Pilot-scale testing of renewable biocatalyst for swine manure treatment and mitigation of odorous VOCs, ammonia and hydrogen sulfide emissions

Devin Maurer¹, Jacek A. Koziel^{1}, Kelsey Bruning¹, David B. Parker²*

¹Dept. of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011

²USDA-ARS, Conservation and Production Research Laboratory, Bushland, TX 79012

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1 ABSTRACT. Comprehensive control of odors, hydrogen sulfide (H₂S), ammonia (NH₃), and
2 greenhouse gas (GHG) emissions associated with swine production is a critical need. A pilot-
3 scale experiment was conducted to evaluate surface-applied soybean peroxidase (SBP) and
4 calcium peroxide (CaO₂) as a manure additive to mitigate emissions of odorous volatile organic
5 compounds (VOC) including dimethyl disulfide/methanethiol (DMDS/MT), dimethyl trisulfide,
6 *n*-butyric acid, valeric acid, isovaleric acid, *p*-cresol, indole, and skatole. The secondary impact
7 on emissions of NH₃, H₂S, and GHG was also measured. The SBP was tested at four treatments
8 (2.28-45.7 kg/m² manure) with CaO₂ (4.2% by weight of SBP) over 137 days. Significant
9 reductions in VOC emissions were observed: DMDS/MT (36.2%-84.7%), *p*-cresol (53.1%-
10 89.5%), and skatole (63.2%-92.5%). There was a corresponding significant reduction in NH₃

(14.6%-67.6%), and significant increases in the greenhouse gases CH₄ (32.7%-232%) and CO₂ (20.8%-124%). The remaining emissions (including N₂O) were not statistically different. At a cost relative to 0.8% of a marketed hog it appears that SBP/CaO₂ treatment could be a promising option at the lowest (2.28 kg/m²) treatment rate for reducing odorous gas and NH₃ emissions at swine operations, and field-scale testing is warranted.

1. Introduction

Emissions of odorous volatile organic compounds (VOCs), ammonia (NH₃), and hydrogen sulfide (H₂S) are a problematic byproduct of pork production (Van Huffel et al., 2016; Cai et al., 2015, Stinn et al., 2014). Mitigation of these emissions is a current issue due to their effect on local and regional air quality. In addition, emissions of greenhouse gases (GHG) (CH₄, N₂O and CO₂) are a potential contribution to climate change.

Manure additives are one of 12 types of technologies that have been proposed to mitigate gaseous emissions from livestock manure storage and handling (Iowa State University Extension and Outreach, 2015; Maurer et al., 2016). However, a limitation of most mitigation technology research is that only about 25% of all research has been evaluated beyond the lab- and pilot-scale and tested in the field. In addition, most research has focused on a limited number of gases. Because of these limitations, it is difficult to rank and adopt mitigation technologies without comprehensive treatment data and an understanding of impact beyond those single or few target gases (Maurer et al., 2016). It is always possible that reducing emissions of one target pollutant can have the opposite effect on other important target pollutants.

The report by Heber et al. (2001) is still the largest pilot-scale study comparing efficacy of swine manure additives for mitigation of NH_3 and H_2S emissions over 42 days. Portejoie et al. (2003) reported a 93% and 92% reduction in NH_3 emissions from swine manure during a 15 d trial using surface-applied kitchen oil and peat, respectively.

Researchers have shown that peroxidase enzymes, when combined with peroxides such as hydrogen peroxide (H_2O_2) or CaO_2 , reduce the concentrations of some odorous phenolic and indolic compounds by polymerization (Morawski et al., 2001; Steevensz et al., 2014; Tonegawa et al., 2003; Ye et al., 2009). The polymerization of the phenolic and indolic compounds increases their molecular weight and thereby decreases their volatility, making them less prevalent in the air. Soybean peroxidase is a bio-based and soybean-derived product. It is produced by shredding soybean hulls, a low value and low utility byproduct. Swine farmers are very familiar with soybeans as they are a common animal feed. Thus, the potential for concerns about adding a treatment of unknown origin to the manure that will eventually be land applied is also low.

This study aimed to investigate the dosage and duration of surface-applied soybean peroxidase/calcium peroxide (SBP/ CaO_2) treatment on finisher pig manure gas emissions over a 5 month period at pilot-scale. Comprehensive evaluation of odorous VOCs (sulfur containing VOCs, volatile fatty acids, phenolics and indolics), NH_3 , H_2S and greenhouse gas (CH_4 , N_2O and CO_2) emissions was completed.

This research builds on our previous proof-of-concept laboratory studies where we evaluated VOC emissions from swine manure treated with SBP/ CaO_2 and either mixed throughout the manure or applied to the surface (Parker et al., 2012; Parker et al., 2016). While the previous lab-

scale studies were limited to VOC emissions, the objective of this larger pilot-scale study was to expand the evaluation to include a comprehensive list of odorous VOCs, NH₃, H₂S, and GHGs while focusing on surficial manure application. As opposed to fully-mixed treatments, potential benefits of surficial application include lower energy requirement, lower labor cost, and no need for specialized mixing equipment. These are important considerations for future farm-scale applications at commercial livestock operations.

2. Materials and Methods

2.1 Experimental Design

The pilot-scale study was designed to simulate manure conditions in a deep pit swine manure system. Deep-pit manure storage is commonly used for manure storage under barns in Midwest U.S. The experimental design was a completely randomized design (CRD) with five treatments and three replications per treatment, for a total of 15 experimental units. The five treatments consisted of SBP application rates of 0, 2.28, 4.57, 22.8 and 45.7 kg/m² (0 to 9.36 lbs/ft²), respectively. CaO₂ was mixed with the SBP at a rate equivalent to 4.2% CaO₂/SBP w/w. The SBP/CaO₂ mixture was surface-applied to the manure. The highest application rate of 45.7 kg/m² (SBP mass/manure surface area) was equivalent to the 50 g/L (SBP mass/volume of manure) application rate tested in the lab-scale studies of Parker et al. (2012; 2016).

2.2 Manure Storage Simulators

Deep pit swine manure from an Iowa State University swine finisher operation was pumped into fifteen 1.22 m (4 ft) tall, 0.38 m (15 in) diameter sealed manure storage simulators (Figure 1; Figure S1). Each simulator initially received 104 L (27.5 gal) of swine manure followed by 9.5 L (2.5 gal) weekly manure additions for two weeks after SBP/CaO₂ addition resulting in a final volume of 123 L (32.5 gal). The ventilation in each simulator was controlled via valves with rotameters (Figure S2) in order to achieve a ventilation rate of 7.5 headspace exchanges per h. The rate was adjusted when fresh manure was added to keep a constant exchange rate. Air exchange rates were consistent with recommended values for less than half-full manure pit storage (assuming cool season; minimum ventilation, growing (34-68 kg) pigs, in swine barn with fully slatted floor) (MWPS, 1983). Storage temperature was kept between 9 and 20 °C (average = 15.6 ± 3.7 °C) to simulate the temperature of deep pit storage (Da Silva Batista et al., 2013).

Baseline gas emissions measurements were taken for two weeks prior to SBP/CaO₂ addition to evaluate emissions from each of the 15 pilot manure storage simulators to make sure that there were no significant difference in emissions due to the system prior to randomly assigning treatments to the experimental units. Total duration of the pilot experiment was 137 days. Manure samples were collected and analyzed before SBP/CaO₂ application and at the end of the experiment to determine effects on manure quality and potential for nutrient retention. Percent relative humidity in manure headspace was monitored via an 850071 Environmental Quality meter (Sper Scientific, Scottsdale, AZ, USA) every day that gas emission samples were collected (Figure S3).

2.3 Volatile Organic Compounds (VOCs)

VOCs were collected in 304-grade stainless steel sorbent tubes (4 mm O.D., 0.10 m long) filled with 65 mg Tenax TA. Air samples were collected using a portable sampling pump (50 mL/min for 15 min), and were analyzed within two days (Figure S4). The sampling flow rates were verified with a NIST-traceable digital flow meter (Bios International, Butler, NJ, USA). Chemical analyses of swine odorants were completed using the TD-MDGC–MS/O system (Agilent 6890 GC; Microanalytics, Round Rock, TX, USA) (Figure S5) using procedures previously described (Zhang et al., 2012) (Olfactometry port was not used in this study). An additional sorbent tube to test for breakthrough was used during each sampling set. Work by Andersen et al. (2012) has shown that methanethiol (MT) can convert to dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) using sorbent tubes and thermal desorption. It is not possible to determine the extent of that conversion with field air samples and determine the exact fraction of DMDS or DMTS that resulted from MT conversion. Additional experiment was conducted post project to determine the extent of MT to DMDS/DMTS conversion using moist and dry air (Cai et al., 2015). It was determined that complete conversion of MT to DMDS (97.5 to 99.5%) and DMTS (0.5 to 2.5%) is possible for moist standard gas stored on sorbent tubes from 1 to 3 days. Thus, caution should be exercised while interpreting measured DMDS and DMTS concentrations as they are likely confounded by conversion of MT and fluxes of these compounds are reported as DMDS/MT and DMTS/MT throughout the manuscript.

2.4 Ammonia and Hydrogen Sulfide

Ammonia and H₂S concentrations were measured via a Drager X-am 5600 portable gas analyzer (Luebeck, Germany) with NH₃ and low range H₂S XS sensors (Figure S6). The analyzer was calibrated using Drager calibration software, Environics 4040 gas dilution system (Tolland, CT, USA) and standard gases (Praxair, Ames, IA, USA) (NH₃: 102 ppm and H₂S: 15.6 ppm). Calibration details are in SI.

2.5 Greenhouse Gases

Gas samples were collected via syringe and 5.9 mL Exetainer vials (Labco Limited, UK) and were analyzed for GHG concentrations on a gas chromatograph (SRI Instruments, Torrance, CA, USA) equipped with FID and ECD detectors, and CO₂-to-CH₄ catalytic convertor (Figures S7 to S9). Gas method detection limits were 1.99 ppm for CO₂, 170 ppb for CH₄, and 20.7 ppb for N₂O. Standard curves were constructed daily using 2 ppm and 10.3 ppm CH₄; 510 ppm, 1010 ppm and 2010 ppm CO₂; and 0.101 ppm, 1.02 ppm and 10.1 ppm N₂O (Air Liquide America, Plumsteadville, PA, USA). Daily standards used for standard curve construction were completed in duplicate for CH₄ and CO₂ while N₂O standards were constructed in triplicate.

2.6 Swine Manure Analysis

Swine manure analysis was completed using standard methods described in greater detail in SI. Total Kjeldahl nitrogen (TKN) was quantified by digestion followed by steam distillation and titrimetric analysis (AOAC, 2000). Ammonia (NH₃) was quantified by steam distillation followed by titrimetric analysis (APHA, 1998). Dissolved reactive phosphorus was quantified by

the ascorbic acid method (APHA, 1998). Total phosphorus was determined by sulfuric acid-nitric acid digestion followed by the ascorbic acid method (APHA, 1998), while pH was determined using the electrode method (APHA, 1998). Total solids/moisture content and volatile solids were determined by oven drying method (APHA, 1998). Percent C, H, N, and S were acquired with a combustion analyzer (Perkin Elmer Inc., Waltham, MA) with a cysteine calibration standard. Conceptual schematics of target gas emissions potential sources and possible fates in the C, N, and S cycles are presented in Figures S10 to S12.

2.7 Soybean Peroxidase Activity

SBP was extracted by 3% acetic acid hydrolysis at room temperature and vortexed periodically over 1 h followed by centrifugation. The extract was then assayed based on modified guaiacol assay (Baker et al., 2002) at 30 °C and 470 nm for 3 min using a UV-Vis spectrometer. Details are presented in SI.

2.8 Estimation of Target Gas Flux

Measured gas concentrations were used for calculation of flux (E), i.e., emissions based on time and emitting surface area of manure ($\text{mass}/(\text{time} \times \text{area})$). Gas concentrations were measured at field conditions and were converted to standard conditions (1 atm, 25 °C, dry air). Methodology of gas flux estimations based on measured variables is presented in SI (equations S3 to S13). Overall mean % reduction (%R) for each target gas was calculated using Eq. 1.

$$\%R = \frac{E_{Con} - E_{Trt}}{E_{Con}} * 100\% \quad \text{Eq. 1}$$

where E_{Con} is the average flux estimate of the desired time interval (day, week, biweekly or overall) of the control, and E_{Trt} is the corresponding average flux estimate of the treatment.

2.9 Statistical Analysis for Pilot-Scale

The General Linear Model procedure, PROC GLM, in SAS System (version 9.3, SAS Institute, Inc., Cary, NC, USA) was used to analyze the data and determine the p values. A significance level of 0.05 was used as the cut off for statistical significance.

3. Results and Discussion

3.1 Visual Observations of the SBP Surface Layer over Time

For the two lowest SBP/CaO₂ application rates of 2.28 and 4.57 kg/m², there was almost complete wetting and absorption of the SBP/CaO₂ into the manure surface progressing from the day of application (Day 1) to Day 137 (Figure 2). At the higher SBP/CaO₂ application rates of 22.8 and 45.7 kg/m², some of the SBP was still visible on the surface of the manure on Day 137 (Figure 3). The 22.8 kg/m² SBP/CaO₂ application rate still had some dry SBP/CaO₂ covering the complete surface of the manure on Day 137 (Figure 3). The highest SBP/CaO₂ application rate of 45.7 kg/m² was ~13 cm thick. The weight of this relatively thick layer of SBP/CaO₂ caused the SBP crust to roll into the top layer during periodic additions of new manure (Figure 3). This resulted in less manure surface coverage with the largest SBP

application rate over time. Assessment of the distribution of dry and wet SBP/CaO₂ layer thickness in the midpoint of treatment (Day 88) is presented in Table 1 and Figure S13. These visual observations of the surface SBP layer are important because they provide a better understanding on the mechanisms responsible for the mitigation effect (e.g., chemical activation of peroxidase, biofilm/biofilter layer interface between manure and air, or the combination of both).

Table 1. Physical characteristics of SBP/CaO₂ layer at Day 1 (application) and “midpoint” (Day 88, Figure S13).

Treatment (kg/m ²)	2.28	4.57	22.8	45.7
Starting (initial application) thickness on d 1 (mm)	6	13	64	127
Dry thickness on d 88 (mm)	2	9	31	15
Wet thickness on d 88 (mm)	24	40	31	15

3.2 Treatment Effects on Odorous Volatile Organic Compounds

The *p*-cresol and skatole fluxes were significantly reduced for all SBP treatments with no apparent benefit with higher SBP application rates (Table 2, Figures 4B,C). These *p*-cresol results compare favorably to the laboratory studies of Ye et al. (2009) and Parker et al. (2012; 2016). Ye et al. (2009) reported almost 100% reduction in *p*-cresol emissions when 2 kg batch of swine slurry was treated with horseradish peroxidase and peroxides. Likewise, Parker et al. (2012) reported reductions in *p*-cresol of 62 to 92% for swine manure treated with SBP/CaO₂ mixed throughout the manure. Parker et al. (2012) also reported a short-term reduction in skatole

and indole emissions that were apparent at 24 h after treatment but negligible after 48 h. However, our surface application demonstrated a longer-term reduction in skatole up to 137 d, an important consideration for possible farm-scale application.

Table 2. Mean (n = 3 reactors) of overall emission reductions and statistical significance associated with SBP/CaO₂ treatment over 137 days.

Pilot-scale test								
SBP/CaO ₂ (kg/m ²)	2.28		4.57		22.8		45.7	
Gas	% Reductions	p value	% Reductions	p value	% Reductions	p value	% Reductions	p value
DMDS/MT	36.2	0.212	67.5	0.021	84.7	0.006	50.3	0.335
DMTS/MT	16.9	0.495	10.7	0.577	-9.2	0.632	-6.2	0.631
<i>n</i> -Butyric Acid	8.5	0.944	19.5	0.225	17.5	0.27	-235	0.0001
Valeric Acid	87.5	0.331	88.5	0.301	79.2	0.362	83.5	0.356
Isovaleric Acid	42.7	0.474	59.2	0.064	58.8	0.071	-354.7	0.0001
<i>p</i> -Cresol	64.9	0.02	53.1	0.04	89.5	0.001	78.2	0.023
Indole	-3.2	0.811	52.6	0.571	81.8	0.093	-24.2	0.544
Skatole	72.6	<.0001	63.2	<.0001	92.5	<.0001	77.2	<.0001
NH ₃	14.6	0.12	25.2	0.008	58.4	<.0001	67.6	<.0001
H ₂ S	10.9	0.688	-105.9	0.062	48.3	0.036	-144.4	0.024
CH ₄	-32.7	0.077	-52.1	0.009	-107.1	<.0001	-231.7	<.0001
CO ₂	-20.8	0.013	-27.4	0.0001	-83.5	<.0001	-123.6	<.0001
N ₂ O	-2.6	0.919	-0.2	0.985	0.4	0.97	-1.3	0.913

Bold font signifies statistical significance ($p < 0.05$) between each treatment and the control. DMDS and DMTS reductions are likely confounded by conversion of MT and are reported as DMDS/MT and DMTS/MT.

There were no significant differences in indole flux for any of the SBP treatments (Figure S14), likely due to the highly variable emission of that compound. DMDS/MT flux was significantly reduced with the 4.57 and 22.8 kg/m² SBP treatments ($p=0.021$ and $p=0.006$, respectively). Treatment application rates of 2.28 and 45.7 kg/m² SBP resulted in reductions of DMDS/MT but were not significant ($p=0.212$ and $p=0.335$, respectively). There was also a trend in application rate level for DMDS/MT with higher dose resulting in higher reduction in flux estimates (Figure 4D). Fluxes of H₂S and DMDS/MT were simultaneously higher in months 2 and 3 of the trial with the H₂S flux peaking at the end of month 1. The resulting DMTS/MT flux did not show any significant effect in regards to any of the SBP treatments (Figure S15). Measured flux for the target short chain organic fatty acids did not result in any significant differences for the SBP treatments with only one exception at the highest application rate (45.7 kg/m² of SBP) with significant ($p=0.0002$ and $p=0.0003$, respectively) increase for *n*-butyric acid and isovaleric acid flux (Figures S16 to S18). Summary of measured gas concentrations and flux ranges of monitored gases are presented in Table S1 and S2.

3.3 Treatment Effects on Ammonia and Hydrogen Sulfide

Ammonia flux was consistently reduced for all SBP/CaO₂ treatment levels, with the higher application rates resulting in more significant reductions (67.6% ($p<0.0001$), 58.4% ($p<0.0001$), and 25.2% ($p=0.008$) for the 45.7, 22.8, and 4.57 kg/m² SBP/CaO₂ application rates,

respectively) (Table 2). NH_3 flux was also reduced by 14.6% for the lowest 2.28 kg/m² SBP/ CaO_2 application rate, but was not significant ($p=0.120$) (Figure 4A).

The higher reductions in NH_3 flux estimates occurred immediately after SBP/ CaO_2 application, with the SBP/ CaO_2 effect wearing off over time. There could be several modes of action responsible for the NH_3 reductions. At least part of the treatment effect could have been the result of the SBP covering the manure surface. Because NH_3 flux is strongly correlated to evaporation rate, anything that inhibits evaporation would also decrease the NH_3 flux (Parker et al., 2013). It is also possible that the SBP binds or absorbs the NH_4^+ or NH_3 in solution. Apparent lowering of pH and relative humidity in simulators headspace is documented in section 3.5. Further research is required to assess the primary mode of action.

Measured concentrations of H_2S in both the control and treatments were between <0.1 to 25 ppm and were highly variable (Figure S19). Thus, the estimates of flux and the mitigation effect were challenging to assess and were not correlated with the application rate. SBP treatments of 2.28 and 22.8 kg/m² resulted in a decrease in H_2S flux estimates while 4.57 and 45.7 kg/m² SBP treatment resulted in an increase in H_2S flux.

3.4 Treatment Effects on Greenhouse Gases

Methane and CO_2 flux were significantly ($p\leq 0.013$) higher overall for all SBP treatments over the 137 day monitoring period except for methane at the lowest application rate ($p=0.077$) (Figure 5A,B). Nitrous oxide flux was not significantly different ($p=0.913$ to $p=0.985$) for all SBP treatment levels over the 137 day period after treatment (Figure 5C). The increase in

generation of CO₂ and CH₄ is likely a result of biochemical breakdown of the SBP. In essence, mitigation of VOCs resulted in increased emissions of CO₂ and CH₄. It is also important to recognize the role of bacteria population that might be controlling the generation of CH₄ vs. N₂O (Montes et al., 2013). For example, manure bacteria can either suppress CH₄ generation at the expense of increased N₂O or vice versa.

Closer inspection of the CH₄ and N₂O flux (Figures 5A and 5C) supports this apparent trend, i.e., increased and re-equilibrating CH₄ and decreased and re-equilibrating N₂O for both the control and treatment. These apparent trends for CH₄ and N₂O in the control suggest that oxygen availability in manure due to pump-out and transfer to storage simulators has influenced variations in bacterial growth early in the experiment. Methane and CO₂ flux was inversely proportional to the SBP application rate.

Emissions of GHGs from stored swine manure occur naturally during storage. The increase in CO₂ and CH₄ flux from SBP treatment is small when put into the context of total GHG emissions from a US commercial swine finisher and swine gestation barn with a deep pit storage. The increase in CO₂ as a result of the SBP/CaO₂ treatments of 2.28, 4.57, 22.8 and 45.7 kg/m² equates to 0.52-0.68, 0.66-0.86, 2.01-2.62, and 2.97-3.88% of the total CO₂ emitted from a typical swine finisher with deep pit, respectively (Sun et al., 2008; Ni et al., 2008). There is much greater CO₂ contribution from pigs' respiration, combustion from natural gas-fired space heaters, and from microbial activity in manure. The increase in CH₄ is small for the two lowest SBP/CaO₂ treatments that could be considered feasible for farm applications, i.e., 2.28 and 4.57 kg/m², with 5.38 and 8.02% of the total CH₄ resulting emitted from a typical deep pit swine finisher barn. The higher, much less practical for farm application SBP/CaO₂ application rates

22.8 and 45.7 kg/m² resulted in a larger increase of CH₄ that equated to 16.5 and 35.6% of the total CH₄ emitted from a typical deep pit swine finisher barn (Ni et al., 2008).

3.5 Treatment Effects on Environmental and Physicochemical Parameters

Relative humidity (RH) in the headspace of the simulators and manure pH were also monitored besides test room temperature and pressure (summarized in Figure S20). The SBP caused a decrease in manure pH coinciding with the application rate (Figure 6A). This decrease in pH was consistent with the observed decrease in NH₃ emissions. Lower pH promotes the formation of the non-volatile ammonium ion leading to lower NH₃ emissions (Figure 4). The lower pH also supported the apparent increase in CO₂ and CH₄ emissions (Figures 5). The RH was also correlated with the application rate (Figure 6B; R²=0.82), the SBP layer seemed to provide a barrier, suppressing evaporation and trapping the moisture in the manure. The effect was greater with higher SBP application rate (with the possible exception of the highest dose and the reasons discussed in 3.1), and faded over time as the SBP became more incorporated into the top layer of the manure. This apparent convergence pattern was consistent with the one observed for NH₃ flux in Figure 4A.

3.6 Soybean Peroxidase Activity

The soybean peroxidase activity was determined on the SBP that had been applied to manure for 88 days to determine if there was still an enzymatic activity after that duration of exposure to the swine manure. The resulting activities for the SBP that had formed a moist crust on the manure, thus being directly in contact with the manure (i.e., doses 2.28, 4.57 and 45.7 kg/m²

SBP), were much lower, i.e., 3.2×10^{-6} , 6.4×10^{-6} and 12×10^{-6} U/mg respectively, than that of the 22.8 kg/m² SBP/CaO₂ application rate (29.6×10^{-6} U/mg), which had dry SBP still floating on the surface of the manure. All SBP activity after 88 days of exposure to swine manure were considerably lower than that of unexposed SBP, which had an activity of 0.0153 U/mg. This suggests that SBP activity is affected by contact with manure and is likely responsible for the treatment effect. Steevensz et al. (2013) reported that grinding/mixing preparation of the SBP and soybean variety affects its useful activity based on the survey of 65 different cultivars. Thus, it is important to consider these variables for optimal treatment effectiveness.

3.7 Manure Analysis

Nutrient conservation is an important parameter for any type of manure treatment that will be later used for field application. As compared to the control, this study resulted in significantly higher TKN levels in the manure for the two highest application rates, but no significant difference for the two lower application rates ($p \geq 0.218$). These differences were not due to the SBP conserving the N that was in the manure but that the SBP was contributing N to the manure. If the TKN levels are adjusted for the amount of N contributed by the level of SBP there was no statistical difference between the SBP treatments. The observed reduction in NH₃ does not add up a high enough percentage to have an effect on the TKN levels in the manure (Table 3).

3.8 Effects of SBP Application Rate

There was a positive correlation between % reduction and SBP application rate for NH₃ and a negative correlation between % reduction and SBP application rate for CH₄, and CO₂. For the rest of the gases, the treatment effect starts to reach an apparent plateau for SBP application rates

314 > 4.57 kg/m² (Figure 7). A strong correlation was also observed for H₂S, DMDS/MT, *n*-butyric
315 acid, valeric acid, isovaleric acid and *p*-cresol for the lowest three SBP application rates, i.e.,
316 when 0, 2.28, and 4.57 kg/m² were taken into account (Table S3). This suggests that larger SBP
317 application rates are not necessarily resulting in a proportional treatment effect. Lower SBP
318 application rate might be sufficiently effective especially when economic feasibility for farm
319 application are considered.

320 **Table 3.** Physical/chemical properties of swine manure.

SBP	TS		VS		Volatility*		COD		pH		NH3-N		TKN		PO ₄ -P		TP	
(kg/m ²)	(%)		(%)		(%)		(mg/L)				(mg/L)		(mg/L)		(mg/L)		(mg/L)	
	Before	D137	Before	D137	Before	D137	Before	D137	Before	D137	Before	D137	Before	D137	Before	D137	Before	D137
0	9.7 ^a	4.3 b	8.4 a	2.9 b	86.0 a	66.8 b	41900 a	17600 a	7.5 a	7.6 a	8550 a	6840 a	11500 a	7320 c	147 a	NA	393 a	NA
2.28	6.6 a	4.0 b	5.3 a	2.7 b	79.7 ab	65.7 b	39400 a	17200 a	7.5 a	7.6 a	11900 a	7300 a	15000 a	7690 bc	146 a	NA	413 a	NA
4.57	8.7 a	3.9 b	7.4 a	2.6 b	82.7 ab	65.5 b	39200 a	17700 a	7.5 a	7.6 a	11200 a	6680 a	15200 a	7690 bc	145 a	NA	395 a	NA
22.8	5.9 a	4.1 b	4.7 a	2.7 b	77.7 b	66.3 b	39100 a	19000 a	7.6 a	7.6 a	10400 a	8000 a	13000 a	7970 b	152 a	NA	413 a	NA
45.7	7.7 a	5.2 a	6.4 a	3.6 a	81.3 ab	69.4 a	41900 a	19600 a	7.5 a	7.4 b	11200 a	7280 a	13000 a	8640 a	157 a	NA	386 a	NA

321 ^a Means for each parameter followed by different letter are significantly different at $p < 0.05$ for each column. D137 = Day 137.
322 Before = before SBP treatment application. TP = total phosphorus. TS = total solids. VS = volatile solids. * volatility =
323 VS/TS×100%.

3.9 Economics of SBP/CaO₂ Treatment

The resulting treatment costs range from \$1.45 to \$29.06 per marketed pig for the lowest to highest SBP application rate, respectively. These costs are based on \$191/metric ton for bulk soybean hulls (University of Missouri Extension, 2014), \$6.00/ton for grinding of bulk soybeans, and \$6.48/kg for bulk CaO₂ (American International Chemical, Inc., Framingham, MA). These costs do not include labor required to apply the treatments, which could vary greatly depending on method of application. Cost estimates for mitigation additives are relatively few and often limited to one or few target gases reported. The \$1.45/marketed pig price estimate is within the range of \$0.01 to \$18.2 price estimates (Table S4) with an average additive cost of \$4.28 ± \$5.80 for manure additive treatment of odor, VOCs, NH₃, and H₂S (Balsari et al., 2006; Heber et al., 2000; Heber et al., 2001; Moreno et al., 2010). With the average carcass weight of a hog from 2010 through 2015 (USDA-AMS, 2016) being 94.9 kg (209.3 lbs) and the average market price over the same period of \$83.60/carcass hundredweight (Iowa State University Extension, 2016), the estimated cost percentage equates to 0.8-16.6% for the range of treatment rates (2.28 to 45.7 kg/m²) in this study.

4. Conclusions

This study provided a comprehensive assessment of a manure additive for mitigation of gaseous emissions of all major compounds of interest from swine manure. Significant reductions in NH₃, DMDS/MT, *p*-cresol and skatole were observed, with corresponding increases in greenhouse gases CH₄ and CO₂ and no effect on N₂O emissions. The SBP/CaO₂ treatment resulted in mitigation of most gases of concern to the swine industry, considering the complex

physical /chemical /biological processes that are at work in swine manure emissions. At a cost relative to 0.8% of a marketed hog it appears that SBP/CaO₂ treatment could be a promising option at the lowest (2.28 kg/m²) treatment rate for reducing odorous gas and NH₃ emissions at swine operations, and field-scale testing is warranted.

ASSOCIATED CONTENT

Supporting Information. Includes photos demonstrating treatment, details of methods and theory, emissions mitigation effects for gases where no significant effect was observed, and explanation of gas emissions from manure as a part of C, N and S cycles.

AUTHOR INFORMATION

Corresponding Author *Corresponding author: tel. (515) 294-4206, koziel@iastate.edu

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally.

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DISCLAIMER

USDA is an equal opportunity provider and employer. The mention of trade names of commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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ABBREVIATIONS

mdGC-MS-O, multidimensional gas chromatography-mass spectrometry-olfactometry; TD, thermal desorption; SBP/CaO₂, soybean peroxidase/calcium peroxide; GHG, greenhouse gas; TKN, total Kjeldahl nitrogen; CRD, completely randomized design; VOCs, volatile organic compounds; AMPAT, Air Management Practices Assessment Tool; RH, relative humidity; FID, flame ionization detector; ECD, electron capture detector; NH₃, ammonia; H₂S, hydrogen sulfide; CO₂, carbon dioxide; CH₄, methane, N₂O, nitrous oxide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide; MT, methanethiol.

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Figure Captions:

Figure 1. Schematic of the pilot-scale simulated deep-pit manure columns.

Figure 2. Top Row: Simulated manure storage – **control** (no SBP). Left: Day 14 after treatment, Middle: Day 88 after treatment, Right: Day 137 after treatment. Middle Row Simulated manure storage treated with **2.28 kg/m²** SBP application rate. Left: Day 14 after treatment, Middle: Day 88 after treatment, Right: Day 137 after treatment. Bottom Row: Simulated manure storage treated with **4.57 kg/m²** SBP application rate. Left: Day 14 after treatment, Middle: Day 88 after treatment, Right: Day 137 after treatment.

Figure 3. Top Row: Simulated manure storage treated with **22.8 kg/m²** SBP application rate. Left: Day 14 after treatment, Middle: Day 88 after treatment, Right: Day 137 after treatment. Bottom Row: Simulated manure storage treated with **45.7 kg/m²** SBP application rate. Left: Day 14 after treatment, Middle: Day 88 after treatment, Right: Day 137 after treatment. This relatively heavy layer of SBP had forced the SBP to crack the crust and roll into the top layer during periodic additions of “fresh” manure. This resulted in less manure surface coverage with the largest SBP application rate over time.

Figure 4. Measured ammonia (A), *p*-cresol (B), skatole (C) and DMDS/MT (D) flux as a function of SBP application rate and time. ◇: Control, ■: 2.28 kg/m², ▲: 4.57 kg/m², ●: 22.8 kg/m², ✱: 45.7 kg/m² SBP/CaO₂ application rate, ----- SBP/CaO₂ application.

Figure 5. Measured methane (A), carbon dioxide (B) and nitrous oxide (C) flux as a function of SBP application rate and time. ◇: Control, ■: 2.28 kg/m², ▲: 4.57 kg/m², ●: 22.8 kg/m², ✱: 45.7 kg/m² SBP/CaO₂ application rates, ----- SBP/CaO₂ application.

Figure 6. Measured pH (A) and relative humidity (B). ◇: Control, ■: 2.28 kg/m², ▲: 4.57 kg/m², ●: 22.8 kg/m², ✱: 45.7 kg/m² SBP/CaO₂ application rates, ----- SBP/CaO₂ application.

Figure 7. Effects of application rate on reduction of target gases. ◇: isovaleric acid, ■: H₂S, ▲: CH₄, ●: N₂O, ✕: skatole, ◆: NH₃, +: DMTS/MT, ■: CO₂, ○: DMDS/MT, —: valeric acid, ✱: *n*-butyric acid, □: *p*-cresol, △: indole. Highest application rate not shown due to no/minor improvement in target gas reduction.

	Day 14	Day 88	Day 137
No SBP
2.28 kg/m ² SBP
4.57 kg/m ² SBP

	Day 14	Day 88	Day 137
22.8 ² kg/m ² SBP
45.7 ² kg/m ² SBP

pH Units

Swine manure

Swine manure treated with
Soybean peroxidase/ CaO_2