Evaluation of additive for reducing gaseous emissions from swine waste

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Abstract: Additives can reduce gaseous emissions from swine waste lagoons and pits. We have demonstrated for the first time that an additive has the potential to reduce methane emission from an anaerobic swine lagoon. ManureMax®, a humate product was evaluated for its ability to reduce gas and odor emissions from swine anaerobic lagoon and barn flush-water. Four treatments, Control (no additive), Low (label dosage), Medium (50% higher than label dosage), and High (200% higher than label dosage) were applied to inclined pipes installed in a swine lagoon. While the Medium and High treatments were not effective, the Low treatment reduced methane, 2-butanone, and tetrahydrofuran concentrations by 34%, 44%, and 57%, respectively vs. the Control in the pipe headspace. Surface treatment was effective on methane for four weeks. ManureMax® was ineffective in reducing nitrous oxide and carbon dioxide concentrations in the headspace. It is unclear why only the Low treatment reduced concentrations of these gases in the lagoon though at least one study also reported similar findings with additive dosage. The Low treatment reduced chemical oxygen demand and total phosphorus in the lagoon supernatant probably due to chelation, flocculation, and/or oxidation. Applying treatments to pipes installed in a lagoon could be cost-effective way to compare treatments in replicated, mesocosm-scale studies in a lagoon. In the lab, all ManureMax® treatments reduced accumulation of three short-chain volatile fatty acids (VFAs) in the headspace of incubated glass bottles but only the High treatment reduced toluene accumulation (by 26%). The ability of ManureMax® to degrade long- and branch-chain VFAs requires further evaluation and its odor reduction potential should be tested using olfactometry.

Keywords: hog, mesocosm-scale, methane, 2-butanone, tetrahydrofuran, volatile fatty acids, toluene

1 Introduction

Swine farms emit several environmentally-important air pollutants such as, ammonia, methane, and volatile organic compounds (VOCs). These pollutants can contribute to odors (e.g., VOCs), climate change (e.g., methane), and eutrophication (e.g., ammonia). The United States Environment Protection Agency (EPA) may regulate air emissions including those of greenhouse gas (GHG) emissions from agriculture under the Clean Air Act. Air emissions from livestock farms can be reduced with dietary modification, improved management, exhaust air treatment, or waste additives. Whereas improved management (e.g., preventing overloading of swine waste lagoons) can have a relatively modest impact, dietary modification and particularly, exhaust air

treatment can be very expensive. If effective, waste additives (or amendments) can also be cost-effective because they require only minor management changes. A waste additive is any substance that when added to animal waste collecting in pits beneath swine barns, in storage tanks, or treatment lagoons, can reduce emission of the target compound/s through physical, chemical, and/or biological means. While new waste additives are regularly introduced into the market, claims of their effectiveness are usually not supported in impartial, controlled studies.

JDMV Holdings of Houston, TX, requested us to evaluate ManureMax® for its ability to reduce gaseous emissions from swine farms and improve lagoon chemical properties. As per the manufacturer, ManureMax® (12.02% humic acids, 1.44% potassium, 0.61% sodium, 0.13% phosphorus, 0.11% nitrogen, 0.004% iron, and 85.35% inert) is a biological activator derived from humates that enhances microbial activity, decreases volatile ammonia and other gas emissions, reduces odor and improves the nitrogen to phosphorus ratio in the waste.

Research on using additives for mitigating pollutant and odor concentrations or emissions from animal waste is not new. WonderTreat[TM], a combination of yeasts reduced odor emissions from livestock waste in the lab but not in the lagoon (Banhazi et al., 2009). A digestive amendment, Bio-Kat applied to swine waste pits improved animal performance, probably due to reduced ammonia concentration in the barn and it also reduced ammonia concentration in the lagoon (Schneegurt et al., 2005). Aluminum chloride solution added to swine waste pits can reduce barn ammonia concentration (Smith et al., 2004). Liao and Bundy (1994) reported that bacterial additives slightly reduced methane concentrations in the headspace of columns containing swine waste. There are no reports in the literature on additives that reduced methane emissions from swine waste lagoons. Additives that may be effective in the lab may not be effective in the lagoon or in different environmental or management conditions (Banhazi et al., 2009). Hence, given the complexity of the lagoon environment, the effectiveness of an additive should be based on its performance in the lagoon.

The label of ManureMax® claimed that that its addition would reduce emissions of ammonia and other gases, including odor. To test the additive's label claims, the research was performed in the lagoon and laboratory. The objectives for the lagoon study were to determine the effect of ManureMax® dosage on (1) concentrations of methane, nitrous oxide (N_2O), carbon dioxide (CO_2), and VOCs near the lagoon liquid surface, and (2) chemical properties of lagoon liquid in a replicated, mesocosm-scale study. The replicated laboratory study was used to evaluate the effect of ManureMax® on headspace VOC concentrations in glass bottles. In the lagoon study, since the open surface area was equal for all the experimental units, we inferred that relative emission would be directly proportional to the headspace concentrations since the main driving force for emission would be the concentration gradient.

2   Materials and methods
This research was conducted at North Carolina State University's (NCSU) Swine Education Unit in Raleigh, NC, during spring 2010. The Unit had ~1,500 pigs (farrowing-to-finish) with a steady-state live weight of ~71,300 kg. The swine barns were flushed four times daily using supernatant from an anaerobic lagoon (~24.9 million L) that was also used for waste treatment.
2.1 Lagoon study

The impact of ManureMax® dosage (discussed below) on lagoon liquid was evaluated in 12 PVC pipes (ID = 102 mm, length = 6.1 m) placed in the lagoon at an angle of 38° from the horizontal (Figure 1); hereafter, each PVC pipe is referred to as an experimental unit (EU). The vertical depth of submergence was ~1.9 m; hence, each EU held a liquid volume of ~25 L. The 12 EUs were placed at a location where the 2009 sludge survey had indicated a relatively uniform sludge depth of ~1 m. All EUs were assumed to have identical volume and composition of lagoon liquid and since, the sludge would plug the bottom the pipe each EU was assumed to represent an isolated lagoon mesocosm.

Figure 1 Placement of the experimental units (EUs) in the lagoon. The EUs are capped at the top with a threaded PVC connector glued to the pipe.

Four treatments (Control, Low, Medium, and High) were assigned to the 12 EUs randomly and each treatment was replicated three times. The Control treatment received only 87.5 mL of barn flush-water twice daily (8:30 AM and 4:00 PM) during the workweek to replicate daily waste loading to the lagoon. The flush-water contained fresh feces and spilled feed in lagoon supernatant. The Low treatment received a label surface loading rate of 204 mL/m^2 on 12 March as well as 0.1 mL of the additive ManureMax® plus 87.5 mL of flush-water twice daily. Compared to Low, the Medium and High treatments received 50% and 200%, respectively, more of initial surface and daily additive applications in flush-water. The initial application was made using 1:19 mixture of ManureMax® in tap water while the daily additive volume (1:39 mixture of additive in tap water) was recommended by JDMV (Suttle, 2010) based on the steady-state live weight of the pigs and size of lagoon. The initial dosage was applied through a 6.1 m long (ID = 20 mm) PVC pipe on the surface and the daily dose was applied using the same pipe with the mixture being released in the top 0.3 m of the lagoon liquid in the EUs. No additive was applied to the rest of the lagoon.

During 16 March through 19 April 2010, methane, ammonia, N_2O, and CO_2 concentrations were measured in each EU at 11.5-min intervals on Mondays, Wednesdays, and Fridays with a photoacoustic (PAS) sensor (Model 1412, Innova Air Tech Instruments, Ballerup, Denmark; detection limits were: methane 0.4 ppm with filter # UA 0969, ammonia 0.2 ppm with filter # 0976, N_2O 0.03 ppm with filter # 0985, CO_2 5.1 ppm with filter # UA0983). The PAS was set to compensate for interferences. Measurements began after addition of flush-water in the morning for an average of 5.3 h per sampling period for a total of 96 h over 17 periods. Air samples were

drawn through sampling lines ~0.3 m above the liquid surface in uncapped EUs to replicate natural conditions. When data from one EU was transferred for storage to the data logger (Campbell Scientific CR 1000), the line was purged to eliminate cross-contamination; thereafter, the data logger switched the sampling line to the next EU. After completing the PAS measurements, flush-water (with or without the additive) was added and the EU was capped (to maximize headspace VOC concentrations) on Monday and Wednesday evenings for collection of air samples in Tedlar bags on Tuesday and Thursday mornings for VOC analyses. Except for those two periods, the EUs were always kept uncapped to allow natural exchange.

Since the sampling periods varied from 2.5 to 7.8 h, the weighted-average concentration was calculated for each sampling period by multiplying the sampling period-average concentration by the sampling period for that day and dividing it by the average sampling period (5.3 h). The weighted-average concentrations of the treatments for the 17 periods were compared using repeated measures analysis of variance (RM-ANOVA) (SAS, 2010) at $\alpha = 0.10$. If at least one treatment was significantly different, least-squares means were compared using Tukey's honestly significant difference (HSD). Hood (2011) observed that the PAS required ~15 min to detect changes in ammonia concentrations but only ~1 min to detect changes in methane, CO_2, and N_2O concentrations. Since the PAS sampling interval was 11.5 min, treatment effects on ammonia concentrations were not evaluated.

To analyze for VOCs, 1 L of air collecting in the capped head space of the EU overnight (on March 17, the EUs had been capped during daytime for 2.5 h) was transferred to a Tedlar bag for analyses of VOCs using the same sampling line used for PAS measurements. These samples were stored in a cooler at 4°C if not immediately analyzed. The samples were analyzed in a gas chromatograph – mass spectrometer (GC-MS) equipped with a HP5-MS column (30 m long × 0.25 mm diameter × 0.25 μm film thickness) (Agilent Technologies, Santa Clara, CA). The chromatographic conditions were: He flow: 1 mL/min, inlet: 250°C, column: 80°C - 325°C @ 10°C/min and held for 2 min at 325°C, and detector: 280°C. All data were acquired using a full scan mode (m/z: 40-450). For each sampling event, the first sample (Low treatment) was run for 30 min and if peaks were not observed after 8 min, the remaining samples were run for 8 min. Outputs were area units and the treatments were compared using RM-ANOVA and Tukey's HSD (SAS, 2010). A total of 10 sets of samples were collected but the last three sets (8, 13, and 15 April 2010) were discarded because of GC-MS breakdown.

Air temperature was measured near the EUs during the study. Just before treatment application, samples of the lagoon supernatant and sludge from the immediate vicinity of the EUs were analyzed to characterize initial properties (Table 1). The supernatant was sampled using a stoppered 250 mL HDPE sample bottle tied to a pole that was un-stoppered with a string at the desired depth. The sludge was sampled using a 13 mm PVC pipe which was stoppered at the top to create a vacuum and hold the sludge sample as it was withdrawn from the lagoon.

Table 1 Properties of the swine anaerobic lagoon sampled on 12 March 2010.

Property[1]	Supernatant[2]	Sludge	Method and reference
TKN (mg/L)	142±5[3]	1040±809	Persulfate digestion and ammonia salicylate method for automated analysis, Standard Methods 4500N$_{org}$ B (APHA, 1998)
TAN (mg/L)	111±0	352±112	Ammonia salicylate method for automated analysis,

			Standard Methods 4500NH3 G (APHA, 1998)
TK (mg/L)	242±6	383±137	HNO₃ digestion followed by emission spectrometry, Standard Methods 3111-B (APHA, 1998)
TP (mg/L)	46±1	1775±621	Persulfate digestion and ascorbic acid method for automated analysis, Standard Method 4500-P F (APHA, 1998)
Ortho-P (mg/L)	32±1	743±163	Ascorbic acid method for automated analysis, Standard Method 4500-P F (APHA, 1998)
COD (mg/L)	756±26	32267 ±15119	Potassium dichromate - H_2SO_4 digestion and colorimetric analysis, Hach Method 8000 (EPA approved)
pH	7.5±0.1	NM[4]	Electrometric method, EPA Method 150.1 (1979)
TS (%)	0.13±0.01	43.56±0.49	Gravimetric, Standard Method 2540 B (APHA, 1998)
FSS (mg/L)	295±39	NM	Glass fiber filtration followed by drying at 103-105°C, Standard Method 2540 D (APHA, 1998)

[1]TKN: total Kjeldahl N, TAN: total ammoniacal N, TK: total potassium, TP: total phosphorus, COD: chemical oxygen demand, TS: total solids, FSS: fixed suspended solids.
[2]Supernatant samples were obtained from ~1.25 m depth; the top layer (0.3 m) was also sampled but its properties are not presented because the column and top layer properties were within 5% of one-another
[3]Mean ± SD (n = 3)
[4]Not measured

At the end of the study, the depths of the lagoon liquid and sludge in each EU were measured and the lagoon surface liquid (top 0.15 m), column liquid (0.6 m below the surface), and sludge were sampled for the same constituents listed in Table 1. The surface and column liquids were sampled as described earlier. The sludge was sampled with a 60 mL plastic hypodermic syringe with its inlet enlarged to ~10 mm and tied to the tip of a pole; the syringe was lowered into the sludge and its plunger was retracted with a string. We observed that the sludge sampling procedure was very satisfactory. Treatment effects on effluent properties were analyzed using ANOVA and Tukey's minimum significant difference (MSD) (SAS, 2010). Comparison of initial (Table 1) and final chemical properties should be undertaken with caution because their sampling methodologies and locations were different.

2.2 Laboratory study
Homogenized swine barn flush-water (90 mL) was poured into a 125 mL narrow-mouth glass bottle; hence, based on headspace volume, ~106 mg-O_2/L of flush-water was available for aerobic activity. ManureMax® was added at the rate of 0, 5.0, 7.5, and 15.0 mL/L to the flush-water in the bottles in the Control, Low, Medium, and High treatments, respectively with each treatment replicated thrice. While the Low treatment received a concentration of 0.5% (v/v) following the label instruction for daily use in swine barn pits, the Medium and High treatments received 50% and 200%, respectively, more additive than the Low treatment. These bottles were immediately sealed with septum caps, crimped, and incubated in an oven at 37°C for 48 h. After 48 h, 500 μL of air from the head space of each bottle was withdrawn with a gas-tight syringe and injected into the GC-MS for analyses of dissolved gases. The first sample (Low treatment) was run for 30 min and since peaks were not observed after 8 min, the remaining samples were run for 8 min. Finally, the dissolved organics were extracted in neat hexane (30 mL) and analyzed using a GC-MS.

3   Results and discussion

3.1 Liquid and sludge volumes in the EUs

The study was designed to ensure that all EUs held equal volumes of lagoon effluent (25 L) but differences in final liquid volumes (Table 2) indicated that the EUs leaked through the sludge 'plugs' at the bottom, at slightly different rates. Difference in leakage rates may have been due to an uneven lagoon bottom underlying the sludge. Difference in sludge volumes, particularly, between Low and other treatments (Table 2) could be due to random undulations in sludge levels. Despite random assignment of treatments, the three EUs receiving the Low treatment were located together; interestingly, while one of those treatments had a final liquid volume of 21 L, the other EUs located with 0.25 m had 26.2 L each. However, the average liquid volumes (Table 2) were within ±6% of the average of the four treatments indicating that with some refinements, such a design could be cost-effective for conducting replicated mesocosm-scale studies in lagoons.

Table 2 Final liquid volumes, final sludge volumes, and average liquid volumes in the EUs.

Treatment	Final liquid volume (L)	Final sludge volume (L)	Average liquid volume (L)
Control	20.0±2.0[1]	6.9±1.8	22.5[2]
Low	24.5±3.0	4.4±0.7	24.7
Medium	22.4±1.9	7.3±1.7	23.7
High	23.9±2.2	7.6±2.0	24.4

[1]Average (n=3) ± standard deviation

[2]Average of initial (25 L) and final liquid volumes

To check if difference between the final liquid surface (due to liquid volumes) and the air sampling tube inlet affected gas concentration measurements, final liquid depths in the individual EUs were plotted against average methane and CO_2 concentrations separately during the last week (three sampling periods) when the difference in liquid levels among the EUs would be higher than average. Methane was the lightest and CO_2 was the heaviest of the gases measured by the PAS; further, as discussed later, methane concentrations were affected by the treatments while CO_2 concentrations were not affected by the treatments. For both gases, the plots of final liquid depth vs. concentration yielded r^2 values of 0.31 to 0.34. When methane and CO_2 concentrations during the last week were plotted vs. sludge depths, r^2 values were 0.03 and 0.30, respectively. Thus, neither liquid nor sludge depth may have confounded the treatment effect.

3.2 ManureMax® concentration effects on headspace methane, N_2O, and CO_2 concentrations

Headspace concentrations of methane, N_2O, and CO_2 in the EUs installed in the lagoon that received different concentrations of ManureMax® are shown in Table 3. Treatment effects were only significant ($p < 0.01$) for methane, but not for N_2O and CO_2. The Low treatment had significantly (Tukey's HSD) lower methane concentrations than all other treatments while High ≈ Control, Control ≈ Medium, High > Medium (Table 3).

Table 3 Headspace methane, N_2O, and CO_2 concentrations in the four treatments over 17 sampling events.

Treatment	Concentration[1] (mg/m^3)

	Methane	N$_2$O	CO$_2$
Control	42.3±11.1ab[2]	0.83±0.08	842±13
Low	27.9±0.3c	0.82±0.04	856±23
Medium	38.4±8.6b	0.77±0.02	843±9
High	50.0±22.0a	0.81±0.03	854±33
p-value[3]	<0.01	0.22	0.20

[1]Grand average concentration (based on 1491 measurements for each treatment) for entire study ± standard deviation (n = 3). The grand average concentration is the arithmetic average for 17 weighted-average concentrations.
[2]Treatment means followed by the same letter in the column are not significantly different at α = 0.1using Tukey's honestly significant difference (HSD) (least square means)
[3]Repeated measures ANOVA (α = 0.1).

Lower methane concentrations in the Low vs. Control treatment may be due to Cu chelation by humates in ManureMax® since Morton et al. (2000) reported that Cu chelation improved methanotrophic activity. Humic substance, an important constituent of the additive could have also acted as terminal electron acceptor, increasing oxidation of organic matter to CO$_2$, resulting in a proportionate decrease in methane formation (Keller et al., 2009). Inhibition of methanogenic bacteria by dissolved ammonia and NH$_4^+$ concentrations (Angelidaki and Ahring, 1993) seemed unlikely since neither specie was in high-enough concentration (based on pH and TAN, Table 1). Higher methane concentrations in the High and Medium treatments (vs. Low) (Table 3) is counterintuitive suggesting either substrate inhibition or other negative interaction between ManureMax® and the methanogens. Banhazi et al. (2009) also measured a modest reduction in odor emission from lagoon effluent at the label application rate of the additive WonderTreat^{TM} but not at double the application rate.

All other conditions being the same, emissions would be proportional to headspace concentrations; hence, it could be reasonably assumed that the Low treatment could reduce methane emissions by 34% vs. Control. Because the Low treatment had the greatest liquid volume (Table 2), it should have produced the most methane since methane production occurs throughout the column (Hamilton et al., 2006). Hence, the 34% reduction in methane concentration in the Low treatment vs. Control might be an underestimation. This is significant, because to our knowledge, this is the first study in which an additive reduced methane emissions in a swine anaerobic lagoon.

The sampling period average (not weighted-average) methane concentrations in the EU headspace, for all the treatments and the average daily air temperature are presented in Figure 2. Methane concentration trends for all the treatments are similar (Figure 2). Methane emissions (and hence, concentrations in the headspace) should increase with temperature due to reduced solubility and greater microbial activity. However, air temperature and methane concentration were not positively correlated (Figure 2) because while the temperature data was based on 24 h, methane concentrations were measured from mid-morning to early-afternoon and the lagoon temperature would lag behind the air temperature. Rapid rise in methane (also other gases) concentrations between 29 and 31 March may be due to two of the coldest mornings (12:00 midnight – 8:39 AM) on March 27 (2.4°C) and 28 (4°C) followed by a very warm morning on March 29 (14.1°C). Because gas solubility increases with decreasing temperature, the generated gases stayed dissolved in the lagoon column and when the column warmed up on March 29, the dissolved gases were likely rapidly emitted. Finally, beginning 9 April, difference between

methane concentrations in the Control and Low treatment seemed to decrease (Figure 2) indicating that the surface application was effective for about four weeks.
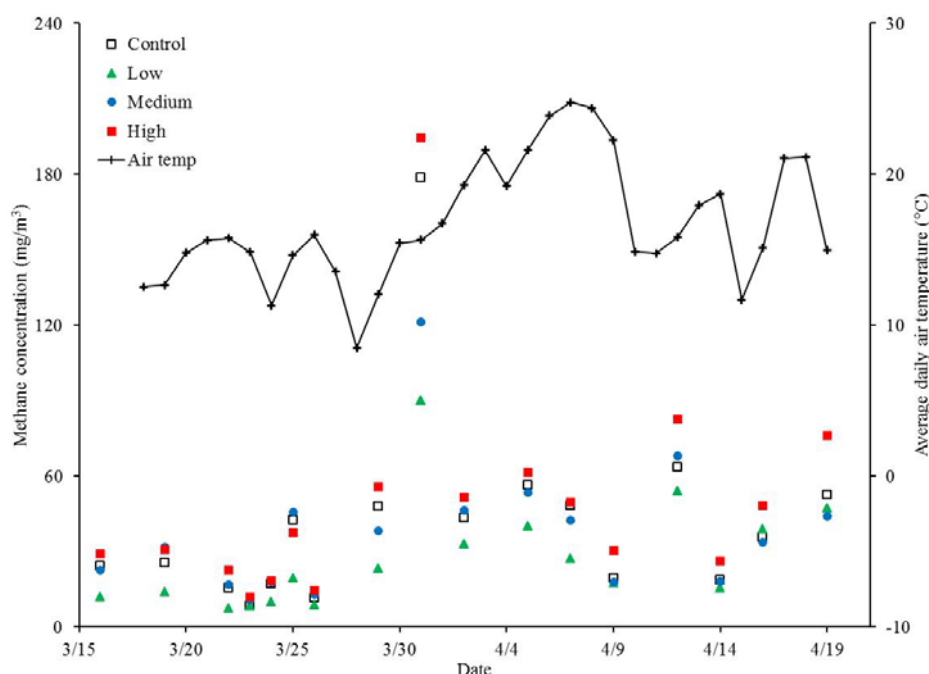

Figure 2 Methane concentrations in the head space of the four treatments during spring 2010. Each concentration data point is the average of sampling period average concentrations of the three replications in a treatment. Average daily air temperature (360 measurements per day during 3/17 to 4/18) is also plotted. Temperature data points are connected only to show trend.

3.3 Effect of ManureMax® dosage on VOCs emissions

Only two VOCs (2-butanone and tetrahydrofuran) were detected by the GC-MS in measurable quantities and with good fits (Table 4) in the seven sets of air samples collected in Tedlar bags from the headspace of the capped EUs (to concentrate VOCs). A hazardous air pollutant (HAP), 2-butanone (phenolic, skunky/ether type of odor) (Lo et al., 2008), is a degradation product of manure. Tetrahydrofuran has a faintly fruity, ether-like odor (OSHA, 2011) and is a constituent of antibiotics and growth promoters fed to pigs (Bioagrimix, 2011).

Table 4 2-butanone and tetrahydofuran in the air samples in the treatments over seven sampling events.

Treatment	Concentration[1] (area units)	
	2-Butanone	Tetrahydrofuran
Control[2]	6437±1307a[3]	4339±945a
Low[2]	3592±1143b	1855±250b
Medium[2]	6472±1280a	3745±638a
High	7486±1440a	4325±678a
p-value[4]	<0.01	<0.01

[1]Grand average based on up to 21 measurements (3 reps per treatment, 7 events) ± standard deviation (based on the grand averages of the three replications).
[2]One replicate was lost for Control, Low, and Medium treatments on 30 March and one replicate was lost for the Low treatment on 1 April.

The Low treatment significantly ($p < 0.01$) reduced concentrations of 2-butanone and tetrahydrofuran vs. other treatments (Table 4). These observations are consistent with reduction in methane concentrations with the Low treatment vs. Control but not the other ManureMax® treatments. Since chelating agents inhibit 2-butanone (Patel et al., 1980), chelation by the humate in ManureMax® may have reduced 2-butanone concentration. The additive may also have created more favorable conditions for the microbial degradation of 2-butanone (Onaca et al., 2007) and tetrahydrofuran (Daye et al., 2003) although require additional work is required to elucidate the precise mechanism of action. Banhazi et al. (2009) also reported reduced livestock waste odor emission in the lab with the low dose of the additive WonderTreat[TM] (combination of yeasts) but none with double the dosage.

Based on average concentrations in the treatments (Table 4), the Low treatment may reduce emissions of 2-butanone and tetrahydrofuran emissions by 44% and 57%, respectively, vs. Control. Average concentrations of 2-butanone and tetrahydrofuran in the four treatments are shown in Figure 3. Because the samples on 17 March were collected after capping the headspace for 2.5 h vs. overnight capping on 19 March, VOC concentrations for all treatments were much higher for 19 March. Changes in concentrations over time for all the treatments were similar (Figure 3). It is unclear why VOC concentrations tracked air temperatures on 23 and 25 March but not on 30 March, 1 April and 6 April (Figure 3). On 6 April, concentrations of both VOCs in the Low treatment exceeded the Control treatment (Figure 3) indicating that the additive might be no longer effective.
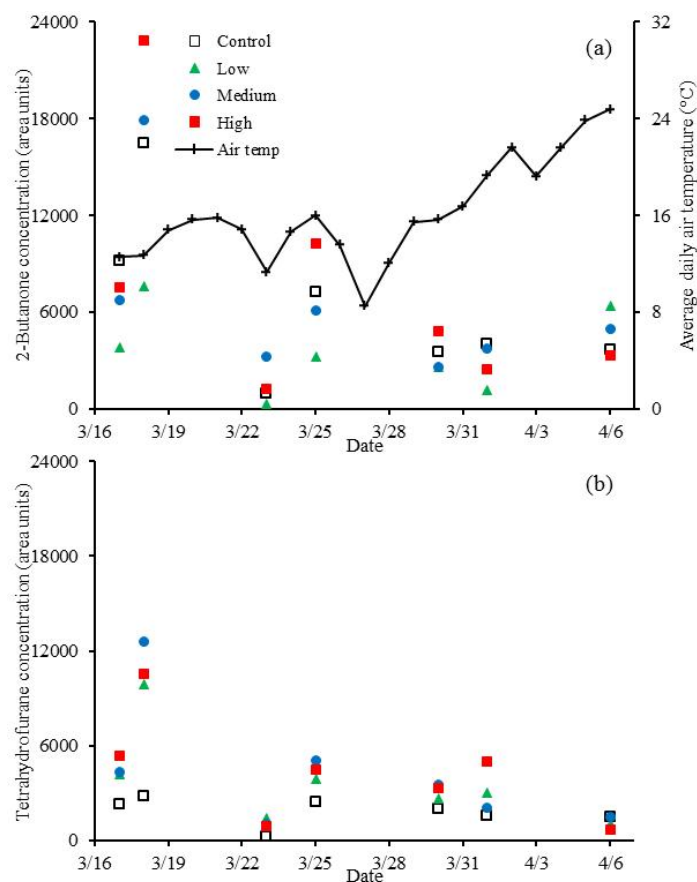
Figure 3 Average daily air temperature and concentrations (area units) of (a) 2-butanone and (b) tetrahydrofuran as affected by treatment during 3/30 – 4/6/2010. Each temperature data point is based on 360 measurements while concentration data point is the mean of three replications except on 3/30 when the averages for the Control, Low, and Medium treatments are based on two replicates; the average on 4/1 for the Low treatment is based on two replicates.

3.4 Effect of ManureMax® on lagoon chemical properties

The ManureMax® treatments did not affect the sludge though they impacted the supernatant (surface and column) to some degree (Table 5). There was no treatment effect on pH in the surface (top 0.15 m), or column (at 0.6 m depth) (Table 5). Virtually identical pH in all treatments (Table 5) precludes the effect of pH on methanogenic or methanotrophic activity. The Low and High treatments significantly reduced COD concentrations than the other treatments in the supernatant (Table 5) which might be due to flocculation and oxidation by ManureMax®, as claimed by the manufacturer. Being negatively-charged colloids, humates tended to flocculate in the presence of cations (Carlsen et al, 1992) and swine anaerobic lagoon effluent has high concentrations of cations, e.g., Ka (NCSU, 2012). But it was unclear why the Medium treatment showed no treatment effect (Table 5). Since COD provides the substrate for methane formation, reducing COD may reduce methane generation.

Table 5 Lagoon chemical properties[1] as affected by treatments at the end of study.

Treatment	pH	COD (mg/L)	TKN (mg/L)	TAN (mg/L)	TP (mg/L)	Ortho-P (mg/L)	TK (mg/L)	FSS (mg/L)	TS (%)
Top layer (top 0.15-m)									
Control	7.4±0.1[2]	839±98a[3]	205±10	164±6	69±4	60±3	250±3	421±103	0.16±0.01
Low	7.4±0.0	652±107b	205±22	175±16	60±3	57±3	255±15	293±49	0.15±0.02
Medium	7.4±0.1	865±38a	205±19	159±7	67±7	61±5	252±10	408±34	0.16±0.01
High	7.5±0.1	648±62b	194±7	157±11	68±4	63±4	237±5	322±76	0.14±0.01
p-value[4]	0.43	0.02	0.77	0.28	0.12	0.29	0.17	0.14	0.13
MSD[5]	NA[6]	180	NA	NA	NA	NA	NA	NA	NA
Column (0.6 m below surface)									
Control	7.6±0.1	836±59a	203±5	160±5	73±4a	53±7	254±8ab	425±50	0.15±0.01
Low	7.6±0.0	671±60b	210±27	172±18	60±4b	54±2	260±5a	331±63	0.15±0.01
Medium	7.6±0.1	871±86a	199±8	159±8	72±2a	60±2	260±4a	406±51	0.16±0.03
High	7.7±0.1	733±33b	194±9	156±10	72±6a	58±6	243±3b	353±49	0.13±0.02
p-value	0.40	0.02	0.66	0.37	0.02	0.31	0.02	0.19	0.25
MSD	NA	138	NA	NA	10	NA	12	NA	NA
Sludge									
Control	7.1±0.0	27267±5052	1945±168	234±61	1524±390	261±38	280±60	-[7]	-
Low	7.3±0.2	26600±2390	1911±260	291±44	1511±170	197±83	300±35	-	-
Medium	7.2±0.2	37700±4838	2652±504	302±116	1673±276	314±271	287±31	-	-
High	7.0±0.0	37967±12440	2597±1183	367±119	2058±403	568±332	253±31	-	-
p-value	0.31	0.20	0.40	0.41	0.21	0.25	0.58	NA	NA
MSD	NA	NA	NA	NA	NA	NA	NA	NA	NA

[1]COD: chemical oxygen demand, TKN: total Kjeldahl N, TAN: total ammoniacal N, TP: total phosphorus, TK: total potassium, FSS: fixed suspended solids, TS: total solids.
[2]Average (n = 3) ± standard deviation.
[3]Treatment means followed by the same letter in the column are not significantly different at α = 0.1 using Tukey's minimum significant difference (MSD).
[4]Repeated measures ANOVA (α = 0.1).
[5]Tukey's minimum significant difference (MSD).
[6]Not applicable
[7]Not measured

Since ManureMax® is claimed to promote flocculation, reduced TKN levels in the surface and column layers had been expected that was not substantiated in any layer (Table 5). No reduction in dissolved ammonia (or TAN) was observed as claimed on the label. Total P concentrations were significantly lower in the Low treatment vs. the other treatments only in the column. While total P concentrations can be reduced through flocculation, the lack of treatment effect in the Medium and High treatment vs. Control was unclear. There was no treatment effect on ortho-P concentrations (Table 5). In the column layer, the High treatment had significantly lower total K concentrations than Low and Medium but not the Control treatment (Table 5) which may be due to flocculation. ManureMax® had been expected to reduce TS and FSS through flocculation but that was not observed in any layer (Table 5).

Reduced COD, total P, or total K concentrations in the supernatant by one of the ManureMax® treatments showed that the additive may have some chelating, oxidizing, and/or flocculating effect. However, lack of a monotonic treatment effect was unclear. We observed relatively high within-and between treatment variability in sludge chemical properties (Table 5) which may be partly attributed to differences in sludge depths. Both within- and between-treatment variability in the column and top layers samples were much lower than the sludge samples, particularly for the dissolved constituents (e.g., TAN, ortho-P).

3.5 Effect of headspace and dissolved VOCs in flush-water in the lab

Gas samples in the headspace of the glass bottles containing barn flush-water analyzed on the GC-MS provided the best match ($\geq$80%) for toluene (plus benzene). Toluene and benzene are both HAPs found in swine manure headspace (Lo et al., 2008). Because the benzene and toluene peaks were contiguous and could not be separated, their mixture is referred to as toluene. Toluene concentration in the headspace was significantly affected by treatment (p = 0.06) and the High treatment significantly reduced toluene accumulation in the headspace of the flush-water vs. Control but not the other treatments (Figure 4).
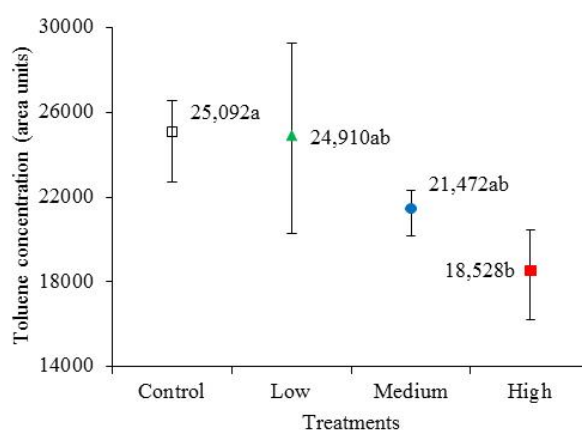

Figure 4 Treatment effects on toluene concentrations in the headspace of swine barn flush-water incubated at 37°C for 48 h. Each data point is the average of three replications. The dashes indicate spread of data. Treatment means followed by the same letter are not significantly different at α = 0.1. Tukey's minimum significant difference (MSD) was 6,123 area units.

Compared to the lagoon study, where mostly the Low treatment was effective, in the lab, the response seemed to be monotonic with toluene accumulation declining with increasing ManureMax® application rate (Figure 4). Since humates provide chelation (Kang and Hua, 2005) and aid oxidation, ManureMax® may have aided some toluene oxidation. However, toluene is very difficult to degrade and that probably explains why only 26% of the toluene was degraded at the highest ManureMax® application rate vs. Control. The degradation pathways of benzene and toluene are similar and yield similar end products. Toluene can be degraded both biotically and abiotically (Anderson et al., 1991) under both aerobic and anaerobic conditions. For example, toluene can be broken down into volatile fatty acids (VFAs) by lithotrophic nitrifiers (Zepeda et al., 2006).

In addition to toluene, three VFAs (acetic, propanoic, and butanoic acids) and acetaldehyde were detected in the headspace of the flush-water samples with matches $\geq$52%. All additive treatments significantly reduced (or eliminated) levels of all VFAs (Table 6). While the Low and Medium treatments completely eliminated the VFAs (Table 6), presence of acetic acid in one High treatment replicate (Table 6) may be due to greater degradation of toluene (vs. other treatments) (Figure 4), resulting in formation of acetic acid. As with toluene, SD values were high in the Control treatment with one of three replicates yielding zero concentrations of propanoic and butanoic acids. Such high SD values were likely due to variability in solids concentrations in the glass bottles.

Table 6 Treatment effects on VFA and acetaldehyde concentrations (area units) in the headspace of incubated swine barn flush-water incubated at 37°C for 48 h.

Constituent	Control	Low	Medium	High	p-value[1]	MSD[2]
Acetic acid	47757±20704[3]a[4]	0b	0b	10786±18682b	<0.01	30867
Propanoic acid	24848±23053a	0b	0b	0b	0.07	25516
Butanoic acid	11451±11356a	0b	0b	0b	0.09	12569
Acetaldehyde	0	6129±10615	1486±1808	659±571	0.53	-

[1]ANOVA ($\alpha = 0.1$).
[2]Tukey's minimum significant difference
[3]Average (n = 3) ± standard deviation
[4]Treatment means followed by the same letter in the row are not significantly different at $\alpha = 0.1$ using Tukey's minimum significant difference (MSD).

Acetaldehyde accumulation in the headspace was unaffected by the treatments (Table 6) despite its absence in the Control treatment. In the ManureMax® treatments, acetaldehyde was detected in one or two replicates and its concentration varied widely, resulting in high SD (Table 6). Since acetaldehyde is an intermediate step in the complete breakdown of VFAs into CO_2 and methane, breakdown of VFAs in the ManureMax® treatments, probably resulted in its accumulation in those treatments.

The GC-MS analyses of the incubated flush-water samples indicated the presence of dissolved toluene in all the samples (Figure 5). However, very high within-treatment variability masked treatment effect. Toluene was detected at 3.19 min and next peak was observed at 13.72 min with additional peaks appearing at 58.49 min. The peaks that appeared after toluene were mostly due to daughter compounds of hexane that was used to extract the liquid phase dissolved gases. Some samples contained benzoic acid, a product of toluene breakdown but the matches were poor and so, no statistical analyses were performed. The 48-h incubation at 37°C may have resulted in the breakdown of most VOCs, with the more recalcitrant toluene persisting both in the air and dissolved phases.
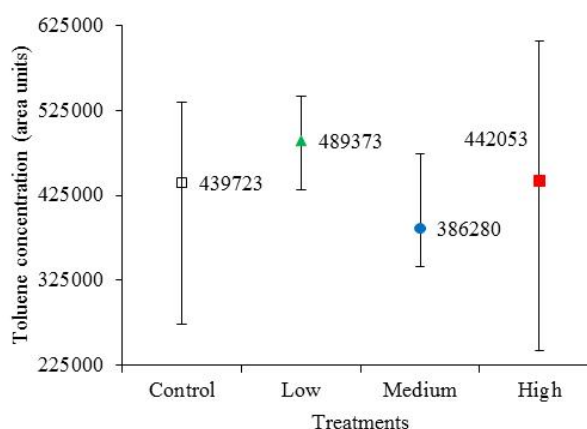

Figure 5 Treatment effects on toluene concentrations in the liquid phase of swine barn flush-water incubated at 37°C for 48 h. Each data point is the average of three replications. The dashes indicate maximum and minimum values.

ManureMax® showed potential, at very high application rates (200% above label dosage) to partially degrade toluene at 37°C in the lab so there is a need to evaluate its effectiveness in the lagoon. ManureMax® seems effective in reducing short-chain VFA concentrations under the test conditions and may reduce odor from hog barns with shallow pit systems. However, lagoon studies using olfactometry are required to quantify odor reduction because odors are predominantly due to long- and branched-chain VFAs (Zhu et al., 1997).

4   Conclusions

Effective lagoon and pit additives could be useful in reducing gaseous emissions from swine farms. A humate product, ManureMax® was evaluated at NCSU's Swine Unit during March - April 2010. The effect of four treatments (Control (no additive), Low (label dosage), Medium (50% higher than label dosage), and high (200% higher than label dosage)) on concentration of methane, N_2O, CO_2, and VOCs was determined. Experiments were performed in triplicate in a system of PVC pipes that was installed in the lagoon. Headspace gas concentrations and the lagoon chemical properties were monitored. At the label dosage rate, ManureMax® reduced methane, 2-butanone, and tetrahydrofuran concentrations by 34%, 44%, and 57%, respectively, when compared to control; emissions of these gases may be reduced comparably. Surface application of the additive at the label dosage rate seemed to be effective for four weeks. But higher dosage rates were not effective. ManureMax® had no effect on N_2O and CO_2 concentrations. We are unaware of any additive that has reduced headspace methane concentrations in a swine anaerobic lagoon. It was unclear why the Medium and High treatments were not effective in the lagoon. The Low treatment was generally the most effective in reducing COD and TP in the lagoon supernatant. With some refinement, applying treatments to pipes installed in lagoons could be a cost-effective way to conduct replicated, mesocosm-scale studies in a lagoon.

In the lab, only the High treatment showed a treatment effect, reducing toluene accumulation in the headspace by 26%. All three ManureMax® treatments reduced accumulation of three short-chain VFAs in the headspace and may thus reduce odor. After incubation, only toluene was detected in the dissolved phase in all treatments but there was no treatment effect.

There is a need to investigate mechanisms by which ManureMax® works. The impact of ManureMax® on odor reduction due to its ability to oxidize short-chain VFAs as well as its impact on long- and branched-chain VFAs needs to be investigated in the lagoon.

References
Anderson, T. A., J. J. Beauchamp, and B. T. Walton. 1991. Fate of volatile and semivolatile organic chemicals in soils: abiotic versus biotic losses. *J. Environ. Qual.,* 20 (2): 420-424.
Angelidaki, I., and B. K. Ahring. 1993. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Appl. Microbiol. Biotechnol.,* 38 (4): 560-564.

Banhazi, T., N. Hudson, M. Dunlop, C. Dyson, and R. Thomas. 2009. Development and testing of an evaluation procedure for commercial manure additive products. *Biosys. Eng.,* 103 (3): 321-328.

Bioagrimix. 2011. Monensin and Salinomycin in Veterinary Medicine - Overview. Available at: http://www.bioagrimix.com/haccp/html/ionophores.html. Accessed 6 December 2011.

Carlsen, L., P. Lassen, and V. Moulin. 1992. Flocculation behavior of humic substances in the presence of cations: consequences on the migration behavior of actinides in the geosphere. *Waste Management,* 12 (1): 1-6.

Daye, K. J., J. C. Groff, A. C. Kirpekar, and R. Mazumdar. 2003. High efficiency degradation of tetrahydrofuran (THF) using a membrane bioreactor: identification of THF-degrading cultures of *Pseudonocardia* sp. strain M1 and *Rhodococcus ruber* isolate M2. *J. Ind. Microbiol. Biotechnol.,* 30 (12): 705-714.

Hamilton, D. W., I. N. Kourtchev, P. M. Ndegwa, H. J. Cumba, F. Gioelli. 2006. Methane and carbon dioxide emissions from simulated anaerobic swine manure treatment lagoons under summer conditions. *Trans. ASAE,* 49 (1): 157-165.

Hood, M. 2011. Design and operation of a biofilter for treatment of swine house pit ventilation exhaust. M.S. thesis, Biological and Agricultural Engineering Dept., North Carolina State Univ., Raleigh.

Kang, N. and I. Hua. 2005. Enhanced chemical oxidation of aromatic hydrocarbons in soil systems. *Chemosphere,* 61 (7): 909-922.

Keller, J. K., Weisenhorn P. B., and Megonigal J. P. 2009. Humic acids as electron acceptors in wetland decomposition. *Soil Biol. Biochem.,* 41 (7): 1518-1522.

Liao, C. M. and D. S. Bundy. 1994. Bacteria additives to the changes in gaseous mass transfer from stored swine manure. *J. Environ. Sci. Health,* B29 (6): 1219-1249.

Lo, Y. C., J. A. Koziel, L. Cai, S. J. Hoff, W. S. Jenks, and H. Xin. 2008. Simultaneous chemical and sensory characterization of VOCs and semi-VOCs emitted from swine manure using SPME and multidimensional gas chromatography-mass spectrometry-olfactometry system. *J. Environ. Qual.,* 37 (2): 521-534.

Morton, J. D., K. F. Hayes, and J. D. Semrau. 2000. Bioavailability of chelated and self-adsorbed copper to *Methylosinus trichosporium* OB3b. Environ. Sci. Technol., 34 (23): 4917-4922.

NCSU. 2012. Livestock Manure Characterization Databases. North Carolina State University: Raleigh, NC. Available at: http://www.bae.ncsu.edu/programs/extension/manure/awm/program/mngmnt_sys.htm. Accessed 26 May 2012.

Onaca, C., M. Kieninger, K-H Engesser, and J. Altenbuchner. 2007. Degradation of alkyl methyl ketones by *Pseudomonas veronii* MEK700. *J. Bacteriol.,* 189 (10): 3759-3767.

OSHA. 2011. Occupational Safety and Health Guideline for Tetrahydrofuran. Occupational Safety and Health Administration: Washington, DC. Available at http://www.osha.gov/SLTC/healthguidelines/tetrahydrofuran/recognition.html. Accessed 6 December 2011.

Patel, R. N., C. T. Hou, A. I. Laskin, A. Felix, and P. Derelanko. 1980. Microbial oxidation of gaseous hydrocarbons: production of methylketones from corresponding *n*-alkanes by methane-utilizing bacteria. *Appl. Environ. Microbiol.,* 39 (4): 727-733.

SAS. 2010. SAS Version 9.2. Cary, NC: SAS Institute.

Schneegurt, M. A., D. L. Weber, S. Ewing, and H. B. Schur. 2005. Evaluating biostimulant effects in swine production facility wastewater. In *Proc. State of the Science of Animal Manure and Waste Management Symposium*, 1-6. San Antonio, TX: National Center for Manure and Animal Waste Management.

Smith, D. R., P. A. Moore, Jr., B. E. Haggard, C. V. Maxwell, T. C. Daniel, K. VanDevander, and M. E. Davis. 2004. Effect of aluminum chloride and dietary phytase on relative ammonia losses from swine manure. *J. Anim. Sci.,* 82 (2): 605-611.

Suttle, M. 2010. Personal communication. JDMV Holdings, Inc., Houston, TX.

Zepeda, A., A.-C. Texier, E. Razo-Flores, and J. Gomez. 2006. Kinetic and metabolic study of benzene, toluene and *m*-xyzlene in nitrifying batch cultures. *Water Res.,* 40 (8): 1643-1649.

Zhu, J., D. S. Bundy, X. Li, and N. Rashid. 1997. Controlling odor and volatile substances in liquid hog manure by amendment. *J. Environ. Qual.,* 26 (3): 740-743.