Final Report
Odor and pathogen management in solids from poultry processing plants

Prepared for:
Alabama Department of Environmental Management

Prepared by:
Edward T. Drabold, Postdoctoral Fellow, Biosystems Engineering, Auburn University
Brendan T. Higgins, Professor, Biosystems Engineering, Auburn University

Date:
September 2, 2026

Executive Summary

The objective of this study was to characterize the chemistry and odor profile of dissolved air flotation (DAF) solids and develop amendment strategies to mitigate odors. A secondary objective was to assess the pathogens present in DAF solids. A variety of analytical techniques, microbiological methods, and mesocosm studies were carried out. The odor profile of fresh and aged poultry DAF differed with the former containing more sulfur organics and the latter containing more volatile fatty acids (VFAs). Sulfur organics like dimethyl disulfide (DMDS) are associated with the smell of rotting meat whereas VFAs give off a fecal odor. Seven out of eight DAF solids samples tested positive for *Salmonella* with enrichment and contained up to 5.6 log10 CFU/g of *E. coli*. However, all *E. coli* and *Salmonella* died out after fermentation for 2 weeks in an anaerobic environment (similar to frac tank storage). Mesocosm trials were carried out to assess odorous emissions after application of DAF solids to soil. A simulated rain event occurred 24 hours after DAF solids application to dry soil to understand how DAF behaves in dry and wet conditions. Five chemical amendments were tested for their effects on DAF solids odor: acidification with sulfuric acid (to pH 2), alkalinization with potassium hydroxide (to pH 12), oxidation with hydrogen peroxide, agricultural liming at both 3% and 10% dry weight mixtures, and quick liming to a pH of 12. Quick lime was tested in two different ways: mixed with the DAF before application or surface application of quick lime after surface application of DAF solids. Alkalinization is one of the acceptable approaches to treating DAF solids in ADEM's current regulations. The best alkalinization technique, pre-mixed quick liming, fully reduced VFA emissions but did not reduce DMDS compared to the control. This was better than agricultural lime which only delayed emissions or potassium hydroxide (KOH) and surface applied quick lime which increased DMDS emission. Subsurface application was performed in shallow (0.75 inch) and conventional (2.75 inch) depths to assess odor control from soil cover. Shallow subsurface application was found to be ineffective whereas conventional subsurface application removed 100% of VFA emissions and reduced DMDS emissions, although the latter was not statistically significant. Tilling DAF solids to fully incorporate them into soil increased odorous emissions but a 'Cut and inject' method performed in the mesocosms significantly reduced both VFA (by 100%) and DMDS (by 70%) emissions. Surface-applied DAF solids with a top dressing of organic amendments were also tested. Specifically, application of grass clippings, pine straw, and biochar were tested and compared to control mesocosms with no amendment. Grass clippings and pine straw suppressed VFA release by 91% and 98%, respectively during the first 24 hours after application and outperformed the control for most of the 320 hour test period. Biochar had no meaningful effect on VFA emissions but suppressed DMDS emissions by 59%. Grass and pine straw also suppressed DMDS emissions but to a lesser extent at 26% and 46%, respectively, during the first 24 hours compared to the control. However, after the rain event, the grass clippings remained saturated with water and resulted in a large spike in DMDS emissions that did not occur in the other mesocosms. Therefore, we concluded that pine straw was the most effective and consistent amendment. It was later proven, in a continuously hydrated mesocosm with surface-applied DAF, that the re-emergence of DMDS was due to grass trapping in moisture and producing an anaerobic microenvironment. This suggests that organic amendment structure (e.g., bulk density) impacts odor control. These results provide a strong foundation from research in a controlled laboratory environment to inform promising odor control practices for outdoor field conditions. Specifically, it is shown that deep subsurface or 'Cut and inject' techniques were able to significantly reduce odor.

Organic amendments like pine straw were also effective. Liming could reduce VFAs and of these, quick lime proved most effective. Nevertheless, all techniques still struggle to fully control putrid sulfurous emissions.

1. Introduction

1.1 Background:

Land application of waste solids from poultry processing and rendering plants has emerged as a critical issue over the last few years in the southeastern US. Solids from DAF systems are being applied to land in the vicinity of growing population centers, resulting in complaints about putrid odors and concerns about pathogens (Fig. 1). DAF solids are considered food processing residuals (FPR) by the state which indicates they may include pathogens. In the words of a local landowner, the odor caused by these DAF solids is significantly more offensive than odors typically associated with litter application: "*We have chicken litter put on our own property, that's not a problem. This is entirely different. And if you live in the country and your used to those smells you can tell the difference*" (Neal, 2019). This issue has caught the attention of local politicians and also state regulatory agencies. In recent years, the Alabama Department of Environmental Management (ADEM) has begun levying fines to applicators who are spraying poultry processing DAF solids on fields (Pillion, 2021). Since then, ADEM has drafted byproduct regulations based on the EPA 503c regulations pertaining to land application of biosolids. Most of these biosolids are sludge from wastewater treatment plants and it is uncertain how effective they are for poultry DAF solids. The two specific practices embedded within ADEM regulations are 1) subsurface application (Figure 1) and 2) material alkalinization to pH 12 prior to surface application.

Figure 1. Subsurface application of DAF solids on pasture (photos courtesy of ADEM).

Continued disciplinary action and the enactment of state and local laws and regulations could effectively end the practice of land application, dramatically raising the cost of disposal of this material for the poultry industry. This would be unfortunate because this material is rich in nutrients that could be beneficial to agriculture. The timeframe is urgent to resolve this issue. Additionally, alternative valorization methods, such as rendering of DAF solids are challenging and done in a limited way which for the foreseeable future cannot replace land application (Drabold et al., 2025).

This project directly addresses this issue by developing amendment and stabilization strategies that applicators could implement to reduce odor and pathogen concerns (Figure 2). These strategies have the potential to eliminate odor or bring it down to a level that nearby landowners find acceptable. Successful completion of the project objectives will provide the industry with options that enable continued land application of solids, thereby reducing the need to switch to more costly treatment and disposal approaches.

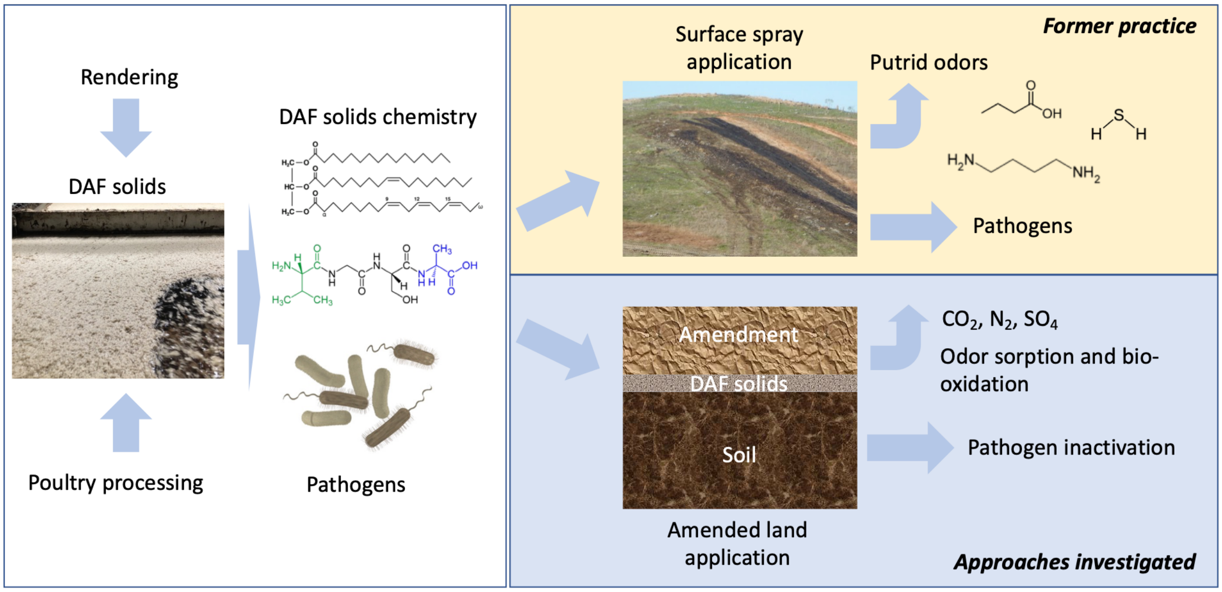

Figure 2. Scheme of former and new approaches to land application of DAF solids. Subsurface application strategies were also investigated as part of this work.

While there is substantial research published on the topic of manure and poultry litter application to land as reviewed by Risse et al. (2006), there is almost no information published on land application of DAF solids from poultry processing. There are of course articles published on using DAF solids captured from manure prior to land application, including amending such material with lime (Porterfield et al., 2021). However, manure DAF solids are expected to be chemically very different from processing plant DAF solids. There are also articles on how to improve operation of DAF units for better solids capture and conversion into feed ingredients (Lee, 2003). Given the lack of relevant literature on strategies associated with controlling odor upon land application of DAF solids, literature was sought on managing odor problems in other industries.

Odor control in waste treatment systems typically fall into three major categories (Shareefdeen, 2005): chemical scrubbing, biofilters/bioscrubbers (Fan et al., 2020), and adsorbers. The latter typically includes activated carbon or similar substrates that can adsorb volatile organic compounds (VOCs). In many biofilter applications, a substrate is used to both sorb odor molecules and also provide a surface for the colonization and growth of microorganisms (Shareefdeen, 2005). These microorganisms can metabolize VOCs and other odor-causing molecules, eliminating the need to regenerate the adsorbent. Furthermore, addition of hydrogen peroxide to biofilters can stimulate the production of the peroxidase enzyme that cleaves the

carbon-carbon bonds of more recalcitrant odor-causing volatile molecules (e.g. those found in petroleum) (Fan et al., 2020).
As previously mentioned, biochar can act as an adsorbent for odorous volatile compounds (Hwang et al., 2018). It can also serve as a scaffold for colonization of beneficial microorganisms (Gul et al., 2015; J.E. Thies et al., 2015; Wang et al., 2020), potentially transforming it into a bioscrubbing technology. Similarly, organic substrates like woodchips have been used extensively as a support for bioscrubbers in composting facilities (Chen et al., 2012; Goldstein, 2007). Finally, application of wheat straw on top of manure storage facilities has also been used to reduce odor (May, 2011). However, it is not clear how effective any of the above strategies are with regard to controlling odor during land application of poultry DAF solids.

1.2 Goal and Objectives:

The goal of this project is to develop amendment and application strategies that significantly lower the levels of nuisance odors and pathogens during soil application of dissolved air flotation (DAF) solids. Effective strategies will allow for continued land application of solid wastes from poultry processing and rendering plants at a low cost to the industry. Our specific objectives are as follows:

1. Characterize the initial chemical composition of DAF waste solids across time and facility
2. Test individual amendment and stabilization strategies for odor and pathogen control of waste solids upon soil application
3. Test combined amendment and stabilization strategies for pathogen control of waste solids upon soil application

2. Materials and Methods

2.1. Collection and aging of DAF solids

DAF solids were collected from poultry and food processing facilities throughout the state of Alabama (Table 1). ADEM staff did most of the collection and retrieved 250 ml samples of slurry in plastic screw cap bottles. These were immediately placed on ice and transported to Auburn University within 8 hours of collection. Collected samples were immediately aliquoted into smaller tubes and frozen at -20 C to preserve odorous molecules.

Table 1. Samples collected for odor analysis.

Sample #	Date	Where	Generator Type	Fresh	Treated
1	6/21/23	LA Storage	Fresh Plant	No	No
2	6/21/23	LA Storage	Fresh Plant	No	No
3	6/21/23	LA Storage	Fresh Plant	No	No
4	6/21/23	LA Storage	Fresh Plant	No	No
5	6/21/23	LA Storage	Fresh Plant	No	No
6	6/21/23	LA Storage	Fresh Plant	No	No
7	6/21/23	LA Storage	Fresh Plant	No	No

8	6/21/23	LA Storage	Fresh Plant	No	No
9	6/21/23	LA Storage	Fresh Plant	No	No
10	6/21/23	LA Storage	Fresh Plant	No	No
11	6/21/23	LA Storage	Fresh Plant	No	No
12	6/21/23	LA Storage	Fresh Plant	No	No
13	6/21/23	DAF	Fresh Plant	Yes	No
14	6/21/23	DAF	Fresh Plant	Yes	No
15	6/21/23	DAF	Fresh Plant	Yes	No
16	6/21/23	DAF	Fresh Plant	Yes	No
17	6/29/23	DAF	Fresh Plant	Yes	No
18	6/29/23	DAF	Fresh Plant	Yes	No
19	6/29/23	DAF	Cook Plant	Yes	No
20	6/29/23	LA Spreader	Fresh Plant	Yes	No
21	6/29/23	LA Spreader	Cook Plant	Yes	No
22	6/29/23	LA Storage	Post Product	No	Yes
23	6/29/23	Fac Storage	Post Product	Yes	No
24	6/29/23	Fac Storage	Post Product	Yes	Yes
25	6/29/23	Fac Storage	Post Product	Yes	Yes
26	8/8/23	LA Storage	Post Product	No	No
27	8/8/23	LA Storage	Post Product	No	No
28	8/8/23	LA	Post Product	No	No
29	8/8/23	LA Spreader	Fresh Plant	Yes	No
30	8/8/23	DAF	Post Product	Yes	No
31	8/8/23	DAF	Post Product	Yes	No
32	8/8/23	DAF Post Press	Post Product	Yes	No
33	8/8/23	DAF Post Press	Post Product	Yes	No
34	8/8/23	Fac Storage	Post Product	No	No
35	8/8/23	Fac Storage	Post Product	No	No
36	8/8/23	Fac Storage	Post Product	No	No

In addition to the above samplings fresh DAF samples were also collected from 8 poultry processing facilities for analysis of poultry pathogens. Samples were collected and transported back to Auburn University on ice for immediate processing.

Finally, a larger volume (5 gallons) of fresh DAF solids were collected from a single poultry processor for the mesocosm studies. Collections occurred on 10/19/23, 6/14/24 and 2/15/25 in 5 gallon buckets with sealing lids. These were placed in a large cooler for transport to Auburn University. For the first collection, the material was sealed in a covered bucket and allowed to sit at room temperature (~25 C) for at least one month to putrefy. For the second batch, the material was sealed in a bucket and allowed to putrefy for 2 weeks, then subdivided in half. One half was stored at -20 C and the other half remained in the original bucket to continue putrefying for >1 month at room temperature. This was done to test the effect of short-term and long term aging of DAF material in future mesocosm experiments. This new line of inquiry resulted from initial findings on the project.

2.2. Characterization of DAF solids

2.2.1 Pathogen identification

Microbial and pathogen analysis was carried out as described previously (Arthur et al., 2024). DAF samples were serially diluted in 9 g/L sterile saline water and 1 mL of selected dilution was plated in duplicate on respective petrifilms (3M, USA) for counts of general indicators: aerobic plate counts (APC), *Escherichia coli* (*E. coli*) and total coliforms (TC). For *Salmonella* enumeration, 0.1 mL of selected dilutions were plated in duplicate on XLT4 agar. Water samples without counts (presumptive negative) were enriched by adding 25 mL of water sample to 25 mL 2X buffered peptone water (ThermoFisher) and incubated for 24 h at 37°C. The pre-enriched samples were inoculated into Rappaport Vassiliadis (Hardy Diagnostics, USA) broth, and Tetrathionate broth supplemented with iodine solution (Hardy Diagnostics, USA), incubated for 24 h at 42 °C and plated in duplicate on XLT4. Presumptive *Salmonella* isolates were confirmed with the 3M Molecular Detection System (MDS). Rapid plate agglutination test was performed on the *Salmonella* isolates by adding one drop (~20 µL) of *Salmonella* O antiserum (Hardy Diagnostics, USA) onto a clean glass slide, mixing with a loopful of isolated colonies and observing the results within one minute. For *Campylobacter* detection, 25 mL of water was added to 25 mL 2X Bolton broth and incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) for 48 h at 42 °C (36). The resultant enrichment was plated in duplicate on Campy Cefex agar (Neogen, Michigan, USA) and incubated under microaerobic conditions for 48 h at 42 °C.

2.2.2 Elemental analysis

Elemental composition of DAF was performed by an external analytical lab at the University of Georgia, Athens, GA, USA. An Elementar vario MAX total combustion analyzer (Elementar, Ronkonkoma, NY, USA) was used for carbon and nitrogen content. Approximately 0.1 g of sample was loaded on a steel crucible in an oxygen atmosphere at 1200°C. The representative oxidized gases were passed through infrared and thermal conductivity cells for carbon and nitrogen.

2.2.3 Protein analysis

Soluble protein analysis of DAF solids was performed with a Thermo Scientific Pierce BCA Protein Assay Kit (Waltham, MA, USA). A BSA standard was prepared within the proper calibration range for the samples. 25 µL were aliquoted in triplicate for each individual sample and standards in a microplate. Then 200 µL aliquots were added to each microplate cell and the microplate was placed in a microplate heater at 37°C for 30 minutes. Afterwards the microplate was cooled to room temperature and read at absorbance 562 nm on a Molecular Devices

SpectraMax M2 microplate spectrophotometer (San Jose, CA, USA). The crude protein content was estimated by multiplying the elemental nitrogen composition by 6.25.

2.2.4 Lipid analysis

A standard Folch extraction was employed to determine the crude fat content of DAF solids. 20mg of freeze-dried material was bead beat in 1.5mL of Folch solvent. A mesh was employed to separate the beads from the liquid phase and washed three times with Folch solvent. Then centrifugation at 4000rpm was undertaken to separate the hydrophobic and hydrophilic phases. The hydrophobic phase was extracted and 1mL was pipetted and evaporated on an aluminum weigh boat to back-calculate the overall fat content. For neutral lipid analysis, 30µL of the same hydrophobic phase was transferred into a polypropylene microplate. 6 corn oil standards were pipetted (2.5µL in 2x increase to 40µL and 60µL) in the same manner. Solvent was evaporated at 90°C. 100µL of sulfuric acid was added to each well and incubated at 90°C for 20 minutes. The microplate was allowed to cool, and background was read on a Molecular Devices SpectraMax M2 microplate spectrophotometer (San Jose, CA, USA) at 540nm. 50µL of vanillin-phosphoric reagent was added to each well and mixed 5x. Color was allowed to develop for 10 minutes then absorbance was tested at 540nm to quantify neutral lipids.

2.3. Analysis of volatile odorous molecules by headspace gas chromatography with mass spectrometry

Analysis of odorous compounds was performed on an Agilent 8890 GC with an Agilent 5977C MS and an 8697 Agilent headspace sampler (Santa Clara, CA, USA). 100mg of SKC Tenax GR sorbent (Eighty-Four, PA, USA) was placed on plastic weighing boats within mesocosms for one hour and covered (see 2.4). After this time the mesocosms were uncovered and the sorbent was poured into Agilent headspace vials and crimped with Agilent aluminum headspace caps. Standards of dimethyl disulfide and butyric acid were prepared under three 6x dilutions of ethanol (6x, 36x and 216x) then 5µL of each solution was pipetted onto aluminum weight boats for individual mesocosms each with only soil. The same Tenax procedure was then applied as for the experimental mesocosms. The crimped headspace vials were then transported to the GCMS-HS for analysis. The

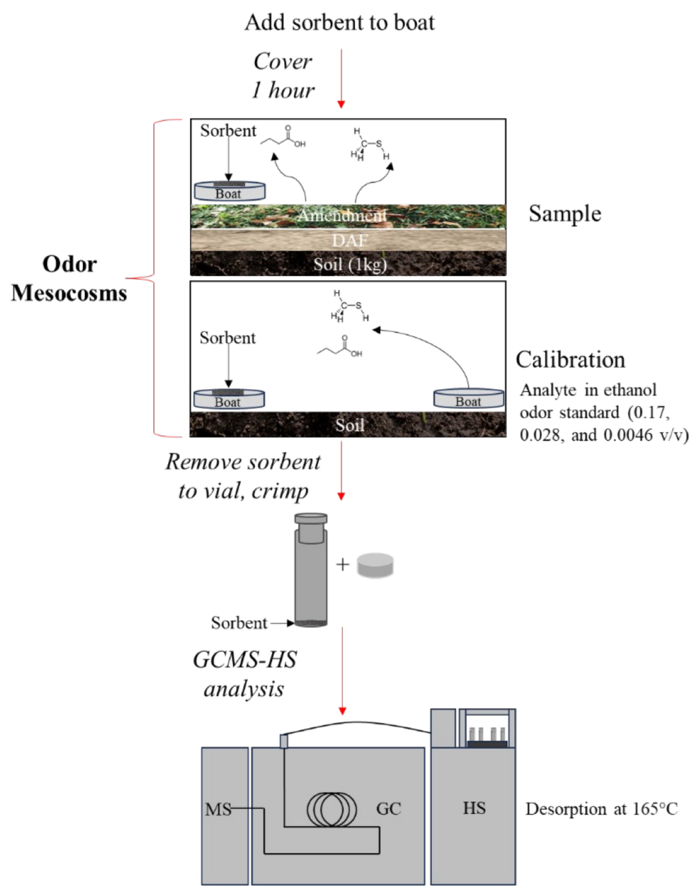

Figure 3. Sampling procedure for odor mesocosms.

headspace oven was set at 165°C and the vial equilibration time was 10 minutes. The GC cycle was set at an initial value of 80°C with a ramp of 10°C/min to 195°C where it held for 10 minutes. For the DAF solids reported in Table 1, 5mL of individual samples were transferred to headspace vials and crimped. The exact same GC method was run except the HS oven was set to 40°C. Figure 3. displays the sampling procedure.

2.4. Mesocosm studies of application strategies

2.4.1. Experimental design:

Three mesocosm experiments were carried out to test different application and amendment strategies for poultry DAF solids. Eight different application strategies were tested in total. These strategies can be broadly characterized into 3 groups: organic amendments (grass clippings, pine straw and biochar), chemical amendments (hydrogen peroxide, sulfuric acid and potassium hydroxide) and subsurface application (0.75 inch and 2.75 inch depths). An experiment was also performed to test the impacts of dry soil versus constant hydration in the mesocosms. Each condition was compared to control mesocosms with DAF solids applied to the soil surface. Three independent replicates of each mesocosm condition was carried out.

2.4.2. Materials:

For the organic amendments, grass clippings were collected from a local Auburn, Alabama lawn, pine straw was purchased at Home Depot and bulk biochar purchased from Waste to Energy Inc. (Slocomb, Alabama, USA). Hydrogen peroxide was a standard 3% solution whereas sulfuric acid and potassium hydroxide were both pure. Soil was collected from E.V. Smith Research Center adjacent to a corn field (Table 2).

Table 2. Characterization of E.V. Smith soil used

Parameters	Soil
pH	4.8 +/- 0.06
Moisture content (%)	2.1 +/- 0.5
Base Saturation (%)	29.8
Lime buffering capacity (ppm $CaCO_3$/pH)	197
Organic matter (%)	1.31 +/- 0.1
Cation exchange capacity (meq/100g soil)	3.35
Texture (%, clay:silt:sand)	9.8:16.3:73.9 +/- 0.1:0.5:2
Soluble salts (mmhos/cm)	0.19 +/- 0.05
Soil type	Sandy Loam

2.4.3. Mesocosm setup:

Mesocosms were composed of Sterilite Gasket Box 7.5 Quart storage bins with latching lids (Fitchburg, MA, USA). 1kg of dry soil was evenly spread across the bottom of mesocosms. Following this, 100g of DAF solids were evenly distributed on top of the soil. The soil and DAF solids alone were considered the control for the experiments. For the organic amendment trial, 10g of biochar or 50g each of grass clippings or pine straw was evenly spread on top of the DAF solids. This resulted in a 3-5 cm depth of organic material above the DAF in the case of grass clippings and pine straw. For the chemical trials, the 3% hydrogen peroxide was diluted with DAF solids to a 1% solution (2:1) and for sulfuric acid and potassium hydroxide the solids were titrated until they reached a pH of 2 and 12, respectively. Agricultural lime experiments were performed in a different batch from the other chemical treatments. This experiment also included a normal control (DAF) and two conditions of 3% and 10% dry weight agricultural lime mixed with DAF. For quick lime experiments, DAF solids were titrated with quick lime to a pH of 12. These quick limed DAF solids were then surface applied in the same manner as the control. Additionally, in the quick lime experiments another treatment was conducted where the same mass of quick lime was surface applied on top of surface applied DAF solids. For the subsurface trials, 1kg of soil was placed in the mesocosms then 100g of DAF solids were evenly spread on top. Another 1 kg of soil (0.75 inch depth) was placed on top of this and a score to the depth of the DAF layer was gently carved through the middle to replicate subsurface injection in the field. Another treatment without the score was also tested. An additional separate subsurface experiment was performed in the same manner as the first except that 3.5 kg (2.75 inch) was used to cover the DAF layer instead. Other techniques that use soil to adsorb odor were also tested: such as tillage into the soil and 'Cut and inject'. These mesocosms had 2.5 kg of soil. In the tilled condition, DAF solids were fully mixed into the top 2 kg of soil. For the 'cut and inject' method, a score line was made into the soil to the 2 kg mark and DAF solids were dropped into this incision. Mesocosms were sampled (section 2.3) at hourly intervals of 1, 24, 30, 48, 72, 144, 196, 240 and 312 hours or until there was no change in odor profile of any mesocosm for at least two samplings. An artificial 'rain event' occurred immediately after sampling at 24 hours. This rain event involved pouring 300mL of water from a graduated cylinder into a colander which uniformly 'rained' across the mesocosms. This volume of water was pre-determined in advance to reach the saturation point of this soil. Lastly, an experiment was conducted to better understand the impact of soil moisture on odor release. Here, DAF solids were surface applied to dry soil, to dry soil with a rain event at 24 hours (as done in previous trials), and to soil that was continuously hydrated from a pan filled with water under the mesocosms.

3. Results and Discussion

3.1. Fresh DAF composition

The first batch of fresh DAF collected in the 5 gallon buckets for mesocosm studies was thoroughly characterized (Table 3). The material is mostly fat followed by protein.

Table 3. Composition of fresh DAF collected for mesocosm studies

Analysis	**Quantification**
Protein content (n=3)	Soluble nitrogen: 2.7% +/- 0.6% Soluble protein: 11% +/- 4.7% Crude protein: 28.3% +/- 0.47%
Total solids content (n=3)	24% +/- 1%
Crude lipid (fat) content (n=3)	80% +/- 15% (crude extract in chloroform)
Neutral lipid (n=3)	57% +/- 4%
pH (n=1)	6.55
Chemical Oxygen Demand (n=3)	1.3 +/- 0.35 mg O_2/mg dry DAF
Elemental composition (CHNS) (n=3)	C [%], H [%], N [%], S [%] avg: 67.7, 9.2, 4.5, 0.5 stdev: 0.4, 0.2, 0.1, 0.1

n values refer to technical replicates for measurements on the same collected batch at the processing plant.

3.2. Odor profile in DAF solids from varying sources

Three major classes of odor molecules were observed in the DAF samples collected from different sites around the state of Alabama (Table 4).

Table 4. Classification of odorous molecules observed in DAF solids

Sulfur organics	**Volatile fatty acids**	**Small amines and amides**
Methanethiol	Propionic acid (-methyl)	2-amino propanediamide
Dimethyl sulfide	Butyric acid (-methyl)	Methanamine, N-methoxy-N-nitroso-
Dimethyl disulfide	Pentanoic acid (-methyl)	Methylamine, N,N-dimethyl-
Dimethyl trisulfide		
Carbon disulfide		

The 5 sulfur organic molecules observed are all part of the same family – they can interconvert amongst themselves upon oxidation or reduction reactions. For example, methanethiol readily converts to DMDS upon oxidation. These particular sulfur organics are associated with the smell of rotting meat, cabbage, and eggs. Methanethiol in particular is the result of the degradation of the amino acid methionine. It creates a very offensive odor to humans. Notably, DMDS is used a commercial soil fumigant, sold under the name "Paladin Technical." It is used prior to planting to kill weeds and soil pathogens; DMDS is intended to volatilize prior to planting.

VFAs are the result of additional breakdown of amino acids and longer chain fatty acids. They convey a pungent fecal odor and are very offensive to humans. The observed amines and amides are also a result of amino acid breakdown by microorganisms and convey a "fishy" odor that is also offensive. The relative abundance of these different classes of molecules is shown in Table 5 across the 36 samples collected by ADEM.

Table 5. Relative abundance of different classes of odor molecules in individual DAF samples

Sample #	Generator Type	Fresh	Treated	S organics abundance	VFAs abundance	Amine & amide abundance
1	Fresh Plant	No	No	0%	37%	7%
2	Fresh Plant	No	No	0%	28%	0%
3	Fresh Plant	No	No	1%	33%	3%
4	Fresh Plant	No	No	3%	28%	5%
5	Fresh Plant	No	No	40%	25%	0%
6	Fresh Plant	No	No	1%	0%	0%
7	Fresh Plant	No	No	1%	65%	0%
8	Fresh Plant	No	No	0%	0%	0%
9	Fresh Plant	No	No	0%	30%	21%
10	Fresh Plant	No	No	10%	8%	0%
11	Fresh Plant	No	No	38%	36%	5%
12	Fresh Plant	No	No	70%	4%	0%
13	Fresh Plant	Yes	No	37%	0%	9%
14	Fresh Plant	Yes	No	14%	0%	7%
15	Fresh Plant	Yes	No	50%	0%	3%
16	Fresh Plant	Yes	No	71%	0%	0%
17	Fresh Plant	Yes	No	0%	0%	0%
18	Fresh Plant	Yes	No	8%	0%	0%
19	Cook Plant	Yes	No	0%	63%	0%
20	Fresh Plant	Yes	No	71%	9%	0%
21	Cook Plant	Yes	No	0%	54%	0%
22	Post Product	No	Yes	0%	1%	0%
23	Post Product	Yes	No	28%	28%	0%
24	Post Product	Yes	Yes	3%	0%	0%
25	Post Product	Yes	Yes	0%	0%	0%
26	Post Product	No	No	7%	8%	0%
27	Post Product	No	No	13%	11%	3%
28	Post Product	No	No	6%	15%	0%
29	Fresh Plant	Yes	No	60%	10%	0%
30	Post Product	Yes	No	5%	0%	0%
31	Post Product	Yes	No	9%	0%	0%
32	Post Product	Yes	No	2%	0%	0%
33	Post Product	Yes	No	0%	0%	0%
34	Post Product	No	No	50%	12%	2%
35	Post Product	No	No	6%	12%	16%
36	Post Product	No	No	17%	16%	6%

Because there is a lot of variation in the data across the different sites and sampling dates, we used principal component analysis (PCA) to observe which site-specific characteristics contribute to clustering. In other words, do samples from a particular type of site, material age, or treatment system have a "characteristic" odor profile? Initial attempts to classify by collection date and collection source (e.g. frac tank, land application site, spreader) led to no clear pattern in the odor profile. Instead, the generator type, age of the material, and use of treatment led to clearer clustering in the data (Figure 4). Samples that had a low PC1 score, such as those from the cook plants, were associated with the fecal odors of VFAs. Samples that scored high on PC1, such as those from fresh kill plants, were associated with the rotting meat odor of the sulfur organics. Overall, aging of the material resulted in more VFAs and fecal order and a reduction in the sulfur organics. This is likely due to conversion of sulfur organics into VFAs by anaerobic microorganisms in the frac tanks. The samples with scores closest to zero on all PCs (least odor molecules) were the two samples that underwent treatment. This suggests that whatever treatment these plants are using was effective in reducing odors in the final material.

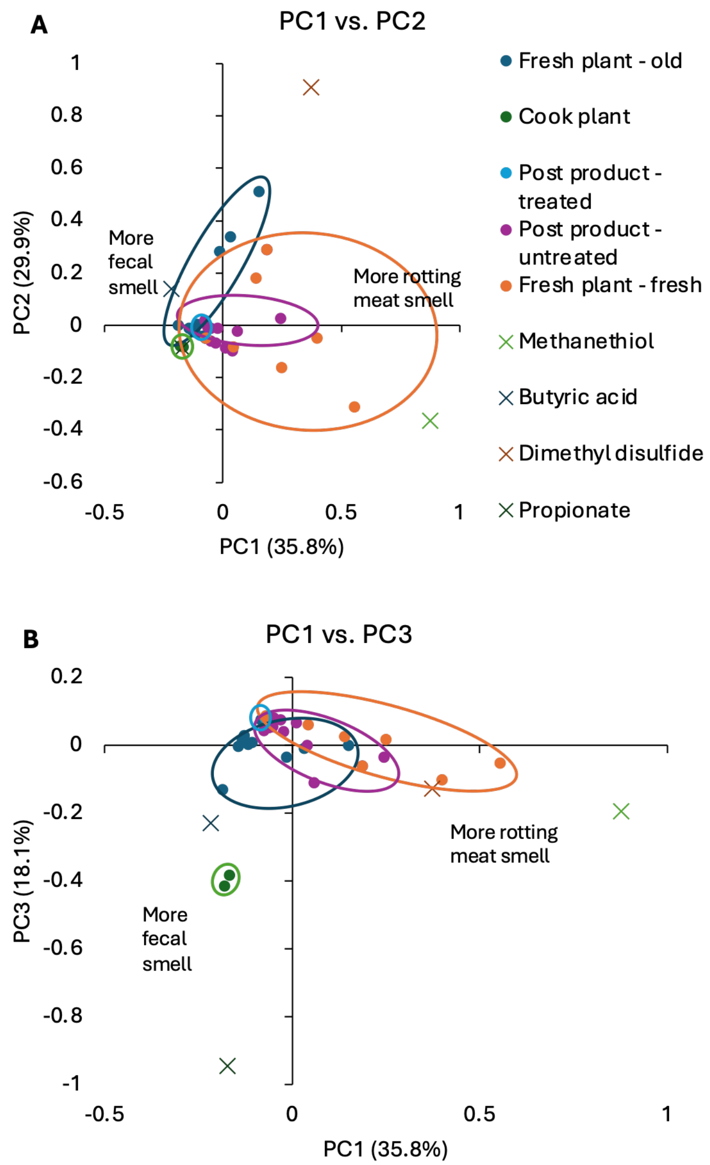

Figure 4. Principle component analysis plot of odor molecules detected in DAF solids samples from different facility and treatment options.

3.3. Pathogen levels in DAF solids

Eight samples each were collected of poultry processing wastewater and the separated DAF solids at processing plants in Alabama and Georgia. These samples underwent microbiological analysis (Table 6). The results showed that seven out of eight samples of DAF tested positive of

Salmonella after enrichment. The specific serotypes of Salmonella are shown in Table 7. *Campylobacter* was not detected with enrichment. *E. coli* levels were up to 5.6 log 10 CFU per gram of DAF solids. These results do raise concern about human pathogens present in freshly collected DAF solids.

Table 6: Summary of bacterial counts in PPW and DAF solids from 8 samples

	E. coli	Total coliforms	APC	*Salmonella*	*Campylobacter*
PPW (log 10 CFU/mL)	0 – 4.5	0.4 – 6.8	2.8 – 7.5	4/8	2/8
DAF (log 10 CFU/g)	0 – 5.6	3.7 – 6.9	2.5 – 8.6	7/8	ND

ND: not detected

Table 7: *Salmonella* serotypes detected in PPW and DAF solids

	Salmonella serotypes	
Plant	PPW	DAF
1	ND	Cerro, Idikan, Mbandaka, Uganda_var._15+.34+
2	Infantis, Kentucky, Enteritidis	Infantis
3	Newport, Rough_O:r:1,5	Tennessee, Kentucky
4	ND	ND
5	ND	Hadar, Kentucky
6	Kentucky, Hadar	Tennessee, Typhimurium
7	ND	Kentucky
8	Hadar	Infantis

ND: not detected

However, the DAF solids collected for the mesocosm studies only tested positive for *E. coli*, not *Salmonella* or *Campylobacter*. However, after 3 months of storage at room temperature and anerobic conditions (similar to a frac tank), all of the detectable *E. coli* had died out (Table 8). Total coliform abundance also declined while aerobic plate counts increased, likely due to the proliferation of bacteria involved in degradation of the DAF organics. This result is consistent with results we have observed in poultry processing wastewater. In batches of freshly collected wastewater that test positive for *Salmonella*, we have consistently seen *Salmonella* die out (become undetectable with enrichment) after just 24 hours of sitting in aerobic conditions at room temperature (Arthur et al., 2024).

Table 8: Microbial analysis of fresh DAF vs. aging for 3 months under anaerobic conditions. Values are log10 CFU/ g dry weight

	E. coli	Total Coliform	Aerobic Plate Count	*Salmonella*	*Campylobacter*
Fresh DAF	4.82 +/- 0.03	6.07 +/- 0.09	6.84 +/- 0.09	ND	ND
Aged DAF	ND	4.10 +/- 0.14	8.48 +/- 0.23	ND	ND

ND: not detected

3.4. Chemical amendments impact odor release in soil mesocosms

Three chemical amendments were tested with DAF solids prior to surface application on dry soil in mesocosms. Emissions were monitored and then a simulated rain event was conducted at 24 hours in which the soil was wetted to saturation. Emissions monitoring from the mesocosm continued for the next 172 hours (Figure 5). Included among these amendments were a strong base (KOH) to raise the pH of the DAF material to 12, consistent with ADEM's currently adopted regulation. However, this trial revealed that this treatment actually made the emissions of DMDS worse. As this is one of the most offensive odors associated with DAF (human detection threshold of 7-12 ppb, (EPA, 2010)), this treatment is an undesirable outcome. Observed DMDS emissions of up to 5-10 ppb/hour would likely be noticeable to nearby populations.

Emissions of VFAs were initially low at pH 12 which is expected given that they are in the anionic (non-volatile) form; however, after the rain event, emissions rose to match those of direct DAF application without amendment. Finally, the alkaline treatment was the only one to release trimethylamine among the four conditions tested here. Thus, we conclude that raising pH of DAF to 12 prior to surface application is not an effective odor control strategy. Acidification of DAF (H_2SO_4) was also tested and found to increase VFA release by an order of magnitude compared to direct DAF application. This too is a highly undesirable outcome and not recommended. Finally, an oxidizer (H_2O_2) was tested and resulted in significantly higher initial VFA release compared to DAF only. More problematic, however, was the large (but inconsistent) increase in DMDS emissions after the rain event that only occurred in the H_2O_2 treatment. In summary, none of the chemical amendments were effective and some were in fact counterproductive at controlling odor during DAF application.

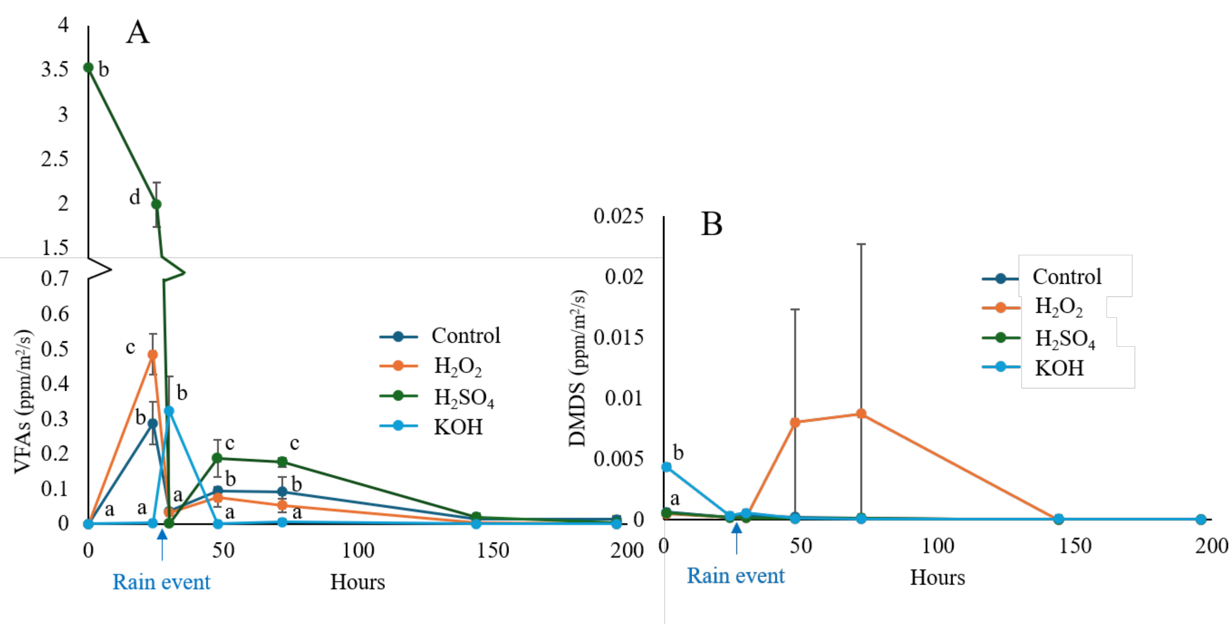

Figure 5. Volatile fatty acids (A) and DMDS (B) emissions were monitored for different chemical amendments and a control (DAF).

The results for odor control from mixing of agricultural lime and quick lime with DAF are presented in Figure 6. The takeaways for these results share some similarities with Figure 5. The agricultural lime chemical amendment at 10% loading delayed the VFA odorous emission, but only modestly reduced them (Figure 6A). There was no effect for DMDS (Figure 6B). The 3% agricultural lime condition was ineffective. The quick lime experiment completely controlled VFA emissions (Figure 6C) but surface application of quick lime (on top of DAF) created significantly more DMDS emissions than the pre-blended quick lime mix with DAF (Figure 6D). This suggests that quick liming may be an effective odor control technique for DAF solids. Pre-processing (mixing) quick lime with DAF solids should occur in a remote location prior to application. This is because DMDS emissions are likely produced during the blending process in the tank rather than after application (emissions during blending were not measured in this trial).

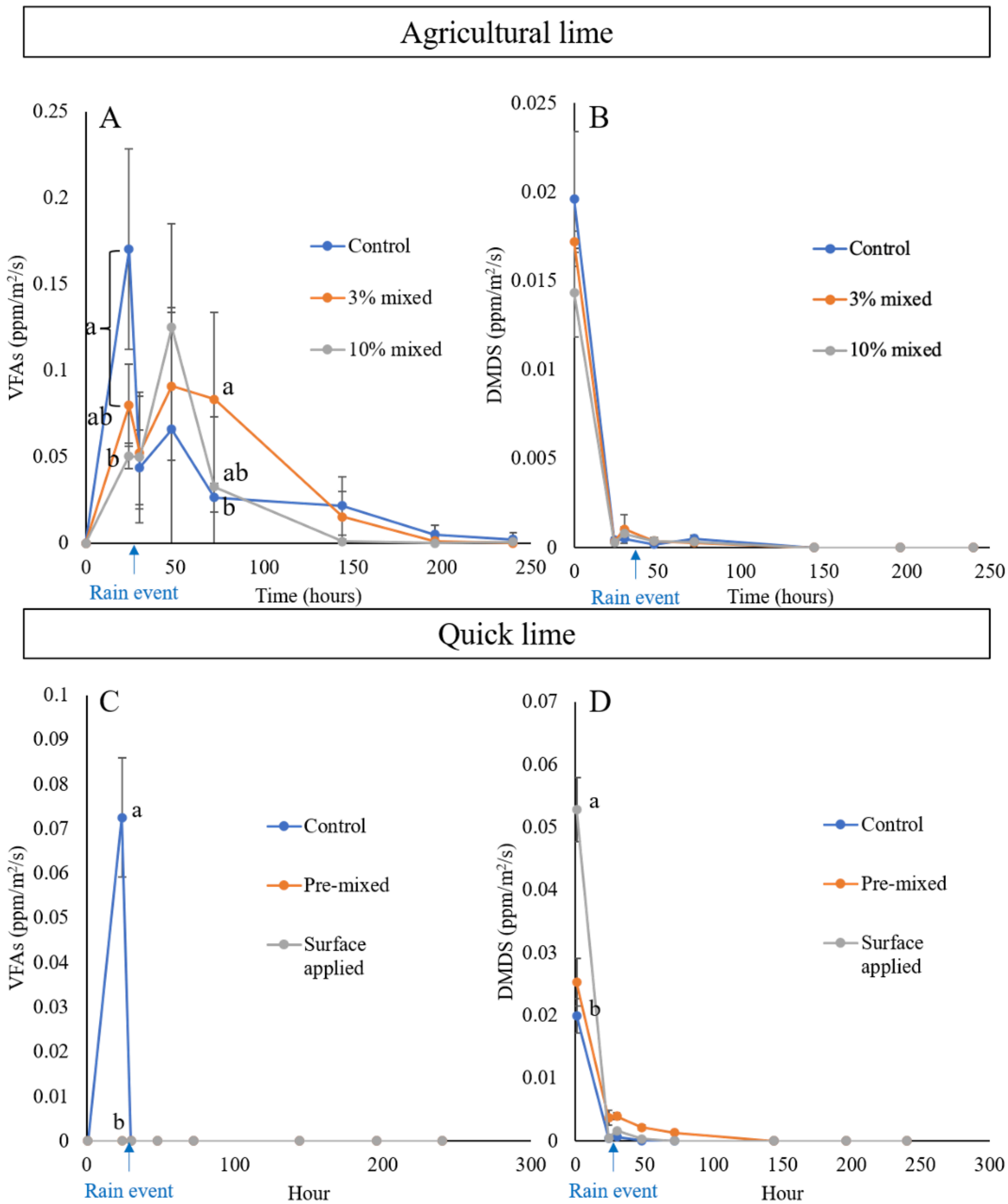

Figure 6. Volatile fatty acids (A and C) and DMDS (B and D) emissions were monitored for different agricultural and quick lime mixtures of DAF and a control (DAF).

3.5. Subsurface application impact on odor release in soil mesocosms

The results for 0.75-inch and 2.75-inch subsurface experiments are provided in Figures 7 and 8, respectively. In the 0.75-inch experiment, the lack of difference in initial DMDS release was surprising given that the soil layer was of similar thickness (3-5 cm) to the organic amendments tested in the previous trial. However, in the 2.75-inch experiment, DMDS was reduced by ~70%, but the reduction was not statistically significant ($p > 0.05$). Both experiments saw a DMDS resurgence later in the experiment, although, the non-scored 'Subsurface' condition did not. This is a promising sign, even though the scored condition likely better replicates actual subsurface injection (with a slit in the soil surface). It should be noted, this particular soil's sorption of DMDS is poor. For VFAs, 0.75-inch subsurface application did not cause major differences in odor control, although it did prevent a resurgence starting at 144 hr. In the case of 2.75-inch subsurface application there was a significant repression of all VFA emissions ($p < 0.05$). Hence, deeper subsurface injection, at 2.75 inches, appears to be a moderately effective odor control strategy whereas shallower techniques are not effective for DAF solids.

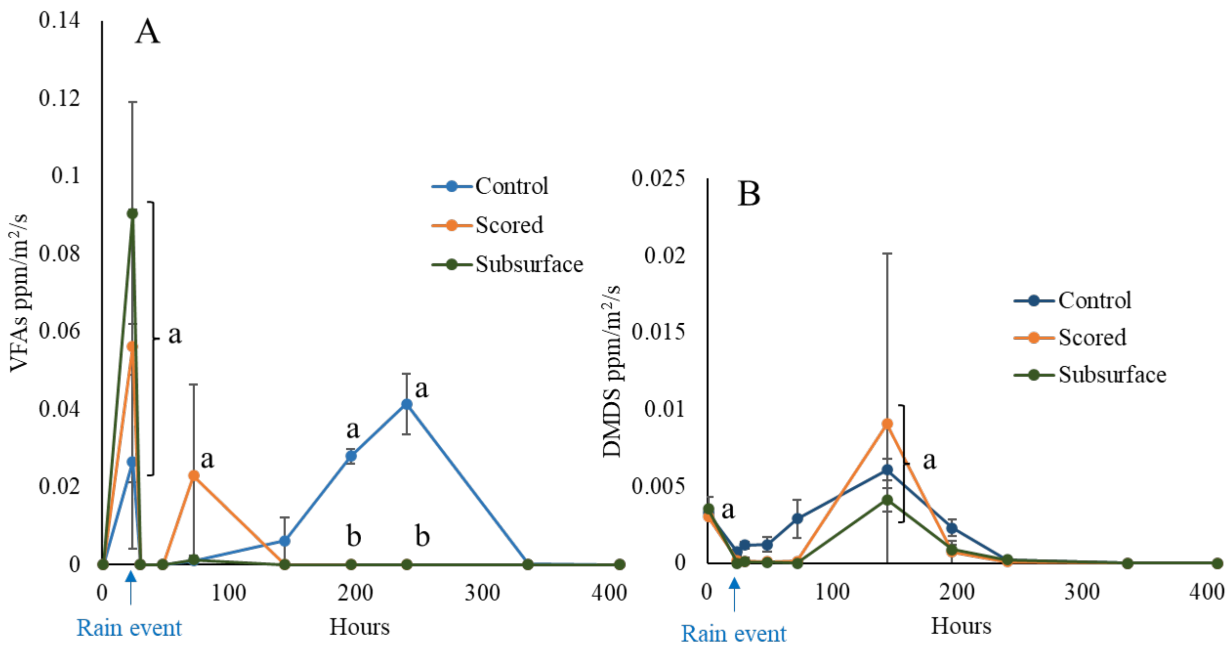

Figure 7. Volatile fatty acids (A) and DMDS (B) emissions were monitored for different subsurface applications with 0.75-inch soil cover and a control (DAF). Letters indicate significance ($p<0.05$).

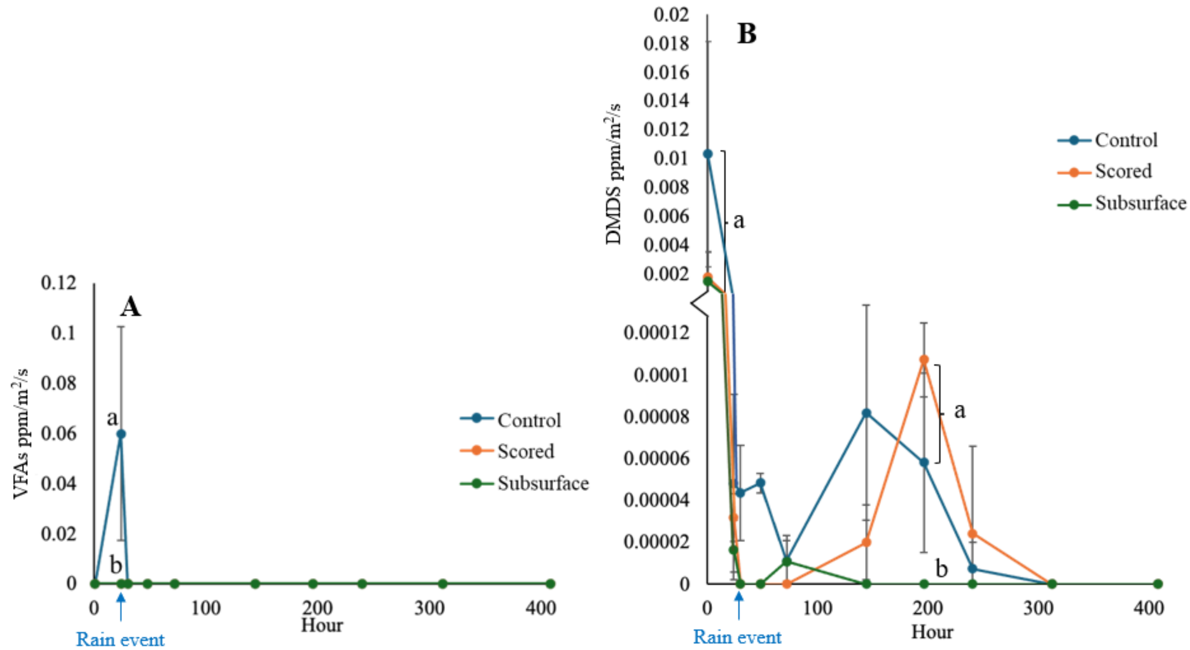

Figure 8. Volatile fatty acids (A) and DMDS (B) emissions were monitored for different subsurface applications with 2.75-inch soil cover and a control (DAF). Letters indicate significance ($p<0.05$).

3.6. Tillage and 'cut and inject' application impact on odor release in soil mesocosms

The results for tilled and 'cut and inject' application methods are displayed in Figure 9. These results support the effectiveness of 'cut and inject' for odor control of DAF solids. The results contrast with the tilled condition which increased odor release compared to the control (surface applied) or 'cut and inject'. This data indicates that a higher soil surface interaction with DAF solids leads to worse odor. The optimal condition appears to be instead for minimum soil interaction wherein DAF solids are placed carefully in or underneath the soil. It should be stressed that soil composition could be an important factor and only one soil type was used (i.e., a drastically different texture or pH soil could lead to different results). The soil used was an acidic (pH 4.8) sandy loam. We hypothesize that extensive soil contact acidifies the DAF solids, shifting the equilibrium of VFAs to the protonated state, and thereby increases their volatility.

Despite similar odor reduction trends, these results alone do not prove that 'cut and inject' is a better technique than the 2.75-inch subsurface experiments performed in Figure 8. A direct experiment comparing 2.75-inch subsurface application and 'cut and inject' would be required. Both treatments controlled VFA emissions. Both treatments reduced DMDS emissions but unfortunately, despite a large DMDS reduction on average, one-hour results from Figure 8B are not statistically significant which would even make percent reduction comparison to respective experimental controls invalid.

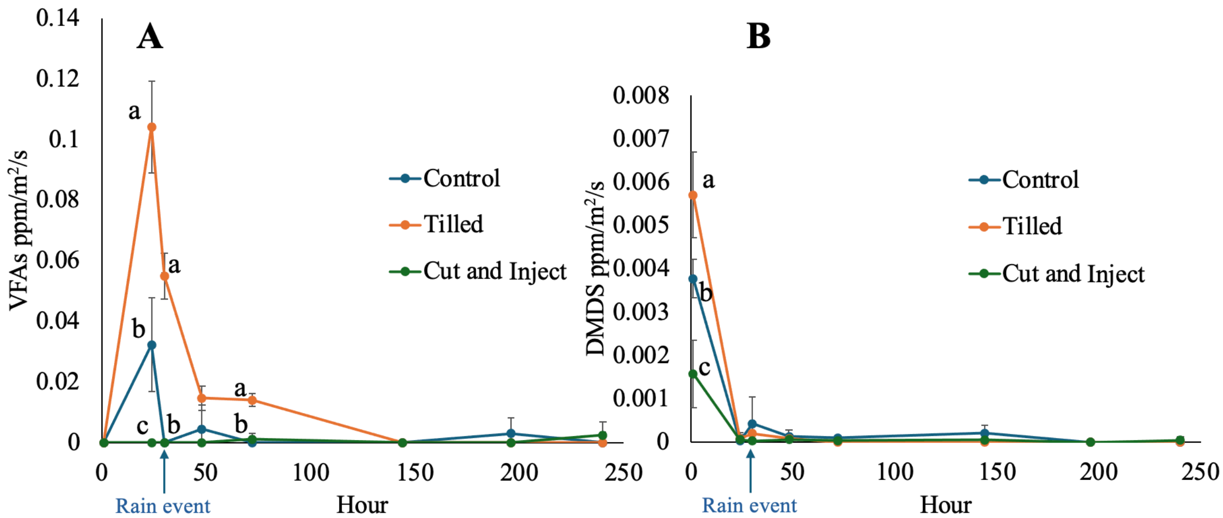

Figure 9. Volatile fatty acids (A) and DMDS (B) emissions were monitored for a Tilled and 'cut and inject' applications. Letters indicate significance ($p<0.05$).

<u>3.7. Organic matter amendments impact odor release in soil mesocosms</u>

Two biomass residues (grass clippings and pine straw) were tested along with biochar as organic amendments (Figure 10). In all cases, the DAF was first applied to the dry soil and the amendment layered over the top. After 24 hours, a simulated rain event took place to wet the mesocosm to saturation and odor emission measurements continued for a total of 320 hours. Both grass and pine suppressed VFA emissions by 91% and 98%, respectively during the first 24 hours after application (before rain event). The suppression was also sustained to varying extents after the rain event. Both grass and pine straw also partially suppressed the initial emission of DMDS by 26% and 46%, respectively. However, the grass clippings later resulted in a very large DMDS emission release after the rain event. This was consistent across all 3 mesocosm replicates for this treatment. We noticed that the grass clippings remained very wet for a sustained time after the rain event whereas the pine straw dried out more quickly. However, this difference could also be due in part to the sorption properties of pine straw. Biochar was effective at suppressing the initial emission of DMDS by 59% and maintaining low DMDS emissions for the duration of the experiment. However, biochar was not effective at controlling VFA emissions: levels were similar to the control. No aminated organics were observed any of the treatments of this trial.

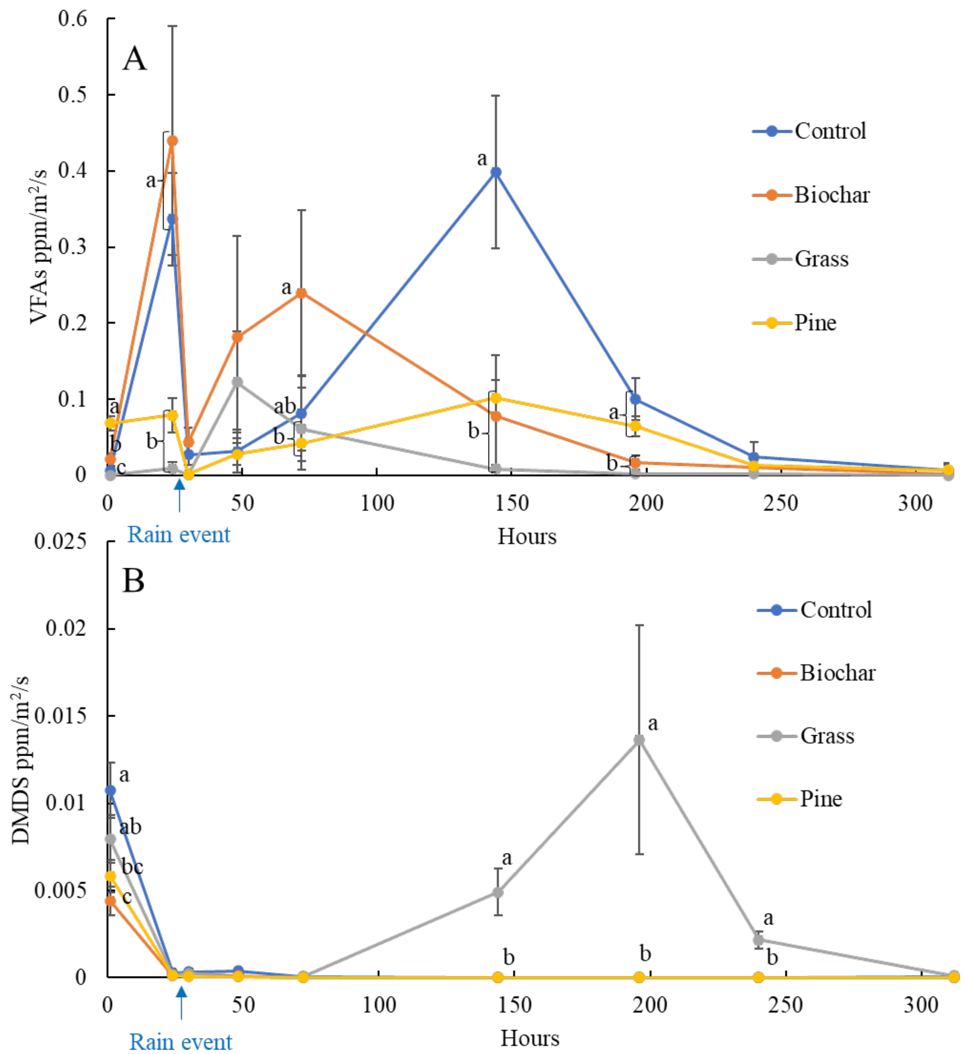

Figure 10. Volatile fatty acids (A) and DMDS (B) emissions were monitored for different organic amendments applied as a drop dressing after surface application of DAF solids. Letters indicate significance ($p<0.05$).

To summarize, the pine straw amendment was most effective among the treatments in this trial. Grass was effective until it became saturated with water and biochar was only effective against the sulfur organic. It is notable that pine needle extracts are sold commercially for odor control associated with smoke damage, garbage rooms, and corpse decontamination (https://www.alron.se/en/butik/luktkontroll/vatdimning/luktkontroll-tallbarr/). Only a single depth of grass and pine straw was tested (3-5 cm) in this trial. However, it is expected that thicker layers would yield more effective odor control, similar to what has been observed with wheat straw covers over manure storage structures (J. Clanton et al., 2001). In that work, 20 cm straw depths reduced odors by 78% whereas 30 cm depths reduced it by 83%. These are much thicker layers than what was used here but cost-effectiveness of transporting large volumes of amendment also needs to be considered.

3.8. Impact of hydration on odorous emissions

The results for the hydrated mesocosm experiment are shown in Figure 11. These results suggest that moisture plays a governing role in odor control, at least for DAF solids. A significant re-emergence of DMDS is observed in hydrated conditions (Fig. 11A) whereas the other conditions did not have a DMDS reemergence. This phenomenon helps to explain why grass-based amendment mesocosms had a reemergence of DMDS: they had similar topsoil moisture contents to the hydrated conditions at 144hr when the reemergence occurred. This same moisture retention phenomenon also likely caused the DMDS reemergence in the subsurface mesocosms in section 3.5. An advantage of high soil moisture contents is that VFA emissions are minimal (they were close to zero, hence not shown) although the production of DMDS as a result makes the concept of controlling odor by increasing soil moisture an unattractive proposition. We later determined (by DO probe) that DMDS was produced due to the anaerobic environment that occurred in saturated soil. Dry soil had better oxygen transfer and higher DO levels. These results indicate that land application of DAF solids should occur in fields with good water drainage (minimize long periods of continuous saturation).

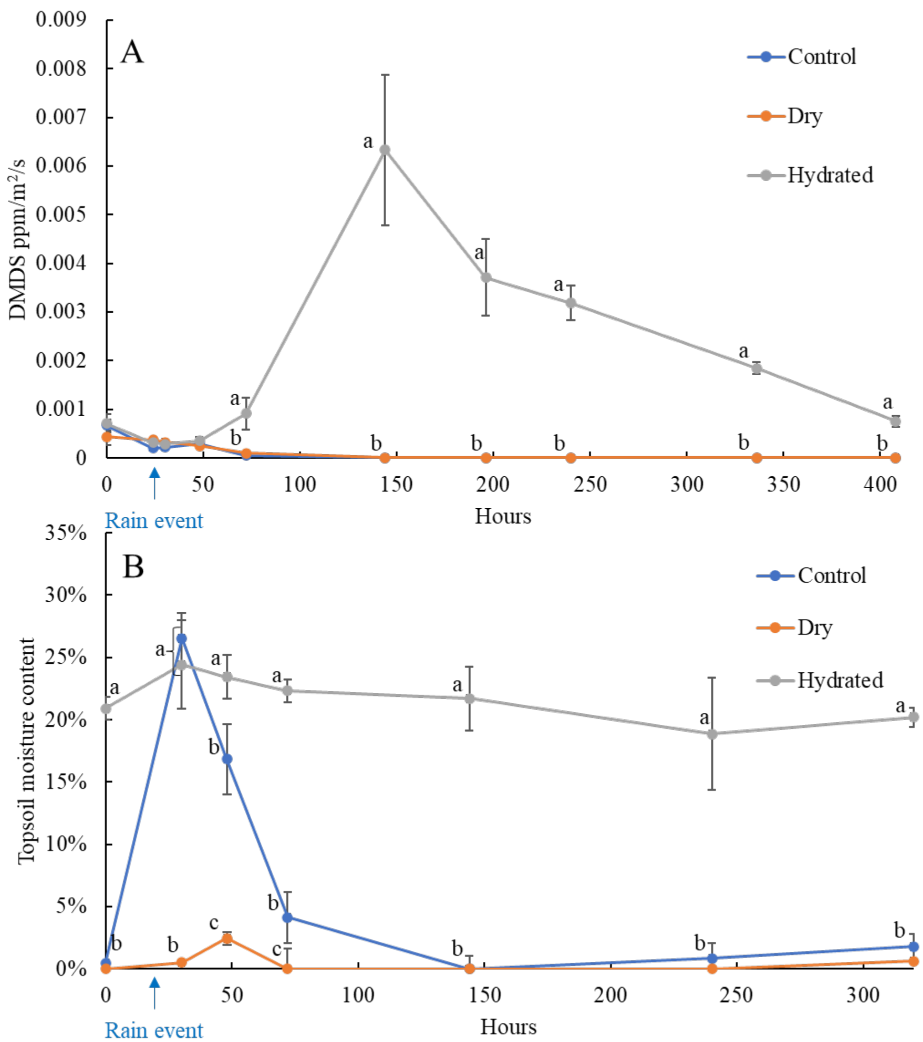

Figure 11. DMDS (A) and topsoil moisture content (B) were monitored for different moisture applications with continuous hydration, dry (no rain) and a control with a single rain event at 24 hr post DAF application. Letters indicate significance ($p<0.05$).

It should be stated that although the same control (DAF only) mesocosm was prepared for all experiments, differences in emission rates were observed between experiments. Beyond natural batch variation, the gas sampling in the chemical trials was delayed 3 hours before the first sampling to prepare pH titrations and reaction products of hydrogen peroxide. In the organic amendment trial, the sampling was carried out within a half hour. Consequently, initial emissions in the latter were higher. However, as treatments were only compared to their respective control mesocosms, conclusions about amendment effectiveness can still be drawn.

4. Conclusions

- DAF source, age, and treatment affects its odor profile with fresher material from poultry plants contributing higher levels of sulfur organics (rotting meat odor) and older material emitting volatile fatty acids (fecal odor).
- Pathogens are initially present in freshly-collected DAF solids but are susceptible to die-out during anaerobic storage.
- Pre-mixing quick lime with DAF reduced VFA emissions, though not DMDS, while other chemical treatments increased odors (KOH, surface-applied quick lime, sulfuric acid and hydrogen peroxide) or only delayed the odor (agricultural lime).
- Adding a top dressing of pine straw significantly improved the odor profile for both VFAs and sulfur organics.
- Grass amendment improved emissions of VFAs and sulfur organics under dry conditions. However, it made DMDS emissions worse after it became saturated with water.
- Biochar was effective against sulfur organics but not VFA emissions.
- Subsurface application at 2.75 inches prevented VFA emissions, but the ~70% reduction in DMDS emissions was not statistically significant. Shallow subsurface application (0.75 inches) was ineffective for controlling both VFAs and DMDS. Tilling DAF into the soil (full incorporation) made odor release worse than the surface applied control, while ‘cut and inject’ significantly reduced all odorous emissions – making it an effective odor control treatment.
- Hydration has an important role on release of odorous emissions. Mesocosm conditions that retain moisture (grass and subsurface) result in a later DMDS reemergence.
- Unlike VFAs, no treatments were able to receive near or 100% reduction of sulfurous odors (DMDS). Although deep subsurface or ‘cut and inject’ techniques controlled DMDS the best (70% emission reduction), it would appear further research is still required to achieve complete odor control of unique poultry DAF material. As a result, without complete, or nearly complete control (>95% reduction) odor complaints for land application of DAF solids will still be possible, though likely significantly lessened.

References

Arthur, W., Morgan, Z., Reina Antillon, M., Drabold, E., Wells, D.E., Bourassa, D.V., Wang, Q., Higgins, B.T. 2024. Pilot-Scale Evaluation of Poultryponics: Insights into Nitrogen Utilization and Food Pathogen Dynamics. *ACS ES&T Water*.

Chen, L., Hoff, S., Cai, L., Koziel, J., Zelle, B. 2012. Evaluation of Wood Chip-Based Biofilters to Reduce Odor, Hydrogen Sulfide, and Ammonia from Swine Barn Ventilation Air. *Journal of the Air & Waste Management Association*, **59**(5), 520-530.

Drabold, E.T., Sakhakarmy, M., Shanmugam, S.R., Adhikari, S., Arthur, W., Rudar, M., Boersma, M., Wang, Q., Higgins, B.T. 2025. Thermal hydrolysis of poultry byproducts for the production of microbial media. *Scientific Reports*, **15**(1), 6107.

EPA. 2010. Pesticide Fact Sheet: Dimethyl disulfide. Office of Chemical Safety and Pollution Prevention.

Fan, F., Xu, R., Wang, D., Meng, F. 2020. Application of activated sludge for odor control in wastewater treatment plants: Approaches, advances and outlooks. *Water Research*, **181**, 115915.

Goldstein, N. 2007. Smart Series Odor Management. in: *BioCycle*, Biocycle Connect, LLC. Emmau, PA.

Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H. 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agriculture, Ecosystems & Environment*, **206**, 46-59.

Hwang, O., Lee, S.-R., Cho, S., Ro, K.S., Spiehs, M., Woodbury, B., Silva, P.J., Han, D.-W., Choi, H., Kim, K.-Y., Jung, M.-W. 2018. Efficacy of Different Biochars in Removing Odorous Volatile Organic Compounds (VOCs) Emitted from Swine Manure. *ACS Sustainable Chemistry & Engineering*, **6**(11), 14239-14247.

J. Clanton, C., R. Schmidt, D., E. Nicolai, R., D. Jacobson, L., R. Goodrich, P., A. Janni, K., R. Bicudo, J. 2001. GEOTEXTILE FABRIC–STRAW MANURE STORAGE COVERS FOR ODOR, HYDROGEN SULFIDE, AND AMMONIA CONTROL. *Applied Engineering in Agriculture*, **17**(6), 849.

J.E. Thies, Rillig, M.C., Graber, E.R. 2015. Biochar effects on the abundance, activity and diversity of the soil biota. in: *Biochar for environmental management: science and technology*, (Eds.) J. Lehmann, S. Joseph, Earthscan Books Ltd. London.

Lee, J.H. 2003. RECOVERY PROCESS FOR FOOD SKIMMINGS FROM DISSOLVED AIR FLOTATION UNITS. *Pp. 237-241 in the Ninth International Animal, Agricultural and Food Processing Wastes Proceedings of the 12-15 October 2003 Symposium (Research Triangle Park, North Carolina USA), Publication Date 12 October 2003.*, St. Joseph, MI. ASABE.

May, G. 2011. Straw covers control odors and other emissions from manure storage structures, Michigan State University Extension. East Lansing, MI.

Neal, M. 2019. North Alabama residents concerned with chicken sludge invading their properties. in: *News19*, Nexstar Media Inc. Huntsville, AL.

Pillion, D. 2021. Alabama fines Arkansas company $34,500 for spraying stinking sludge. in: *AL*, Alabama Media Group. Birmingham, AL.

Porterfield, K.K., VanOrnum, D., Roy, E.D. 2021. Assessment of lime-conditioned dairy manure fine solids captured using dissolved air flotation for fertilization in horticulture. *Journal of Environmental Quality*, **n/a**(n/a).

Risse, L.M., Cabrera, M.L., Franzluebbers, A.J., Gaskin, J.W., Gilley, J.E., Killorn, R., Radcliffe, D.E., Tollner, W., Zhang, H. 2006. Land Application of Manure for Beneficial Reuse. *Animal Agriculture and the Environment*.

Shareefdeen, Z. 2005. *Biotechnology for Odor and Air Pollution Control*. Springer Science & Business Media.

Wang, P., Peng, H., Adhikari, S., Higgins, B., Roy, P., Dai, W., Shi, X. 2020. Enhancement of biogas production from wastewater sludge via anaerobic digestion assisted with biochar amendment. *Bioresource Technology*, **309**, 123368.