volatile fatty acids were in the optimal range recommended for AnD. Reflecting on these operating parameters can help explaining poor process performance. Free ammonia (generally considered bactericidal)

dominates that pH > pKa, which was the case in the reactor with added quicklime. Measured ammonia concentrations were well above (bacterial) inhibitory concentrations. There was no measurable bulk biogas (CO_2, CH_4) production. VOCs identified in the reactors headspace were offensive odorants many of which increased in concentration concomitant with treatment. It was concluded that successful emergency carcass disposal using AnD would probably require implementation of physical and chemical feed conditioning and operating practices (grinding of carcasses, the addition of carbon source to achieve favorable C:N ratios, pH control, and gas harvesting) typical of industrial AnD operations. Such measures are complicated, costly, time-consuming and unlikely to be practical during emergencies associated with livestock or poultry mass mortality. Failure to achieve practical endpoints with the anaerobic digestion set the stage for experiments with AeD in a burial-AeD hybrid context.

2.2. Aerobic reactor design and experimental conditions

Four lab-scale aerobic reactors were constructed using a 7.6 L glass container (desiccation jar, 27 cm height × 23 cm wide) with a sealed lid (Anchor Hocking Company, Lancaster, OH) (Fig. 1, Fig. S2-S3). These (replicate) reactors were heated and maintained at 34 ±1 °C (digestate temperature) by a warm-water jacket to expedite the digestion. Before entering the aeration system, the fresh pumped air was passed through a 0.2 μm air filter and then through a VOC filter to remove airborne bacteria and to minimize background VOCs. The filtered air was then distributed to the reactors through Teflon (PTFE) tubes equipped with individual flow controllers. A ring-shaped aeration tube was placed at the bottom of each reactor to provide homogeneous air flow inside the reactor. Each reactor was continuously aerated at a fixed flow rate of 3 L/min. The airflow rate was determined in a preliminary trial to reach DO levels in the

reactor that are above the recommended 1 to 2 mg/L for aerobic digesters (Tchobanoglous et al., 2003). Higher DO levels were desired considering that the whole chickens are being treated (not a fairly uniform input of a sludge or slurry). Due to the variable release rate of body fluids and associated organics produced by anaerobic decay inside the carcass, this fixed aeration rate was not expected to sustain uniform or optimal O_2 concentrations. Periods of zero or low O_2 concentrations were anticipated but this is also likely to be the case for crude aeration systems used in emergency burial situations.

<Figure 1>

Fig. 1. Aerobic digestion 7.6 L reactor for treatment of whole poultry carcasses.

Three holes were drilled through the glass lid. The first hole (~25 mm) located in the center of the lid was designed for safe gas and digestate sampling as well as temperature and dissolved O_2 (DO) monitoring. The second hole (~3 mm diameter) located on the side of the lid and plugged by a Thermogreen septum was used for gas (VOCs) sampling. The third hole (~3 mm) was for air supply tubing. To prevent microbial and VOC contamination, these reactors were placed in a NuAire Class II, type B2 biological safety cabinet (NuAire, Plymouth, MN). An initial volume of sterile distilled water (6 L) and one inoculated poultry carcass were added to each reactor. To meet study time constraints a relatively high water-to-carcass ratio (~10:1) was used to facilitate submergence of the carcass and initiate decay of its outer surface. A similar water-to-carcass ratio was used in a study of AeD of sheep carcasses in a high-density polyester/fiberglass vessel by Williams et al. (2009). During emergencies when a large mass of mortalities is placed in a burial trench, less water may be needed as greater amounts of liquid will be contributed by the carcasses which are 60-70% water by weight. If results of this

laboratory-scale research are encouraging, field-scale studies will be necessary to explore optimal: water-to-carcass ratios; aeration rates; trench liner materials and installation options; etc. Removal of carcass leachate from the trench and secondary treatment to lower the risk of pathogen reemergence as described by Koziel et al. (2017b) could also be considered. All analytical results were reported as means of n=4 reactors.

2.3. Carcass decomposition

The % of carcass decomposition was measured to assess the effectiveness of AeD of whole carcasses during treatment. The percent of the mass loss during AeD was determined based on the mass of carcass recorded at the start and the end of the experiment (post 13-week AeD). White Leghorn chicken carcasses (8 weeks old, 585 ± 10 g weights) were provided by the Poultry Research Farm of Iowa State University (ISU, Ames, IA) (Fig. S4). Poultry carcasses were stored in a freezer at -20 °C and were moved to a cold room (at 4 °C) for 24 h for defrosting before the bacterial inoculation process. Gastrointestinal swabs were conducted before inoculation. The purpose of this test was to detect the presence of selected *Salmonella* and *Staphylococcus* organisms in the digestion systems of the sampled poultry carcasses. A sterile cotton swab (BBL CultureSwab, Becton, Dickinson, and Company, Franklin Lakes, NJ) was inserted approximately 2 cm into the lower gastrointestinal tract of each poultry carcass. The gastrointestinal swabs were transported in an ice cooler to the Veterinary Diagnostic Lab (ISU, Ames, IA) for analysis. A 2.5 cm incision was made in the middle of the abdomen of each chicken carcass before pathogen inoculation to facilitate pathogen release into the water in each digester. This incision only cut the skin and the meat and did not pierce the gastrointestinal system.

2.4. Digestate quality

Digestate quality was assessed to determine if the digestate (specifically TSS and BOD) meet U.S. EPA effluent guidelines for a secondary treatment currently set at 45 mg/L each for weekly average concentration. This is important to assess because burial trench liners may eventually rupture and contaminate groundwater. Pump-out and discharge of digestate may also be needed to either remediate or reuse the disposal site. Also, monitoring ancillary parameters (DO; volatile suspended solids, VSS; total ammoniacal nitrogen, TAN; pH) was useful to assess process completion and overall AeD process effectiveness. A 200-mL digestate sample was collected weekly from each reactor. Values of pH (Accumet AB15 pH meter, Thermo Fisher Scientific, Waltham, MA), and DO (YSI DO200 DO meter, YSI, Yellow Springs, OH) were measured immediately after digestate sample collection. Samples were kept cold using dry ice and then immediately transported to the Environmental Engineering Research Lab (ISU) for analysis of BOD_5 (5-d test) using Standard Methods 5210A and 5210B (APHA, 2005). Analyses of TSS, VSS, and TAN were conducted at Water Quality Research Lab (ISU) using Standard Methods 2540A, D, and E, and 4500-NH_3-N, respectively (APHA, 2005). Digestate temperatures were measured directly in liquid using K-type thermocouples and thermometer model HH501DK (Omega Engineering, Stanford, CT).

2.5. Gaseous process biomarkers

Off-gas from each reactor was monitored to answer key questions regarding the most common and unique VOCs in the off-gas of AeD, their temporal patterns, and their similarity to biomarkers found in earlier swine carcass composting research. Headspace gas samples were collected to identify potential biomarker VOCs. Gas samples were collected weekly from

headspace gas of the four aerobic reactors (n = 4 replicates) using time-weighted averaging and solid-phase microextraction (TWA-SPME) with 0.5 cm retracted fiber (85 µm Carboxen/polydimethylsiloxane, Supelco, Bellefonte, PA), with 30 min sampling time (Fig. S3). All gas samples collected from the gaseous effluent of reactors were analyzed using a gas chromatography - mass spectrometry (GC-MS) system consisting of 6890N GC and 5973 MS (Agilent Technologies, Santa Clara, CA). Method detection limits of pyrimidine, DMDS, DMTS, phenol and *p*-cresol were 1.50, 0.70, 1.50, 8.44, and 5.76 ppbv, respectively. Interfering VOCs were identified in the preliminary trial and were eliminated from analyzed gasses. These included 2-aziridinylethyl amine, 1-butanol, benzaldehyde, methoxy-phenyl oxime, and octaethylene glycol which were consistently found as emitted by the aeration system and the reactors filled with distilled water. Three of these (2-aziridinylethyl amine, 1-butanol, and benzaldehyde) are used widely in the plastic industry (Dikshith, 2010; Patnaik, 2007). Details of TWA-SPME method development, optimized gas sampling conditions, gas sample analysis, and benchmarking of newly developed methods to sorbent tube-based VOCs sampling are discussed elsewhere (Koziel et al., 2017a).

2.6. Improved process monitoring

Gaseous biomarkers were also monitored to determine if they could be used to monitor process completion in a minimally invasive and biosecure manner (an important concern when dealing with infectious carcasses). This was done to answer specific questions how do the VOC data compare with traditional digestate parameters (TSS, VSS, BOD, DO, TAN, and pH). Statistical analyses were completed to determine if gaseous biomarkers could be correlated with physicochemical parameters for liquid digestate. Data were analyzed using statistical analysis

software SigmaPlot v11.0.0.77 (Systat Software Inc., San Jose, CA). Data were subjected to one-way analysis of variance (ANOVA). Treatment means at different time points were compared using either Tukey's honest significant test at 95% confidence level ($p \leq 0.05$ and n = 4) if normal or Wilcoxon signed-rank test if non-normal. The relationships between physicochemical, biological parameters and VOCs emissions were determined using Pearson's product moment correlation. All microbiological data were $\log_{10}$ transformed to improve normality.

2.7. Managing the risk of pathogen re-emergence

2.7.1. Marker strains of common pathogens

Two model pathogens were used to address key questions whether the AeD process reliably inactivates common pathogens such as Salmonella and Staphylococcus. This was done to further inform practical concerns about potential pathogen re-emergence and a need of a post-digestion disinfection and to inform if other pathogens are likely to have similar inactivation characteristics. Salmonella and Staphylococcus were selected as representative pathogens that have the capacity to survive in a wide variety of different foods and environments. They are representative of a broad category of foodborne pathogens related to infections of man and animals (Persoons et al., 2009). They are two common organisms that are often present both externally (surface of the body) and intestinally (gastrointestinal system) in poultry (Bolder, 1997).

Marker strains were used to improve the capability to quantify their survival by using selective growth media that suppresses non-study-related bacteria. Nalidixic acid-resistant ST4232 (Gram-negative bacteria) were obtained from the U.S Dept. of Agriculture, Agricultural

Research Service (USDA-ARS, Ames, IA), and MRSA43300 (Gram-positive bacteria) were obtained from the Veterinary Diagnostic and Production Animal Medicine (VDPAM), ISU. Each selected bacterial species was separately grown overnight on different agar plates. To make Salmonella and Staphylococcus suspensions, 3 to 5 colonies of each selected bacterium were mixed thoroughly in separated glass tubes containing 5 mL of phosphate buffered saline (PBS) solution. McFarland standard No. 0.5 was used as a reference to adjust the turbidity of bacterial suspensions. Standard plate counts were also conducted to determine the cell count of each bacterial suspension, which was approximately 1×10^8 CFU/mL. Each poultry carcass was initially (week #0) inoculated with 5 mL of Salmonella suspension on the right side of its abdomen and 5 mL of Staphylococcus suspension on the left side of its abdomen by sterile syringes. Resulting initial tracer pathogen concentrations were 1.85×10^9 CFU/carcass (ST4232) and 8.75×10^8 CFU/carcass (MSRA) (Koziel et al., 2017b).

2.7.2. Microbiological analyses

To determine the extent of pathogen inactivation caused by AeD, digestate samples (5 mL) were collected weekly from each aerobic reactor using a 10 mL sterile pipette and analyzed on the same day of sampling. Samples were homogenized by an analog vortex mixer (Thermo Fisher Scientific, Waltham, MA). Each eluate was subjected to 7 serial 10-fold (100 µL in 900 µL) dilutions in PBS solution. Three selected media were chosen for enumeration: (1) XLT4-nalidixic acid agar (prepared and provided by VDPAM, ISU, Ames, IA) for ST4232; (2) chrome agar (Bio-Rad Laboratories, Hercules, CA) for MRSA43300; and (3) blood agar (Trypticase soy agar with 5% sheep blood) (Remel, Lenexa, KS) for aerobes and anaerobes. The concentration of each inoculum was obtained by spreading dilutions (100 µL) on selected media and then

inoculating at 35 °C for 48 h. Total colony counts were enumerated using Q-Count automated colony counter (Spiral Biotech Advanced Instruments Inc., Norwood, MA). The matrix–assisted laser desorption-ionization time-of-flight mass spectrometry MALDI-TOF MS Biotyper System (Bruker Corporation, Fremont, CA) was used for identification and confirmation of the two selected bacteria. In cases where ST4232 and MRSA43300 were not detected by enumeration, enrichment culture technique was used to confirm the absence of these bacteria. Enrichment for ST4232 was conducted as described in ISO standard 6579:2002 (E) (ISO, 2002) and enrichment for MRSA43300 was conducted as described in Frana et al. (2013).

3. Results and Discussion

3.1. Carcass decomposition

Carcass decomposition using AeD was much faster and more complete than AnD where no visible carcass breakdown was observed during the 22-week preliminary trial. After 3 months of AeD, the mean mass of poultry carcass was reduced by 95.0 ± 0.9%. Poultry carcasses in aerobic reactors started to visibly break down within less than a week. After four weeks of the trial, there was no visual evidence of an intact poultry carcass in any reactor (Fig. 2). At the end of the trial (after digestate removal), the only solid materials remaining were partially decomposed feather tissue and small fragments of bones (Fig. S5). These findings are generally in agreement with significant carcass reduction reported in previous studies of aerobic bio-reduction of sheep (Williams et al., 2009). These findings suggest that in-trench AeD may significantly reduce the time that land used for burial sites is diverted from other productive uses.

<Figure 2>

Fig. 2. Progression of poultry carcass decomposition with aerobic digestion time, i.e., from whole poultry carcass floating in water (Day 0) to the brown suspension of undistinguishable digested tissues on Day 85.

3.2. Digestate quality

Significant reductions in peak TSS (99%), VSS (99%), BOD (99%) and TAN (98%) were measured (Table 1). The TSS and BOD met the U.S.EPA discharge limits of 45 mg/L (weekly average for both parameters) for treated wastewater discharge from a secondary treatment system (USEPA, 1987). Peak TSS and VSS in weeks #1to #3 was supported by visual observations (Fig. 2) and digestate samples (Fig. S6). The carcass broke down (spilling internal organs) at ~ week #3 which coincided with increased opacity in the digestate. Peaking of TSS and VSS was 'mirrored' by nearly opposite trends in DO which started at 6.0 ± 0.1 mg/L, fell to a minimum during weeks #1 to #4 (between 0.2 to 0.4 mg/L) and then slowly recovered to 5.5 ± 0.04 mg/L at week #13. Utilization of available DO was consistent with the BOD (7,250 ± 1888 mg/L) in week #1, and with subsequent peaking of VSS and TSS levels, concomitant with the dissolution of the carcass. As the digestate became more transparent, (Fig. S6), the BOD declined to 7 ± 2 in week #13. TAN levels decreased by 98% from 609 ± 77 to 8 ± 1 mg N/L. pH levels varied from initial 6.9 to as high as 8.7 in week #7, resulting in a negligible fraction of bactericidal ammonia throughout most of the study.

<Table 1>

Table 1. Physicochemical characteristics of digestate during aerobic digestion of pathogen-inoculated poultry carcasses.

High concentrations of BOD, VSS, TSS, and nutrients (TAN) in the 1st four weeks of the trial were expected and consistent with other poultry carcass disposal studies (Brglez and Hahn,

2008) and general trends associated with use of AeD for treatment of meat processing and household wastewater (Buendía et al., 2008; Ichinari et al., 2008).

Increased microbial activity associated with excessive nutrient loading (due to decomposition of the poultry carcass) requires greater amounts of O_2 to maintain the aerobic conditions in the reactors (Tchobanoglous et al., 2003). In this study, the aeration system was set to provide a constant air flow rate of 3 L/min (due to constraints for VOC emissions measurement), causing a temporary decrease in DO concentrations in the first 4 weeks. This is consistent with observations that high concentrations of BOD can reduce the DO in digestate to levels insufficient to maintain the aerobic environment (Tchobanoglous et al., 2003). Thus, a fixed DO level (variable aeration rate based on DO feedback) AeD treatment may be a desirable option for further acceleration of the decay process.

The results showed the digestate with high TAN concentration. This is in agreement with previous AeD work in high N loads associated with sheep carcass (Gwyther et al., 2012; Williams et al., 2009). Carcasses contain significant amounts of organic N that can be hydrolyzed to ammonia under aerobic conditions, and which then can be further oxidized to nitrite (by nitrosomonas) and then nitrate (by nitrobacters) (Anthonisen et al., 1976). High-throughput nucleotide sequence analysis can provide insight in bacterial community successions for buried animal carcasses (Yang et al., 2015).

3.3. Managing the risk of pathogen re-emergence

No MRSA43300 were detected from any digestate samples from all inoculated reactors. *Salmonella* ST4232 was only detected in the first three weeks of the trial. Total viable counts of

ST4232 decreased by 2-log values from the original starting concentration within the first 3 weeks and was not detected after week #3 (Fig. 3).

<Figure 3>

Fig. 3. Survival and growth of *Salmonella* (ST4232) and *Staphylococcus* (MRSA43300), aerobes, and anaerobes in digestate during aerobic digestion of poultry carcass. Presented data are means of measured bacteria concentrations in 4 aerobic reactors ± SD (marked as error bars).

Rapid disappearance of lab-grade MRSA43300 could be explained by microbial competition and predation reducing the population of pathogens within aerobic reactors (Williams et al., 2009). In the AeD process, the microorganisms are being starved and may utilize metabolic by-products and other bacteria (Sincero and Sincero, 1996). Studies have found that the reduction of pathogens in the AeD process is promoted by competition from naturally occurring microorganisms (Ceustermans et al., 2007; Pietronave et al., 2004) which were also found and measured in this study (Fig. 3).

The digestate samples of aerobic reactors were found to have naturally occurring populations of aerobes and anaerobes that are consistent with poultry carcass disposal (Brglez and Hahn, 2008). A significant ($p < 0.05$) increase in numbers of anaerobes within the first three weeks of the trial was observed in all aerobic reactors, possibly due to their facultative nature. In the first four weeks of AeD, naturally occurring aerobes and anaerobes were detected at levels of 10^6 to 10^8 CFU m/L. The aerobes consumed oxygen rapidly, causing the depletion of O_2 in the digestate. Previous research shows that even if the initial O_2 concentration is adequate for aerobic metabolism, the exponential growth and efficient chemotaxis of bacteria can result in depletion of O_2 in the aerobic system (Taylor, 1983). On week #7, concentrations of naturally occurring aerobes and anaerobes were reduced by > ~2-log values, slowly declining until the end of the

trial (Fig. 3). Except for the peak of anaerobe activity at week #3, the concentration of naturally occurring aerobes was ~ 1 log-value > than that of anaerobes.

Naturally occurring aerobic bacteria can decompose organic materials to harmless or stable forms by mineralizing them to CO_2 and water (Liu et al., 2000). Although the poultry carcasses were not tested for the presence of naturally occurring bacteria before placing into the reactors, it is known that significant numbers of aerobes, anaerobes, and facultative bacteria can be found on the skin surface and in the respiratory tract of dressed and eviscerated poultry (Avens and Miller, 1970; Berrang et al., 2003). Under aerobic conditions, the bacteria can survive and grow rapidly (Tchobanoglous et al., 2003). Many of them are obligate aerobes that can grow under limited O_2 conditions. Some species such as *Enterococcus,* can produce antibacterial peptides called 'bacteriocins,' giving them a competitive advantage over other microorganisms (Fisher and Phillips, 2009; Nishie et al., 2012). Combined with the ability to survive in harsh environmental conditions (Fisher and Phillips, 2009), this may explain why both aerobes and anaerobes were detected at the end of the trial, although they had been reduced by > 3-logs. Of course, many of the bacteria are facultative and can thus operate in both types of environments.

Due to their high cost, virus detection tests were not in the scope of this study. Viruses need live cells for propagation, so viral survival should be minimized during AeD because, other than bacteria, there should be no living cells. However, the fate of Newcastle Disease, low pathogenic avian influenza (LPAI), and highly pathogenic avian influenza (HPAI) should be tested in future trials of AeD of poultry carcasses (Koopmans et al., 2004). Also, further work needs to be done for a better understanding of the nature of bioaerosols emitted from the AeD system.

3.4. Gaseous process biomarkers

3.4.1. Identification of gaseous biomarkers

Screening for VOCs present in off-gas during 13 weeks of AeD yielded 48 VOCs from nine organic chemical groups that are consistent with a signature composition for gases emitted from decaying organic matter (Table 2). Many of identified chemicals (and chemical groups) appear to have a distinct temporal pattern, e.g., appearing only in the initial and/or mid-phase of the 13 week-long AeD process. More importantly, only five VOCs (pyrimidine, DMDS, DMTS, phenol, and *p*-cresol) were found consistently throughout the 13 week trial in all reactors. Thus, these five compounds are considered to be biomarkers whose absence signals completion of poultry carcass decomposition.

<Table 2>

Table 2. VOCs emitted from poultry carcass aerobic digestion.

The absence of pyrimidine, DMDS, DMTS, phenol and *p*-cresol as biomarker compounds of decomposition of poultry carcasses is consistent with previous research of swine carcass decay during composting (Akdeniz et al., 2009; 2010a; 2010b), where pyrimidine, DMDS, and DMTS were identified as biomarkers. Pyrimidine is the principal N-containing heterocyclic compound known as one of the 'building blocks' for the formation of nucleic acids and proteins (Lagoja, 2005). The proteins break down into peptides, which are reduced to amino acids (Forbes, 2008; Vass, 2008). During the AeD process, proteins are broken down into amino acids. Studies reported that in many bacterial, animal, and plant processes, pyrimidine degradation occurs via either reductive or oxidative pathways (Piškur et al., 2007; Rawls, 2006). Pyrimidine is likely to be degraded to pyrimidine bases via their nucleotides to the end products of NH_3 and CO_2 (Moffatt and Ashihara, 2002). Sulfur-containing VOCs such as mercaptans and dimethyl sulfide

are one of the products of decomposition of organic matter by rumen microorganisms (Keenan et al., 1967) and bacteria (Lo Cantore et al., 2015). Certain aerobic bacteria could break down S-containing amino acids cysteine and methionine, which are intermediate products of microbial protein degradation, into methyl mercaptan, part of which was oxidized to DMDS (Segal and Starkey, 1969). These S-containing VOCs escape from the digestion system, resulting in a decrease in the organic S proportional to the amount of methionine decomposition (Segal and Starkey, 1969).

3.4.2. Temporal emission rates of biomarker VOCs

Temporal patterns of biomarker VOC emission rates were consistent with high initial microbial activity and generally similar to the pattern of emissions reported by Akdeniz et al. (2009, 2010a, 2010b) for decaying swine tissues and carcasses during composting. The VOC emission rates were highest during the initial 4 weeks when populations of aerobes and anaerobes both exceeded 10^8 CFU/mL. Measured emission rates (i.e., a product of measured concentrations and aeration rate) of the five biomarker VOCs from aerobic reactors of inoculated poultry carcasses are shown in Fig. 4. These VOCs were found in the headspace of the reactors as early as on the 4th day of the AeD process. The highest emission rates of DMDS, DMTS, pyrimidine, and phenol were measured in the 1st and 2nd week. The emission rates were 3.65×10^{-2}, 3.32×10^{-3}, 1.46×10^{-4}, and 1.50×10^{-3} µg/kg/h (i.e., VOC mass/initial carcass mass/time) for pyrimidine, DMDS, DMTS, and phenol, respectively. The highest emission rate of *p*-cresol was measured on week #3 and was 1.25×10^{-3} µg/kg/h. After week #3 (coinciding with high microbial activity), emission rates of biomarker VOCs significantly decreased (*p*

<0.05). In the final week of AeD process, the emission rates were 3.16×10^{-8}, 4.81×10^{-5}, 2.78×10^{-8}, 8.50×10^{-6}, and 1.47×10^{-6} µg/kg/h for pyrimidine, DMDS, DMTS, phenol, and *p*-cresol, respectively. Relatively low emissions late in the process were still quantifiable due to the use of a more sensitive sampling and sample preparation technology useful for measurement of very low VOC concentrations (Koziel et al., 2017a).

<Figure 4>

Fig. 4. Measured emissions rates of 5 target volatile organic compounds (VOCs) at standard conditions (T = 25 °C, P = 1 atm, RH = 0%). Presented data are means of VOC emissions (µg VOC/kg carcass/h) ± SD (n = 4 reactors).

3.4.3. Odor mitigation

Inspection of observed (Table 2) temporal patterns reveals a significant reduction in the presence of several highly odorous VOCs (e.g., *p*-cresol, indole, trimethylamine, methyl mercaptan, DMDS, DMTS, butyric acid) during the first 2 months of AeD. The practical odor implications of these reductions depend not only on a chemical's concentration in the air but also on how these concentrations are perceived by the human nose. This differs from chemical to chemical and is characterized in part by a chemical's odor detection threshold (ODT) which is the lowest concentration of the chemical that is detectable by the 'average' human nose (Devos et al., 1990). Significant reduction of odor observed during the course of AeD is depicted in Fig. 5 which shows temporal trends in odor activity value (OAV) which is the ratio of a measured VOC concentration to its odor detection threshold, ODT (Parker et al., 2012). Pyrimidine (5th biomarker) is not included because it does not have a published ODT. Table 2 and Fig. 5 show that three important odorants are reduced below their ODT by AeD in 5 to 9 weeks. Preliminary studies of AnD treatment (similar to the decay process in unaerated burial trenches) showed the

same chemicals persisting at 22 weeks at concentrations greater than measured at 7 to 8 weeks. Scrubbing of the effluent gas stream from a burial-AeD hybrid system with a passive biofilter could aid in minimizing the impact of emergency carcass disposal on local air quality during the 5 to 9 weeks when OAVs are likely to exceed 1.0 as shown in Fig. 5. Additional gas stream treatment for bioaerosol (if warranted) is also a feasible option.

<Figure 5>

Fig. 5. Odor activity values vs. time of four key odorants released during aerobic digestion of poultry carcasses.

3.5 Improved process monitoring

Minimally invasive and biosecure method utilizing gaseous effluent for process monitoring may be an attractive alternative to the invasive and inherently risky collection, handling and analysis of potentially infectious digestate. Observed correlations between physicochemical, biological parameters and VOCs emissions during AeD are summarized in Table 3 (Fig. S7). Phenol, *p*-cresol, and DMTS appear to be of greatest potential utility for minimally-invasive process monitoring, Phenol was the best predictor of TAN (R = 0.96), BOD (R = 0.92), and DO (R = -0.91). Second best predictor for BOD (R = 0.89) was DMDS. Phenol was also the best predictor for *Salmonella* (R = 0.95) and aerobes (R = 0.88). The second best predictor for *Salmonella* (R = 0.91) was DMTS. *P-C*resol was the best predictor for anaerobes (R = 0.88). Thus, minimally invasive and biosecure gas sampling should be further researched at full-scale as a viable alternative to traditional digestate analyses.

The physicochemical parameters that best correlated with the survival of *Salmonella* (ST4232) were BOD (R = 0.96) and TAN (R = 0.88). The naturally occurring aerobe abundance displayed a negative correlation with DO (R = -0.98) and a positive correlation with TAN (R =

0.95). The results also show that the population of naturally occurring anaerobes was correlated with TSS (R = 0.89) and VSS (R = 0.89), followed by DO (R = -0.88).

<Table 3>

Table 3. The matrix of Pearson correlation coefficients between physicochemical, biological parameters and VOCs emissions during aerobic digestion (n=52). Numbers across the top of the table correspond to chemicals associated with the same number in left-hand column.

Results shown in Table 3 and Fig. S7 are based on the constant airflow to reactors. Research is warranted on testing the effects of varied airflow delivery for maintaining optimized DO and for improved performance of AeD including the physical, chemical and biological processes described here, the energy savings (or costs), and the scaling up to in-trench pilot field trials.

4. Conclusions

In conclusion, answers to questions 1-5 posed in the study objectives are as follows.

1. Carcass digestion: With aeration, carcass decomposition of 95% was achieved in 13 weeks. Under anaerobic conditions similar to those within a heavily loaded burial trench, no visible decomposition was observed in 22 weeks.

2) Digestate quality: During 3 months of aerated decomposition peak values of common pollutants ((biochemical oxygen demand, total suspended solids, volatile suspended solids, total ammoniacal nitrogen; BOD, TSS, VSS, TAN, respectively) in decomposing carcass leachate that threaten the quality of shallow groundwater and hydrologically connected streams were reduced by 99%, 99%, 99%, and 98%, respectively. The aerated digestate met U.S. Environmental Protection Agency effluent guidelines for TSS and BOD by week 7 of the study.

3) Gaseous process biomarkers: Of 48 volatile organic compounds (VOC) identified in the off-gas produced during 13 weeks of decomposition only pyrimidine, dimethyl sulfide

(DMDS), dimethyl trisulfide (DMTS), phenol, and *p*-cresol persisted throughout the trials and thus are considered as biomarkers whose absence indicates completion of decomposition. Three of these, pyrimidine, DMDS, and DMTS, also were identified as biomarkers of process completion in previous swine mortality composting research. Three highly odorous VOCs, DMDS, DMTS, and *p*-cresol, were present in the aerated decomposition off-gas at odor activity values (OAV) >1 for 5-9 weeks. The same odorous compounds, however, were present in high concentrations in the off-gas of anaerobically decomposed carcasses for at least 22 weeks. Neither process is odor free, but the off-gas from aerated decomposition will require biofiltration or other odor control measures for a much shorter time than anaerobic decomposition.

4) **Improved process monitoring:** Phenol in the off-gas was the best predictor of TAN (R = 0.96), BOD (R = 0.92), dissolved oxygen (DO) (R = -0.91), *Salmonella* (R = 0.95) and aerobes (R = 0.88). *P-C*resol was the best predictor for anaerobes (R = 0.88). Since collection, handling, and analysis of potentially infectious digestate poses biosecurity hazards, the minimally invasive and relatively biosecure technique of sampling the aeration gas stream should be further researched in the field to evaluate its viability as an alternative to messy, difficult, and biohazardous sampling of burial trench digestate.

5) Managing risk of pathogen re-emergence: Marker strains of *Salmonella* (ST4232) and *Staphylococcus* (MRSA43300) were quickly inactivated. No MRSA43300 were detected in any of the digestate samples. *Salmonella* ST4232, initially present in digestate at 10^5 CFU/mL were detected only during the first three weeks of decomposition. Naturally occurring populations of aerobes and anaerobes peaked at about 10^8 CFU/mL during the first 4 weeks, declining to around 10^6 CFU/mL at the end of the 13-week trials.

Further work is needed to explore the broad range of pathogens that may be significantly reduced by aerated digestion. Results of this preliminary study are believed sufficiently positive to justify field studies of trench liner and aeration equipment options, operation and maintenance issues, and costs associated with the aerated burial disposal concept.

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Table 1. Physicochemical characteristics of digestate during aerobic digestion of pathogen-inoculated poultry carcasses.

Digestion time (weeks)	pH	DO (mg/L)	BOD (mg/L)	VSS (mg/L)	TSS (mg/L)	TAN (mg N/L)
0	6.9 ± 0.1	**6.0 ± 0.1**	n/a	n/a	n/a	n/a
1	8.5 ± 0.1	0.2 ± 0.02	**7,250** ± 1888	293 ± 74	335 ± 68	609 ± 77
2	8.4 ± 0.2	0.3 ± 0.04	5,650 ± 1940	936 ± 19	993 ± 26	**672** ± 73
3	8.3 ± 0.1	0.3 ± 0.04	5,875 ± 206	**1,026** ± 62	**1,099** ± 25	544 ± 16
4	8.5 ± 0.1	0.4 ± 0.05	1,055 ± 168	737 ± 69	789 ± 70	413 ± 13
5	8.6 ± 0.1	3.6 ± 0.06	105 ± 13	408 ± 14	453 ± 32	221 ± 8
6	**8.7 ± 0.1**	3.6 ± 0.05	24 ± 5	123 ± 14	133 ± 21	118 ± 4
7	8.4 ± 0.1	4.3 ± 0.04	17 ± 2	28 ± 5	32 ± 5	39 ± 2
8	8.2 ± 0.1	5.0 ± 0.06	19 ± 2	29 ± 6	35 ± 3	34 ± 2
9	8.1 ± 0.1	5.1 ± 0.05	11 ± 4	14 ± 2	23 ± 3	26 ± 1
10	8.0 ± 0.1	5.2 ± 0.10	12 ± 4	15 ± 1	19 ± 4	17 ± 2
11	7.9 ± 0.1	5.3 ± 0.05	12 ± 1	11 ± 2	14 ± 1	17 ± 1
12	7.9 ± 0.1	5.4 ± 0.03	11 ± 1	10 ± 2	13 ± 2	13 ± 1
13	7.8 ± 0.1	5.5 ± 0.04	7 ± 2	8 ± 2	10 ± 2	8 ± 1

Note: data represent means ± SD (n = 4). BOD, VSS, TSS, and TAN were not available in week #0. **Bold** font highlights peak values. DO = dissolved oxygen, BOD = biochemical oxygen demand, VSS = volatile suspended solids, TSS = total suspended solids, TAN = total ammoniacal nitrogen.

Table 2. VOCs emitted from poultry carcass aerobic digestion.

Compound name	CAS No.	Week No.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Acids or volatile fatty acids														
Acetic acid	64-19-7	+						+	+					
Butyric acid	107-92-6	+	+	+	+	+	+	+	+					
Propionic acid	79-09-4	+												
α-Aminoxypropionic acid	56-41-7													+
Alcohols														
Ethanol	64-17-5						+							
Isobutanol	78-83-1						+							
1-Butanol	71-36-3	+					+							
1-Heptanol	111-70-6						+							
1-Hexanol	111-27-3						+							
1-Propanol	71-23-8						+							
3-Methyl-2-butanol	598-75-4		+											
1-Pentanol	71-41-0		+	+	+	+	+							
Aldehydes														
Acetaldehyde	75-07-0							+	+					
Benzaldehyde	100-52-7								+	+	+			
Hexanaldehyde	66-25-1			+	+				+					
Heptanaldehyde	111-71-7								+					
Nonanaldehyde	124-19-6								+					
Pentanaldehyde	110-62-3			+	+		+							
Alkanes														
Dodecane	112-40-3				+									
Heptane	142-82-5		+											
Octane	111-65-9		+	+			+							
Pentane	109-66-0				+		+				+			
Propane	74-98-6								+					
Tetradecane	629-59-4				+						+			
Aromatics														
Benzene	71-43-2								+					
Pyrrole	109-97-7		+											
Toluene	108-88-3		+						+					
Styrene	100-42-5		+											
p-Xylene	106-42-3								+					
Ketones														
Acetone	67-64-1		+			+			+					
2- Butanone	78-93-3		+											
2-Heptanone	110-43-0						+							
2-Pentanone	107-87-9		+											
3-Pentanone	96-22-0					+								
Nitrogen-containing compounds														
Butylamine	109-73-9								+					
Dimethylamine	124-40-3			+	+						+	+	+	

Ethylamine	75-04-7					+	+							
Ethylenediamine	107-15-3				+		+	+						
Formamide	75-12-7								+					
Trimethylamine	75-50-3				+	+	+	+	+	+				
Pyrimidine	**289-95-2**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Phenols & Indoles														
Phenol	**108-95-2**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
***p*-Cresol**	**106-44-5**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Indole	120-72-9	+	+	+	+	+	+	+	+					
Sulfur-containing compounds														
Methyl mercaptan	74-93-1	+	+	+	+	+								
Dimethyl disulfide	**624-92-0**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Dimethyl trisulfide	**3658-90-8**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Dimethyl sulfone	67-71-0													+

Note: (+) compounds detected in the VOC gas samples (in all n =4 reactors); Separated compounds were identified using mass spectral matches with ChemStation's NIST 11 Mass Spectral Library (2011) (probability-based matching ≥ 70%). For pyrimidine, DMDS (dimethyl disulfide), DMTS (dimethyl trisulfide), phenol and *p*-cresol, spectral matches and column retention times were compared with those of standard analytes. **Bold** font signifies biomarkers whose absence signals completion of poultry carcass decomposition.

Table 3. The matrix of Pearson correlation coefficients between physicochemical, biological parameters and VOCs emissions during aerobic digestion (n=52). Numbers across the top of the table correspond to chemicals associated with the same number in left-hand column.

		1	2	3	**4**	**5**	**6**	7	8	9	10	11	12	13
1	DMDS	1												
2	Pyrimidine	0.96**	1											
3	**DMTS**	0.94**	0.93**	1										
4	**Phenol**	0.66**	0.71**	0.85**	1									
5	***p*-Cresol**			0.45**	0.72**	1								
6	VSS			0.38**	0.77**	0.83***	1							
7	TSS			0.39**	0.77**	0.83***	0.99**	1						
8	DO	-0.55**	-0.67**	-0.71**	**-0.91****	-0.72***	-0.87**	-0.87**	1					
9	BOD	0.69**	0.74**	**0.89****	**0.92****	0.76***	0.67**	0.67**	-0.82***	1				
10	TAN	0.59**	0.69**	0.78**	**0.96****	0.74***	0.86**	0.87**	-0.96***	0.91**	1			
11	*Salmonella*	0.74**	0.75**	**0.91****	**0.95****	0.71***	0.61**	0.62**	-0.79***	0.96**	0.88***	1		
12	Aerobes	0.52**	0.67**	0.68**	**0.88****	0.69***	0.86**	0.87**	-0.98***	0.79**	0.95***	0.75***	1	
13	Anaerobes	0.33*	0.49**	0.53**	0.79**	**0.88*****	0.89**	0.89**	-0.88***	0.75**	0.85***	0.71***	0.89***	1

Note: *p<0.05; **p<0.01; ***p<0.001 (two-tailed). **Bold** font highlights correlations useful for minimally invasive AeD process monitoring via gas monitoring. AeD = aerobic digestion, VOCs = volatile organic compounds.