Final Report

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Evaluation of Biological-Based Additive for Pollution Abatement

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Abstract

Odor emission has always been a challenge for intensive animal operations. Various pit additives continue to be developed and improved to mitigate odor and manure solids. A commercial, biological-based additive was evaluated in a laboratory setting for effectiveness in reducing manure solids, and gas and odor concentrations. In a semi-long-term test, twelve 3.79-L (1-gallon) glass jars were used to mimic semi-long-term manure storage. Manure was added into jars every week at a rate of 3.8-cm/week (1.5-inch/week) until the jars were full. Jars were arranged into four groups, each had three jars: untreated (control) group, and pit additive treated groups at different additive concentrations. There was no significant difference in ammonia concentrations ($p < 0.05$), while hydrogen sulfide could not be detected at 0.1 ppm level. However, total and volatile solids were significantly reduced although the reductions were relatively low. After the additive application method and dosages were verified, a six-month and deep manure storage test, or long-term test, followed. Nine, 15 cm (6") ID, 1.52 m (5') long PVC tubes were used to simulate different treatments: 3 for control, 3 for normal (100%) dosage, and 3 for 200% dosage. Each reactor was ventilated at 2 L/min of filtered room air using a piston air pump. Concentrations of ammonia and hydrogen sulfide of the exhaust were measured every month, while odor concentrations were measured at the middle and end of the test. No significant difference was observed for pH, ammonia and hydrogen sulfide concentrations between the control and treatment groups, while hydrogen sulfide concentrations were more variable. Reduction of total solid and volatile solid was observed for the 200% dosage group. Odor reductions for the 100% and 200% treatment groups were reduced (although not significantly different) by 21.6% and 11.2% for the third month sampling, and were reduced by 56.0% and 80.0%, for the sixth month sampling, respectively. Odor concentration, after logarithm transformation, was significantly reduced by the 200% dosage treatment at the end of the test ($p = 0.013$). Nutrient contents were similar among the groups, which confirmed that the additive did not alter manure nutrients during the six-month testing period. Future research should consider improved manure loading and storage conditions, and include field tests.

Introduction

Manure management on the swine finishing farms is one of the most important challenges many farmers face due to environmental protection and odor nuisance issues. For some animal farms, especially those who are larger and without enough distances to neighbors, odor released from manure storage pits can become pollution nuisance. In addition, large amount of emission from manure can be dangerous to worker's and animal's health. Ammonia (NH_3) and hydrogen sulfide (H_2S) are two common gases produced from manure storages. Emissions of NH_3 and H_2S used to trigger emission reporting (EPA, 2018) and are often measured as surrogate of odor. These gases also have relatively low permissible limit of exposure to ensure personnel safety. The Occupational Safety and Health Administration recommends exposures of H_2S not exceeding 20 parts per million (ppm, ceiling) (OSHA, n.d.), and the Short Term Exposure Limit (STEL) of NH_3 in 15 minutes is 35ppm (CDC, 2014). High concentrations of these gases could cause several health problems to workers, ranging from eye irritation to loss of consciousness or death (Higuchi et al., 2009).

Many approaches have been tested to resolve the problems from long-term manure storage and prevent potential sources of air pollution. Manure could be transferred to other farms (Ali et al., 2012) for additional treatment. Economic aspect, however, is often the major barrier for the successes of off-site management. There have been many commercially available pit additives for mitigating odors and reducing manure solids. One of the largest comprehensive pit additive effectiveness research was conducted in 2001 to evaluate 35 types of additives (Heber et al., 2001). Since then, there have been more additive products developed and marketed. Newly developed products such as biological-based additive can be a novel approach to reduce the odors and solids, while maintaining the valuable nutrients over the long-term manure storage.

This study aims to test effectiveness of a biological-based pit additive that was marketed in Missouri. The objective of this study is to conduct short-term, laboratory-scale tests to optimize application dosage and method, and characterize the potential of product in reducing solids. Based on the short-term results, a long-term test was conducted to verify effectiveness of the product in reducing manure solids, and gas and odor concentrations. Through the investigation of this manure additive, small and local business could benefit from this technical evaluation and recommendations.

Materials and Methods

The laboratory tests were conducted at the Agricultural Engineering Building laboratory, University of Missouri, Columbia, MO. Slurry manure was prepared to contain 8-9% of total solids, and started with 20% volume at the beginning of the tests. Solid manure was collected from a commercial finishing farm located at central Missouri, slurry manure was collected from University of Missouri Swine Research Center. All storage tests were conducted in the laboratory at normal room temperature.

Semi-long term test

In the semi-long-term laboratory test, 12 glass jars were set up in four groups (each contained 3 jars as replication): control (raw manure only), 50% dosage, 100% dosage (recommendation of company), and 200% dosage. All jars were started with 20% volume of pre-mixed manure at the beginning week. Additive was added to the respective groups at week 3 after the manure had a chance to settle. Manure were added weekly at the rate of 3.8 cm (1.5”) per week until the jars were full (Figure 1). Value of pH was checked weekly to help monitor microbial activity in each group.

Figure 1: Semi-long-term pit additive test, with 12 glass jars set up in laboratory

All jars were kept together under room temperature, and monitored for three months to mimic semi-long-term manure storages. Gas measurement was measured every month for NH_3 and H_2S concentrations. The glass jars were not ventilated but were capped. Each of the plastic lid had a 1.3 cm (0.5") diameter opening, allowing the manure to off-gas to the lab atmosphere. Gas concentrations were measured by using individual chemical tubes (Draeger Gas Detection Tubes, Draeger Safety, Sugarland, TX) and a hand-held bellows pump (Draeger Accuro pump, Draeger Safety).

Grab samples of the manure were collected at the end of the test for total solid, volatile solid total ammonia, and pH measurements. This was to test the hypothesis that the manure additive does reduce the solid contents and gas concentrations during semi-long-term manure accumulation and storage period (Figure 1). The first semi-long-term test was conducted from September 2017 to January 2018. A second test was started in April 2018 and completed in September 2018.

Long-term reactor test

Long-term storage and treatment of manure was conducted to mimic deep-pit storage of commercial finishing farms, using 15 cm ID x 1.52 m long (6" ID x 5' long) schedule-40 PVC tubes as reactors. A total of nine (9) reactors were set up as controlled and treated groups (Figure 2). Control group contained raw manure addition and storage only, while two treatment groups were added with additive at 100% dosage (recommendation of company) and 200% dosage, respectively. Solid manure was diluted with liquid manure to 8-9% of total solid, and started with 20% volume at the beginning of the long-term test. Manure was added every week at a rate of 3.8 cm (1.5") per week to mimic the manure accumulation in a commercial finishing barn. The use of additive was started at week 6 at 100ppm (100%, as recommended by the company) and 200 ppm (200%) dosages.

Figure 2: Reactor set up using schedule 40 PVC tube and cap, and ventilation system, for long-term additive test.

Caps of reactors were attached, not glue, to PVC tubes, and sealed with petroleum jelly to prevent air leakage, so that they could be detached for weekly manure loading. Each cap was installed with straight-through wall connector (1/4" polyethylene, McMaster-Carr, Elmhurst, IL) for ventilation. Airflow was provided by a piston air pump (3000Lph, 18W, EcoPlus, Vancouver, WA) that went into each reactor through a 12-outlet manifold. Room air was pumped through two in-line strainers (1/2" and 3/4" Female NPT Strainers, VacMotion Inc., Plymouth, MA) with 50 mesh stainless steel screen. The first strainer contained activated carbon pallets (Acurel LLC, Cranbury, NJ) to filter particulate matter and odors before entering the reactors. The second strainer was installed in series to ensure filtering out potential materials from the first strainer. Airflow was maintained at the rate of two liter per minute. Rotometer (RMA-16, Dwyer, Waco, TX) was used to check airflow rate of each rector daily. For more accurate measurement, an airflow calibrator system (Gilibrator-2, Sensidyne, LP, St. Petersburg, FL) was used to monitor airflow of each reactor once per week. When the exhaust airflow fluctuated more than 200 mL/min, the ventilation system, especially the cap and fittings were checked carefully for potential leakage and obstruction, and the airflow was adjusted by the manifold valve when needed.

The effectiveness of the additive in reducing odor was evaluated by comparing the treated and untreated (control) odor and gas concentrations. Concentrations of NH_3 and H_2S was measured every month by using individual chemical (Draeger) tubes and a hand-held bellows pump as described above. Exhaust air released from each reactor was sampled for odor evaluation, using 10-liter Tedlar bags in July, and again in October, 2018. New Tedlar bags were flushed three times with compressed air and conditioned with exhaust air as mentioned in previous odor sampling method (Lim et al., 2003). Samples were overnighted to St. Croix Sensory, Inc (MN) for odor concentration and hedonic tone evaluation. According to the European odor standard, the frequency distribution for detection

thresholds for odorants is log-normal (CEN Standard, 2003).

Measurements of H_2S was continued into the 7th and 8th months of the test, because there was indication that the monthly and grab measurements were not able to monitor the fluctuations of the H_2S emissions. Additional gas samples from each of the reactor were collected three times per week (on Monday, Wednesday, and Friday) for two weeks in the 8th month of measurement. Exhaust air samples of the reactors were collected using 25L-Tedlar bags over 10 min. Bags were pre-flushed once with compressed air and conditioned once with the reactor exhausts before collection (Lim et al., 2003). Concentrations of H_2S were measured using a pulsed fluorescence analyzer (Model 450i, Thermo Scientific, Waltham, MA). The analyzer was checked using zero air and calibration gas (2348 ppb) before and after the bag measurements to monitor the accuracy of the analyzer. The zero air readings of the analyzer ranged from 2 to 49 ppm and average 24.2 ppb, while the calibration gas reading ranged from 2153 to 2330 ppb and averaged 2229 ppb. No adjust of the measurements were made because the zero air and calibration gas readings were relatively consistent over the two weeks, and the differences were relatively low compared with the exhaust concentrations.

Manure levels in the reactor was measured weekly to monitor the manure addition and potential leakages. Manure nutrient variables including total solids, total nitrogen, phosphorus, potassium, pH, total carbon, volatile solids, and electrical conductivity were sampled and measured at the end of the test. Sampling was conducted by emptying each of the reactor into a 76-L (20-gallon) container, and mixed using an 81-cm (32") drywall mud mixer (Pro-Grade 32" Mixer, Amazon, Seattle, WA) driven by an electric drill. Manure samples were frozen at -20°C immediately and sent to the Soil and Plant Analysis Laboratory at University of Missouri for analysis (Nogueira et al., 2019). This lab is a certificated lab following standard analysis methods. The test is to verify the hypothesis that the manure additive does not alter the manure nutrients significantly, which is critical to the value of the stored manure, which will be land applied as crop fertilizer.

Data analysis

Raw data was analyzed by using R version 3.5.1 (free software, Free Software Foundation, Boston, MA). Analysis of variance (ANOVA) test was conducted to compare the significant differences of the different mean values among the groups. Tukey's Honest Significant Difference test (Tukey Test) was used to confirm the significant different between groups in the comparison. Reported values were given as standard deviation to the means. All averages of odor concentrations were reported as geometric means because they typically exhibit lognormal distributions (European Committee for Standardization, 2003). Logarithm transformation was applied for odor concentrations to determine statistical significant difference between treatment and control groups. Readings were considered significantly different when p value was less than 0.05.

Results

Semi-long-term pit additive test

Test 1 (September 2017 to January 2018)

Table 1 summarizes variables measured during the first semi-long-term test. After three months, pH of all four groups showed a general increasing trend, although not statistically different, the means ranged from 7.3 ± 0.3 to 7.8 ± 0.3. Ammonia concentrations showed relatively low variance, and there was no significant difference in ammonia concentrations among the four groups ($p = 0.949$). Hydrogen sulfide could not be detected at 0.1 ppm level. This may be caused by the jar's small volume and the direct contact with environment. Emission of H_2S could be released quickly to the air at certain occasions, and could be more variable than NH_3 as reported in the literature (Ni et al., 2000; Ni et al., 2009). During this test, the glass jars were placed in ventilation hood, and inconsistent airflow has resulted in various level of evaporation among the jars. Because of evaporation from jars, the TS and VS of control and 50% groups were lower than the other groups.

Table 1: Summary of variables measured in the first semi-long-term test.

	Control	50%	100%	200%
n	3	3	3	3
pH	7.5 ± 0.2	7.8 ± 0.3	7.3 ± 0.3	7.4 ± 0.0
Ammonia (ppm)	133.3 ± 0.3	125.0 ± 0.3	116.7 ± 0.0	140.0 ± 0.1
Hydrogen sulfide (ppm)	N/D[a]	N/D[a]	N/D[a]	N/D[a]
Total solid	8.3% ± 0.2%	8.3% ± 0.0%	9.1% ± 0.3%	9.3% ± 0.2%
Volatile solid	6.0% ± 0.2%	6.0% ± 0.0%	6.6% ± 0.2%	6.8% ± 0.1%

[a] N/D = not detectable (bellow detection level)

Test 2 (April 2018 to September 2018)

Initial pH of each groups at the beginning of the test was 6.8. After adding the manure weekly, pH (measured 5 cm, or 2" from manure surface) was slowly increasing and reached 8.2 after 16 weeks of storage (Figure 3). However, the difference between each group was barely distinguishable. Fluctuations of the pH can be considered as an indicator of microbial activity. The similar pH between the four groups revealed that the pit additive did not impact the pH and bacterial communities within the treated manure samples. The fact that the control and treatment groups had such similar trend in pH, suggests that the additive did not alter the chemical components of the manure storage. The effectiveness of additive might come from enzymes, proteins or other bacteria species in the solution.

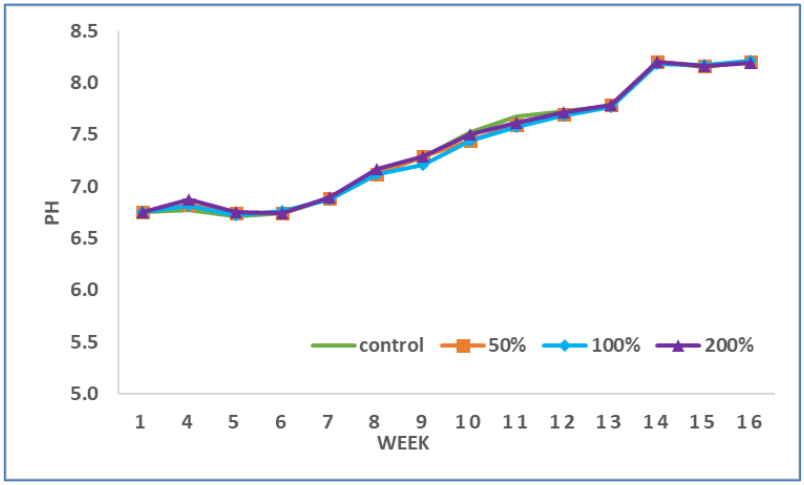


Figure 3: Mean pH of different groups in the semi-long-term test

Table 2 summarizes variables of the second semi-long-term test. Values of pH of all four groups showed a general increasing trend, and the overall mean values ranged from 8.05 ± 0.11 to 8.24 ± 0.03 over the long-term test. Ammonia concentrations measured at the headspace of the glass jars were relatively high, averaged above 240 ppm. Concentration of the 50% dosage group was the lowest while all NH_3 concentrations were in the range of 240 to 287 ppm, there was no significant difference in ammonia concentrations among the four groups ($p = 0.246$). Again, hydrogen sulfide could not be detected at 0.1 ppm level. However, the differences of total solid and volatile solid between the four groups were statistically significant, with *p*-values equal to 0.007 and 0.003, respectively. The lowest total solid and volatile solid were observed for the group treated with 200% additive dosage, at 5.30% ± 0.16% and 3.45% ± 0.13%, in comparison with 5.74% ± 0.07% and 3.83% ± 0.08% for the control group, respectively. In general, the additive reduced the amount of TS and VS with increasing dosage, during the semi-long-term test (Table 2).

Table 2: Summary of variables measured in the second semi-long-term test.

	Control	50%	100%	200%
n	3	3	3	3
pH	8.24 ± 0.03	8.05 ± 0.11	8.22 ± 0.03	8.10 ± 0.07
Ammonia (ppm)	250.0 ± 36.1	240.0 ± 17.3	286.7 ± 20.8	271.1 ± 33.1
Hydrogen sulfide (ppm)	N/D[a]	N/D[a]	N/D[a]	N/D[a]
Total solid	5.74% [a] ± 0.07%	5.69% [a,b] ± 0.16%	5.39% [b,c] ± 0.10%	5.30% [c] ± 0.16%
Volatile solid	3.83% [a] ± 0.08%	3.80% [a] ± 0.13%	3.49% [b] ± 0.06%	3.45% [b] ± 0.13%

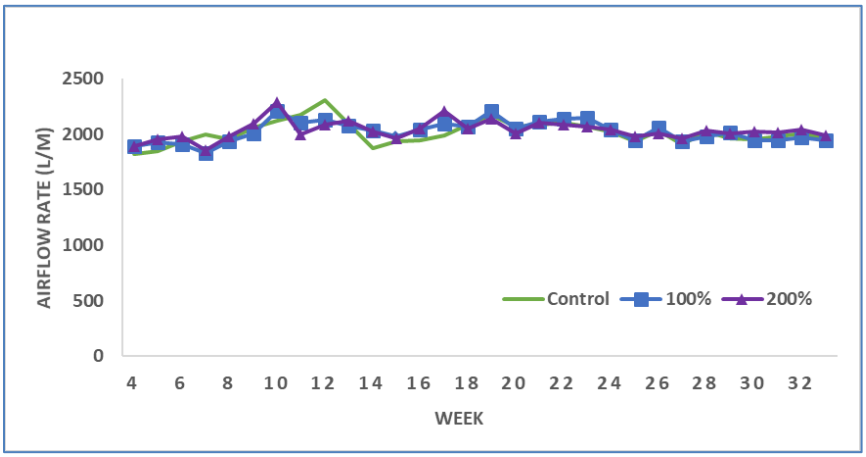
[a] N/D = not detectable (bellow detection level)

The mean values were displayed with lowercase (a, b and c) superscript to distinguish significant difference ($p<0.05$)

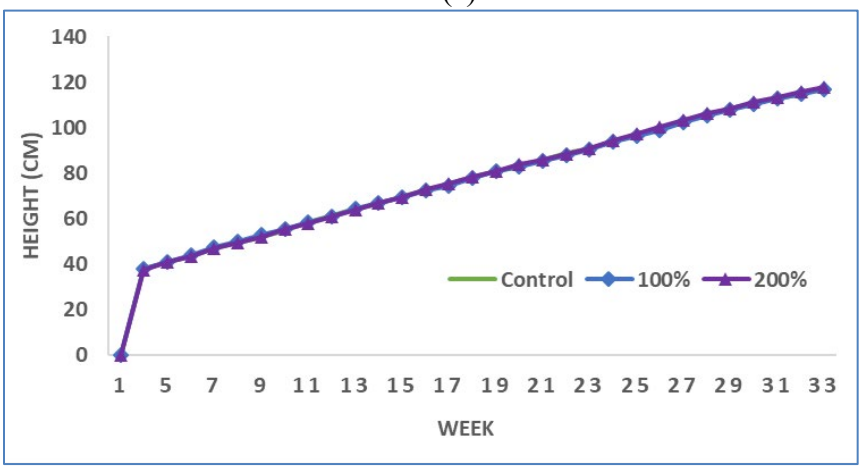
Long-term pit additive test

Reactor Airflow Rate, Manure Level, and pH values

Airflow rate fluctuation can be an indicator of air leakage or clogging within the ventilation system. Therefore, airflow rates of each of the reactors were measured to analyze any abnormal performance of the ventilation system and reactors. Over the eight months test period, airflow rates were fluctuating between 1800 to 2200 cc/minute (Figure 4a), and in general maintained at 2000 cc/minute throughout the test period without major adjustment. Manure levels of the reactors were shown to have a steady weekly increase due to the consistent manure addition (Figure 4b).



(a)



(b)

Figure 4: Height of manure (a) and airflow rate (b) of the three treatment groups, over a 33 weeks test period.

Similar to the semi-long-term test using glass jars, pH was recorded weekly (Figure 5). The difference of pH in each group inside tall reactors was again relatively minor. Interestingly, the pH of all reactors did not go over 7.0 during the first 23 weeks, while pH in glass jars surpassed 7.0 after only 6-7 weeks, and were above 7.5 after five months or more of loading. This suggested that the airflow system may be critical to maintaining the pH in the reactors, and better represent the deep-pit manure storages. The constant air exchange might have affected the dissolved oxygen amount in the manure (although only at the top layer of the manure), and helped removing significant amount of emissions from the headspace that was not experienced by the jar tests.

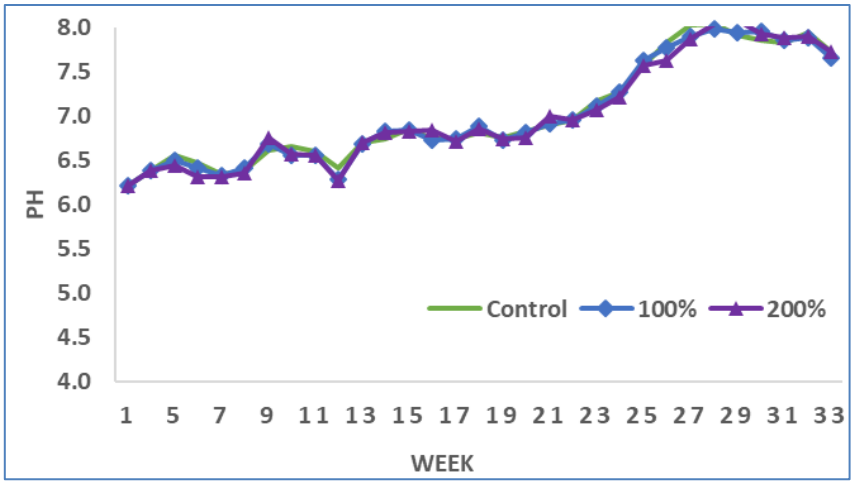
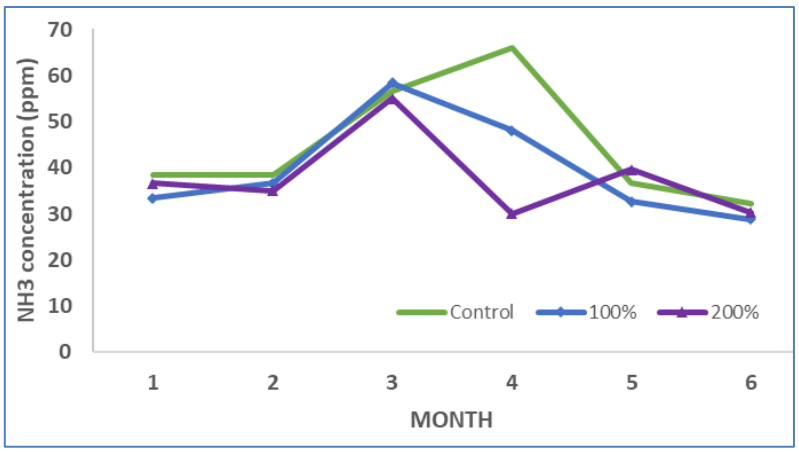


Figure 5: Mean pH values of the long-term test.

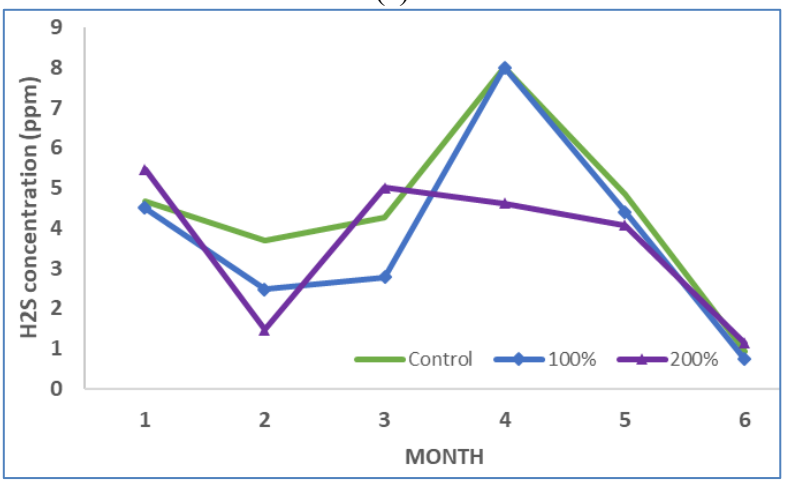
Gas Concentrations

In general, the gas concentrations increased over the first four months, most likely due to manure addition, and increasing amount of organic materials inside the reactors, Figure 6. It was interesting to observe a downward trend for both gases after the fourth month. The overall mean NH_3 concentrations for the control, 100%, and 200% treatment groups were 44.7 ± 13.4, 39.6 ± 11.3, and 37.8 ± 9.1 ppm, respectively, and there was no significantly difference (p = 0.566) between the groups during the six months of monitoring. Manure added with 200% dosage of additive released relatively lower ammonia than others, and did not peak at the 4th month. The overall mean concentration was 30.0 ± 5.2 ppm, in comparison with 66.0 ± 4.4 ppm from control reactors, and 48.0 ± 18.7 ppm from 100% dosage columns. It is interesting to note that the exhaust concentrations did not continue to increase during the last two months, for both the NH_3 and H_2S concentrations. However, the gas concentration measurements were very limited, and very likely not capturing the fluctuation over time.

Concentrations of H_2S appeared to be more variable and difficult to evaluate, most likely due to the nature of how the gas was slowly released by the microbial activities (mostly anaerobic bacteria) and trapped at the bottom portion of the reactors. There have been research showing sporadic releases of H_2S from manure storages (Ni et al., 2000; Ni et al., 2009). We have conducted tests by manually tapping the glass jars (of the semi-long-term test), and the H_2S concentration of the jar headspace increased from below detection limit to over 10 ppm, which confirmed the sudden H_2S release, and that the grab sampling was not able to monitor such releases. The overall mean H_2S concentrations for the control, and 100%, and 200% treatment groups were 4.4 ± 2.3, 3.8 ± 2.5, and 3.6 ± 1.9 ppm, respectively. The treated groups of 100% and 200% dosages had 21% and 18% lower H_2S concentrations than the control reactors, respectively, but over the six months the concentrations were not statistically significant different from each other (p = 0.822).



(a)



(b)

Figure 6: Concentrations (ppm) of Ammonia (a) and Hydrogen sulfide (b) in the exhaust of the reactors.

Table 3: Ammonia and Hydrogen sulfide concentrations (ppm) in the exhaust of the reactors.

Gas	Month	Control	100%	200%
NH_3 concentration (ppm)	1	38.3 ± 7.6	33.3 ± 5.8	36.7 ± 2.9
	2	38.3 ± 2.9	36.7 ± 2.9	35.0 ± 5.0
	3	56.7 ± 7.6	58.3 ± 5.8	55.0 ± 5.0
	4	66.0 ± 4.4	48.0 ± 18.7	30.0 ± 5.2
	5	36.7 ± 4.7	32.7 ± 2.1	39.7 ± 4.2
	6	32.2 ± 3.5	28.8 ± 1.7	30.3 ± 2.9
	Average	***44.7 ± 13.4***	***39.6 ± 11.3***	***37.8 ± 9.1***
H_2S concentration (ppm)	1	4.7 ± 3.3	4.5 ± 0.5	5.5 ± 4.1
	2	3.7 ± 2.0	2.5 ± 1.7	1.5 ± 0.7
	3	4.3 ± 0.7	2.8 ± 0.5	5.0 ± 1.7
	4	8.0 ± 1.7	8.0 ± 3.6	4.6 ± 1.6
	5	4.8 ± 1.7	4.4 ± 4.2	4.1 ± 1.2
	6	0.9 ± 0.5	0.7 ± 0.7	1.1 ± 0.9
	Average	***4.4 ± 2.3***	***3.8 ± 2.5***	***3.6 ± 1.9***

Due to the fluctuation of H_2S concentrations, the long-term test was extended into 8th month to allowed additional sampling and monitoring of the H_2S concentrations using a pulsed fluorescence analyzer. Overall, the 10-minute sampling of H_2S concentrations showed higher concentrations after the manure loading (days 2) and followed by a lower trend in the days 4 and 6 measurements. Again, there was no significant difference in H_2S level released from each groups ($p = 0.933$). The dataset shows improvement of the H_2S measurement, but no conclusion can be made about the sporadic releases, that more frequent monitoring of the exhaust concentrations is needed to better characterize the diurnal and other variations over the entire test period.

Table 4: Hydrogen sulfide concentrations (ppm) measured by bag sampling and pulsed fluorescence analyzer during the seventh month of test.

Week	Day	Control	100%	200%
1	2	12.0 ± 4.4	10.6 ± 2.4	10.4 ± 4.0
	4	4.6 ± 2.7	4.0 ± 2.6	3.0 ± 1.0
	6	1.5 ± 1.1	1.1 ± 1.2	3.6 ± 1.8
	Average	***6.0 ± 5.4***	***5.2 ± 4.8***	***5.7 ± 4.1***
2	2	9.4 ± 1.6	12.0 ± 1.6	9.0 ± 4.4
	4	3.7 ± 1.4	5.4 ± 1.2	4.1 ± 3.7
	6	2.3 ± 2.7	4.8 ± 2.7	3.4 ± 2.7
	Average	***5.1 ± 3.8***	***7.4 ± 4.0***	***5.5 ± 3.0***
Average in two weeks		**5.6 ± 4.2**	**6.3 ± 4.2**	**5.6 ± 3.2**

Odor Evaluations

Odor concentrations and hedonic tones of the reactor exhaust samples were summarized in Table 5. Overall, lower concentrations (but not statistically different) were observed in the treatment groups, when compared with the untreated reactors, especially during the sixth month sampling. For the third month odor samples, odor concentrations for the 100% and 200% treatment groups were reduced by 21.6% and 11.2%, respectively (Table 5). More reductions were observed for the sixth month sampling, that the odor concentrations for the 100% and 200% treatment groups were reduced by 56.0% ($p = 0.245$) and 80.0% ($p = 0.154$), respectively. Although the average odor reductions were relatively high, the low number of samples and high variances likely resulted in the low p value. The odor concentrations of the second group samples were so high that the olfactometry laboratory had to pre-dilute the three control and two treated samples before the olfactometer was able to provide enough dilution during the olfactometry evaluation. Because of the dilution, no hedonic tone could be evaluated for the samples that were pre-diluted. After logarithm transformation, no significant difference was observed for the odor concentrations between groups during the 3-month period ($p = 0.944$). However, after 6-month of treatment period, Tukey Test showed no difference for the 100% dosage group, but a significant reduction was observed for the 200% dosage group ($p = 0.013$).

The hedonic tones averaged -5.8, -5.4, and -5.6 for the 3-month control, 100%, and 200% groups, respectively. All the hedonic tone values were in the negative range, indicating unpleasant characteristics experienced by the olfactometry panelists. For the sixth month samples, the hedonic

tone of control reactors and two first reactors of 100% group could not be detected at 10:1 dilution level. The hedonic tone value for the last reactor treated by 100% dosage was -4.2, while the average value for the 200% group was -6.30 ± 1.66.

Table 5: Mean odor concentration and hedonic tone of exhaust samples, after 3 and 6 months of storage.

Odor Variables	Month	Control	100%	200%
Detection Threshold	3	13,945 ± 4,163[a]	10,933 ± 1,050[a]	12,387 ± 3,099[a]
	6	137,936 ± 99,785[a]	60,736 ± 25,106[a]	27,651 ± 5,196[b]
Hedonic tones	3	-5.77 ± 0.35	-5.43 ± 0.40	-5.63 ± 0.42
	6	N/D[a]	-4.2[b]	-6.30 ± 1.66

[a] N/D: not detectable at 10:1 dilution level

[b] Data observed for only one reactor in the group, the other two could not be detected at 10:1 dilution level

The mean values were displayed with lowercase ([a, b and c]) superscript to distinguish significant difference ($p<0.05$)

Manure Nutrient Evaluations

Manure samples were collected at the end of the test and analyzed for important nutrients and characteristics. Total nitrogen, ammonium, phosphorus, potassium, moisture, pH and electrical conductivity were measured to determine the effects (if any) of additive to the stored manure. In general, manure stored inside reactors for over eight months, nutrient levels and characteristics including moisture, pH and electrical conductivity were similar to each other (Table 6). The pH and total solid values agreed with the measurements listed in previous section. There was slight reduction in nitrogen, ammonium, phosphorus and potassium when double amount of additive was applied. However, the difference between three groups is not significant with *p*-value for each indicator range from 0.109 to 0.679. Only *p*-value of Electrical Conductivity is lower than 0.05 but it is not an important criterion to evaluate the nutrient components. Results indicated that the manure nutrients were preserved similarly when additive was added. Similar to the semi-long term tests, reactors treated with 200% dosage of additive showed a low reduction in total solid and volatile solid. However, the statistical data analysis did not indicate significant difference with *p*-values of 0.145 and 0.182 for TS and VS comparison, respectively.

Table 6: Manure nutrients and total solids and volatile solids at the end of the test.

Test	Unit	Control	100%	200%
Nitrogen (N)	ppm	5214± 472	5315 ± 116	5067 ± 319
	lb/ac-in	1180 ± 107	1204 ± 26	1147 ± 72
	lb/1000 gal	43.4 ± 3.9	44.3 ± 1.0	42.2 ± 2.7
Ammonium (NH_4)	ppm	4462 ± 63	4522 ± 37	4184 ± 439
Phosphorus (P)	ppm	1064 ± 14	1082 ± 22	954 ± 159
	lb P_2O_5/ac-in	552 ± 7	561 ± 11	495 ± 83
	lb P_2O_5/1000 gal	20.3 ± 0.3	20.6 ± 0.4	18.2 ± 3.0
Potassium (K)	ppm	1688 ± 9	1604 ± 81	1415 ± 215
	lb K_2O/ac-in	459 ± 3	436 ± 22	385 ± 59
	lb K_2O/1000 gal	16.9 ± 0.1	16.0 ± 0.8	14.2 ± 2.1
Moisture	%	93.03 ± 0.12	93.07 ± 0.25	93.23 ± 0.42
pH	7.81	7.88 ± 0.08	7.83 ± 0.04	7.91 ± 0.06
Electrical Conductivity	mmhom/cm	10.93 ± 0.70	12.20 ± 0.56	10.97 ± 0.21
Total solid	%	6.94 ± 0.14	7.08 ± 0.10	6.69 ± 0.32
Volatile solid	%	4.98 ± 0.13	5.05 ± 0.08	4.73 ± 0.30

Discussion

Results from the semi-long-term glass jars did not show significant difference in NH_3 and H_2S concentrations. This may be due to a combination of the lack of airflow, small working volume and relatively shallow storage depth. The total solid and volatile solid, however, showed significant difference between the control and groups treated with 200% dosage of additive. Meanwhile, the long-term storage reactors showed more promising outcomes. The observed pH values were relatively stable, which was likely due to the consistent ventilation within the reactors for long-term manure storage and treatments. Similar to the semi-long-term- test, NH_3 and H_2S concentrations were not significantly reduced when additive was applied at either the recommended dosage or double the dosage. However, 56% and 80% odor reductions (although not significantly different) were observed for the reactors treated with additive, and logarithm transformation of detection threshold showed a significant difference between the 200% dosage treatment group with others at the end of the test (sixth month odor sampling),, confirming that the biological additive was able to mitigate some odor although the gas concentrations measured were not affected. More frequent and semi-continuous measurement of the gas concentrations is recommended for future evaluation tests. For important manure nutrients and characteristics, no significant differences were not observed between the control and treated groups. Therefore, the risk of nutrient loss because of additive applied can be eliminated.

Effectiveness of biological additives can also be proven by increasing number of bacterial community inside the manure pit. In another study, *Bacillus sp. TAT105* was added to reduce ammonia emission (Kuroda et al., 2017). The bacteria used ammonia as its own nitrogen source for metabolism. The tested pit additive solution consisted of a combination of enzymes, proteins and bacteria working together as unique complex for manure treatment. Nitrogen was trapped inside the tank and therefore,

ammonia releasing was decreased. The specific mechanism, however, has not been revealed yet. The manure used in this research was kept frozen until use, it is not sure if the freezing and thawing had affected the manure and microbial in ways that reduce the additive effectiveness. Metagenomics evaluation of the control and treated samples could also be performed to better analyze the microbial differences in future study. Components of the volatile organic compounds and volatile fatty acids should also be sampled and analyzed to correlate with the odor results. In addition, no field test of the additive was conducted (which was originally included in the project) due to budget reduction. Future study may focus on the test of additive application in the commercial barns where the interaction between additive and various environmental conditions can be fully evaluated.

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