THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ANIMAL SCIENCES

ACCUMULATION OF HYDROGEN SULFIDE IN DAIRY MANURE IN RELATION TO GYPSUM USE AND POSSIBLE SOLUTIONS

VANCE BROWN
FALL 2014

A thesis
submitted in partial fulfillment
of the requirements
for baccalaureate degrees
in Animal Sciences and Agricultural Systems Management
with honors in Animal Sciences

Reviewed and approved* by the following:

Eileen Fabian Wheeler
Professor of Agricultural Engineering
Thesis Supervisor

Troy Ott
Professor of Reproductive Physiology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Hydrogen sulfide is a deadly gas that has recently become an issue on dairy farms across the country. Upon agitation of manure storages, large quantities of hydrogen sulfide have been released into the air and caused morbidity and mortality to farmers and their animals. Anecdotal evidence reveals that the use of recycled gypsum in the dairy barn as a bedding amendment appears to be the cause of this elevated hydrogen sulfide accumulation in manure storage systems. Recycled gypsum, or $CaSO_4$, contains sulfate. Certain bacteria have the ability to reduce sulfate in an anaerobic environment and acquire energy. Given a long enough storage time, limited disturbance, and the formation of a surface layer crust, an anaerobic environment is created below the surface of a large storage pit. When the environment becomes anoxic enough, sulfate-reducing bacteria convert the sulfate into hydrogen sulfide gas. This gas can then build up in the manure and remain trapped until agitation. This study investigated the relationship between gypsum presence in the manure and hydrogen sulfide accumulation in a laboratory setting. It was determined that gypsum presence did, in fact, cause significant hydrogen sulfide accumulation. Secondly, solutions to mitigate this risk were tested. The first solution was Vital Breakdown™ (Homestead Nutrition, New Holland, PA), a proprietary product manufactured by Homestead Nutrition, Inc. designed to accelerate microbial decomposition and reduce odors from manure storages. The second product contains iron oxide, a recycled material that has the potential to bind to hydrogen sulfide and render it insoluble. Both solutions had a promising effect on reducing blooms of hydrogen sulfide emissions in this study. Iron oxide appeared to reduce hydrogen sulfide dramatically when used at a specific inclusion rate. Vital Breakdown™ promoted the slow release of hydrogen sulfide from the manure rather than accumulation. Large scale investigations on dairy farms are the next step to determining the effectiveness and practicality of the proposed solutions.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

There are many people who have helped me in the course of this research and in the development of this thesis. To begin, I would like to thank the Animal Sciences administrative faculty and Jana Peters for helping me find a thesis supervisor which would allow me to combine my interests in Animal Sciences and Agricultural Systems Management. I would also like to thank my academic advisor, Dr. Troy Ott, for making sure I was on track throughout my Honors career and helping me wrap my head around how to produce a thesis.

My thesis supervisor, Dr. Eileen Fabian, deserves a great deal of credit and appreciation. I came to her with little idea as to what to study for my thesis research. She was very welcoming and generated several ideas for me, all of which would have made great and practical thesis research projects. We decided on this study because of its critical importance to farm safety, the lack of laboratory research, and the ability for the study to be done in a laboratory setting. Dr. Fabian was extremely helpful and instrumental in the construction of my thesis as well. She has been nothing but a joy to work for and I hope that my efforts here make her effort worthwhile.

Lastly, I need to give substantial credit and the greatest thanks to Mike Hile, a PhD student under Dr. Fabian. Mr. Hile has been my direct supervisor and mentor throughout this whole process. From designing the experiment, using the equipment, and collecting the samples, Mike has been there with me. While Mr. Hile is extremely busy completing his own research work, Mike was always extremely approachable and willing to help me with this project. Mike was just as involved in this project as I was, and invested significant time and effort to ensure I was on the right track and doing things correctly. We were always bouncing ideas off of each other and I think we made a great team. Through the combined efforts of Mr. Hile and Dr. Fabian, I was provided with everything I needed to accomplish this project. From funding to product, they were there to help me out. Without these instrumental faculty, my project

would not have come to fruition. For that, I say thank you, and I sincerely hope that my research and work here validates your efforts and leads to greater things. Thank you!

Chapter 1

Introduction

Hydrogen sulfide (H_2S) is a deadly gas which has recently become an issue in dairy farms across the country. Following the patterns of human history, applying new methods and improvements in animal agriculture yields unforeseen consequences. This study represents one example. For decades, dairy farms have been storing manure in outdoor, open-air storage pits. Hydrogen sulfide poisoning has never been a significant problem in relation to dairy manure storage, however, over the last few years, a serious issue has arisen. While problems with hydrogen sulfide accumulation in manure have been investigated in swine operations, research has not been warranted in dairy manure. The scenario that some manure handlers have become all too familiar with follows a pattern. A hypothetical situation follows.

In the normal course of events on the typical modern dairy, the farm owner or his laborers (possibly even his children) scrape the manure out of the barns and into the outdoor storage. In addition to feces and urine, the manure enters the pit containing the inorganic (sand) or organic (straw, shavings) bedding material as well as the new gypsum they have been adding to their bedding. The wash water from the parlor, as well as the waste from the footbaths they use to decrease hoof infections also flows into the outdoor storage. Nothing seems out of the ordinary. When spring finally arrives and the ground is suitable for manure application, the farm's manure hauler arrives to haul the manure out of the storage. In preparation for removal, the farmer drops an agitator into the pit, and begins to mix and homogenize the slurry. He steps out of the cab, to feel a slight breeze coming from over the pit. All of a sudden, he drops to the ground, and immediately loses consciousness. It is possible this man could lose his life, and not from what we typically expect. He was not in a confined space, he did not smell methane or ammonia,

and he has been agitating his dairy manure the same way for years. Further investigation determines hydrogen sulfide as the cause.

Hydrogen sulfide is an extremely poisonous gas that is capable of killing humans and animals (Meinen et al., 2013). The gas is heavier than air, which means it can accumulate in low lying areas and dissipate slower than lighter gases (Cornell PRO-DAIRY 2013). At low exposure levels, hydrogen sulfide is detectable by a rotten egg smell typical of sulfur compounds (OSHA 2014). However, even at these low levels, effects to human health have been noted, such as eye and throat irritation and headaches (OSHA 2014). When the concentration of hydrogen sulfide reaches 100-150 parts per million (ppm), the sense of smell is desensitized by the gas and it becomes extremely dangerous (OSHA 2014). By the time the gas concentration reaches 500 ppm, collapse and death can occur within an hour (OSHA 2014). The Occupational Health and Safety Administration (OSHA) recognizes hydrogen sulfide as a hazardous gas. The official OSHA sustained exposure limit for an eight hour work shift is 10 ppm, while the general industry upper level is 20 ppm (OSHA 2014). The general industry peak limit according to OSHA is 50 ppm for up to 10 minutes of exposure (OSHA 2014). What is of most concern to this investigation is the OSHA immediate danger to life and health (IDLH) level of 100 ppm (OSHA 2014). As described above, it is at this point that the gas no longer is detectable by smell.

For decades we have known that manure generates dangerous gasses. Whether in confined spaces or in open air pits, manure emits a plethora of compounds including methane and nitrous oxide (Owen, JJ and WL Silver, 2014). Through efforts made by academic institutions and their Extension educators, greater care is practiced when handling manure (Meinen et al., 2013). This includes using signs warning of dangerous gasses and securely barricading storages. This heightened awareness of the danger has created a safer working environment for farmers across the country (Meinen et al., 2013). Technology has also allowed us to better recognize dangers. For example, personal gas meters have become an option for farmers across the country. Companies like Industrial Scientific (Oakland, PA) manufacture personal monitors like the Tango TX1 that monitor hydrogen sulfide concentrations (Industrial Scientific 2014).

Worn by the worker, the small monitor alerts the individual of gas concentrations above 10 ppm using an audible, visual, and vibrational alarm (Industrial Scientific 2014). This allows workers to clear themselves of danger before it is too late. The effectiveness of these monitors will no doubt make them more commonplace.

Increased incidences of lethal exposure to hydrogen sulfide in dairy manure in recent years has been attributed anecdotally to increased use of gypsum bedding amendments (Fabian, Eileen E., personal communication). Limited observations with gas meters (some conducted by Penn State faculty) and anecdotal evidence supports this relationship in open-air type storage systems (Fabian, Eileen E., personal communication). Recycled gypsum has become more popular as a bedding amendment in dairy barns (Fabian, Eileen E., personal communication). The product is sourced as waste from the drywall and construction industries (USA Gypsum 2014). The suppliers of this product and their customers claim some important benefits of its use. They claim cows remain cleaner in the barns and are more comfortable (USA Gypsum 2014). Because recycled gypsum is inorganic, it does not facilitate bacterial growth which leads to a lower incidence of infections (USA Gypsum 2014). Gypsum is also more absorbent than most bedding materials, and so it helps cows stay clean without drying out their skin or feet (USA Gypsum 2014).

To understand why the relationship between gypsum bedding use and increased hydrogen sulfide accumulation is entirely plausible, it is necessary to investigate the microbial metabolism reactions occurring in the stored manure. It is important to understand the concept of anaerobic respiration in manure and how an anaerobic environment is formed. Respiration is the process by which organisms harvest electrons from high-energy compounds to create their own usable high energy compounds (Madigan et al., 2012). Respiration requires an electron donor and an electron acceptor (Madigan et al., 2012). The electron acceptor must have an affinity for gaining electrons, while the electron donor must have the affinity for losing them. The difference in the electronegativity (the electron affinities) between the electron donor and acceptor dictates the amount of energy that can be harvested from this reaction

(Madigan et al., 2012). These reactions are known as reduction (gain of electrons)-oxidation (loss of electrons) reactions because it brings these two processes together. There are many different compounds which can serve as electron donors or acceptors (Madigan et al., 2012). Those that have been observed have been organized into a "redox" table which allows us to visualize the energy producing capability of various reduction-oxidation combinations (Madigan et al., 2012). For example, the combination of H_2 as an electron donor and O_2 as an electron acceptor, forming H_2O, yields the greatest redox potential, or the greatest energy yield (Madigan et al., 2012). This pair is used by many "higher" animals, including humans. In this way, we breathe oxygen to use it as an electron acceptor throughout our body. Humans have evolved as obligate aerobes, meaning we can only use oxygen as our electron acceptor. However, microorganisms have generated a vast diversity of adaptations which allow them to use different compounds for electron acceptors (Madigan et al., 2012). The most important to this investigation are those which are able to use sulfite (SO_3^{-2}) as an electron acceptor.

In the stored manure, many different microorganisms thrive (Pell, 1997). Given the microbial complexity of the ruminant excreta and the variety of nutrients available for continued microbial growth in manure, the manure environment has the ability to change throughout storage. The most important is the development of an anaerobic environment. The microorganisms that we are most interested in are obligate anaerobes, meaning they can only survive in an environment devoid of oxygen. When manure is first pumped into storage, it is infused with oxygen through its exposure with the air and agitation. However, microorganisms generate an anaerobic environment below the surface of the manure by using up the oxygen first (Madigan et al., 2012). These aerobic microorganisms are the microbes which thrive in the beginning (Madigan et al., 2012). As the manure becomes more anoxic due to microbial aerobic respiration and lack of aeration, microorganisms begin to use the "next best" electron acceptor to generate energy until it is depleted (Madigan et al., 2012). This process continues until microbes have depleted all of the available electron acceptors (Madigan et al., 2012). The redox schematic (below) shows the relative

order of electron acceptors in terms of energy potential, which dictates the order in which they are used (Madigan et al., 2012):

$$\frac{1}{2} O_2 \rightarrow Mn^{+4} \text{ (Manganese)} \rightarrow NO_3^- \text{ (Nitrate)} \rightarrow Fe^{+3} \text{ (Iron)} \rightarrow SO_3^{-2} \text{ (Sulfite)}$$

The microbes that are the focus of this thesis use sulfite (SO_3^{-2}) as an electron acceptor. They require an anoxic environment and thrive after the above electron acceptors have been depleted. Recycled gypsum has a chemical formula of $CaSO_4$, which means that when gypsum is present in the manure, sulfate (SO_4) is available for reduction, while compounds like hydrogen and fatty acids are readily available to be used an electron donors. Hydrogen (H_2) is available to sulfate-reducing bacteria due to a process called syntrophy (Madigan et al., 2012). Syntroph bacteria can metabolize fatty acids and other compounds and create H_2 (Madigan et al., 2012). While this reaction is energetically unfavorable, when it is paired with an energetically favorable use of H_2 by another species of bacteria (like sulfate-reducing bacteria), it becomes a net energy gain (Madigan et al., 2012). These syntrophic bacteria are important in generating the substrate that sulfate-reducing bacteria use in combination with sulfate as an electron acceptor (Madigan et al., 2012). Some syntroph species of bacteria include *Syntrophobacter, Syntrophomonas, Pelotomaculum, and Syntrophus* (Madigan et al., 2012). Sulfate-reduction, which generates H_2S (hydrogen sulfide) from SO_4^{-2}, can be separated into assimilative and dissimilative reduction (Madigan et al., 2012). Assimilative reduction incorporates the H_2S product into organic compounds like sulfur-containing amino acids (Madigan et al., 2012). This results in little accumulation of H_2S in the environment. Dissimilative reduction simply releases the H_2S into the environment where it can accumulate in significant concentrations (Madigan et al., 2012). The reduction of sulfate (SO_4^{-2}) to hydrogen sulfide (H_2S) requires some intermediate steps (Madigan et al., 2012). Careful observation reveals that sulfite (SO_3^{-2}) is the true electron acceptor as opposed to sulfate (SO_4^{-2}). Conversion of stable sulfate to sulfite is accomplished in an energy-consuming reaction carried out by the microorganism (Madigan et al., 2012). The reaction is shown below and is catalyzed by ATP sulfurylase (Madigan et al., 2012):

$$SO_4^{-2} + ATP \rightarrow APS + PP_i$$

In the reaction above, ATP (adenosine triphosphate) is bound to sulfate where it donates the phosphates that make up this high energy compound (Madigan et al., 2012). Adenosine phosphosulfate (APS) is formed as an intermediate. The APS is then reduced to sulfite in dissimilative reduction in the reaction below catalyzed by APS reductase (Madigan et al., 2012):

$$APS + 2e^- \rightarrow AMP + SO_3^{-2}$$

With the presence of sulfite (SO_3^{-2}), sulfite reduction can take place. Notice the presence of AMP (Adenosine monophosphate) which contains much less energy than ATP. Through the transfer of electrons and generation of proton motive force (proton concentration differential across a membrane), sulfite is reduced to hydrogen sulfide by the enzyme sulfite reductase (Madigan et al., 2012). The resulting proton motive force generates ATP via ATPase (Madigan et al., 2012). The following reaction summarizes the reduction of sulfate (Madigan et al., 2012):

$$SO_4^{-2} + ATP + 8e^- \rightarrow AMP + H_2S$$

The resulting hydrogen sulfide is excreted into the environment in dissimilative reduction, where it can accumulate. Some of the most well-known species of sulfate reducing bacteria include *Desulfovibrio, Desulfomonas, Desulfotomaculum, and Desulfobulbus*, all of which can use hydrogen as an electron donor (Madigan et al., 2012). In addition, these bacteria are all obligate anaerobes (Madigan et al., 2012). The creation of an anaerobic environment is essential for this process to occur (Madigan et al 2012). As manure sits in storage with little agitation, a solid crust can form on the surface of the manure, further limiting oxygen penetration into the liquid manure. It is well documented that the environment within stagnant dairy manure storages is highly anoxic (Fabian, Eileen E., personal communication). It appears the link between gypsum bedding use and production of hydrogen sulfide in the stored manure is plausible given adequate conditions. As hydrogen sulfide is produced, the unaerated and liquid environment of the manure storage coupled with the heavy weight of hydrogen sulfide gas (heavier than air) causes the gas to remain at the bottom of the manure storage and accumulate. This accumulation is

then quickly released when the manure is agitated. Agitation is often done before emptying the pit. It is therefore crucial to investigate this risk. While anecdotal evidence has affirmed this relationship, repetition in a lab environment is necessary and to date, very little laboratory data is available (Fabian, Eileen E., personal communication). Therefore, the first hypothesis of this study is that the presence of recycled gypsum in stored manure causes the production and accumulation of elevated levels of hydrogen sulfide gas compared to manure without gypsum addition.

While laboratory research is limited in this issue, solutions have already been proposed to mitigate this hydrogen sulfide risk. While some anecdotal evidence has validated these solutions, in-depth research is once again limited. Some on-farm success has been seen so far (Fabian, Eileen E., personal communication). The two solutions to be investigated in this study are the commercially available Vital Breakdown™ (Homestead Nutrition, Inc., New Holland, PA), and an iron oxide product (Iron Oxide Recovery, Inc., Pittsburgh, PA).

Vital Breakdown™ is a manure amendment manufactured by Homestead Nutrition, Inc. that is marketed for a purpose other than hydrogen sulfide mitigation (Homestead Nutrition 2014). The proposed mechanism of Vital Breakdown™ is to accelerate the microbial decomposition process in the manure by providing certain species of bacteria to the manure storage environment (Homestead Nutrition 2014). Often, manure begins to settle into various fractions before microbial decomposition can break down the organic compounds (Hile, Mike, personal communication). Thick accumulation of manure at the bottom of storage tanks represents a challenge to pump and clean out (Fabian, Eileen E., personal communication). In addition, some bacteria generate more volatile compounds (odors) than others (Hile, Mike, personal communication). Vital Breakdown™ contains a proprietary mixture of bacteria intended to outgrow and limit the proliferation of bacteria which generate unfavorable odors (Hile, Mike, personal communication). Vital Breakdown™ contains a mixture of microorganisms including species from the genera *Saccharomyces, Bacillus, Pseudomonas, Esherichia, Enterobacter, and Aspergillus* (Homestead Nutrition 2014). The initial treatment rate for Vital Breakdown™ is two pounds per 10,000 gallons of

liquid manure (Homestead Nutrition 2014). It has been proposed that the increased microbial decomposition will reduce accumulation of thick manure deposits at the bottom of the pit which trap gasses like hydrogen sulfide. In addition, a more uniform and active manure environment will reduce the process of crust formation with its additional gas emission blockage. While it may not be the primary reason for using the product, it has been proposed that reduced hydrogen sulfide accumulation may be a side effect of its use.

The second proposed solution for reducing hydrogen sulfide gas is an iron oxide product (Iron Oxide Recovery, Inc., Pittsburgh, PA). The iron oxide products investigated in this study were sourced from Iron Oxide Recovery, Inc. based out of Pittsburgh, PA (Hedin Environmental 2014). The chemical formula for iron oxide is FeOOH. The reason this product has been proposed as a solution is its ability to interfere with hydrogen sulfide accumulation. In the laboratory isolation of sulfate-reducing bacteria, a medium which contains ferrous iron (Fe^{+2}) is used to culture the microorganisms (Madigan et al 2012). The principle behind this is the reaction of H_2S with ferrous iron (Madigan et al 2012). As the sulfate-reducing bacteria produce hydrogen sulfide and secrete it into the medium, the ferrous iron combines with the hydrogen sulfide to produce an insoluble ferrous sulfide (Madigan et al 2012). The precipitate is very black in color, which allows microbiologists to identify the presence of sulfate reducing bacteria (Madigan et al 2012). In addition, the diminished accumulation of hydrogen sulfide removes the inhibitory (poisonous) effect that high concentrations of hydrogen sulfide have on the sulfate-reducing bacteria (Madigan et al 2012). This allows for more bacterial growth for culturing (Madigan et al 2012). By using an iron oxide product in the manure, the goal is to mimic this laboratory effect for culturing sulfate-reducing bacteria. In its native form, the iron oxide product contains ferric iron (Fe^{+3}), while ferrous iron (Fe^{+2}) is the form which binds to hydrogen sulfide (Reade 2014). However, the use of iron as an electron acceptor precedes sulfate according to the redox schematic. Therefore, it is reasonable to anticipate that the conversion of ferric iron to ferrous iron will be accomplished through microbial anaerobic respiration, leaving ferrous iron available to bind with hydrogen sulfide. Currently, iron oxide

products are used in the decontamination of water, including removing poisons from drinking water, and for treating wastewater in certain industries such as mining and refining (Reade 2014). The product is a readily available and recycled product, much like the gypsum bedding additive (Hile, Mike, personal communication). The supplier, Iron Oxide Recovery, Inc., collects the iron oxide product from coal mine drainage (Hedin Environmental 2014). Not only does this product eliminate contamination of freshwater sources, it represents a usable byproduct (Hedin Environmental 2014).

As the second part of this study, the two proposed solutions will be tested under laboratory conditions to determine their effect on hydrogen sulfide generation. This secondary investigation leads to a secondary hypothesis that given a clear causal relationship between gypsum presence and hydrogen sulfide accumulation in dairy manure, supplementing a manure additive such as Vital Breakdown™ or iron oxide at a prescribed rate will reduce the accumulation of hydrogen sulfide in dairy manure and therefore reduce the risk of harmful exposure upon manure agitation.

Chapter 2

Materials and Methods

To test both presented hypotheses, manure samples were collected and stored under laboratory conditions. To mimic the on-farm environment, samples were stored for at least six months to allow microbial activity to proceed. However, for practical reasons, not all characteristics of an outdoor open-air storage were able to be replicated in the laboratory. Two separate trials were run in a series. Each trial contained eight samples (four treatments x 2 samples/treatment). In the first trial, gypsum presence, gypsum inclusion rate, and the addition of Vital Breakdown™ additive were tested for effects on

hydrogen sulfide emissions. The second trial tested gypsum presence, and two different iron oxide for effects on hydrogen sulfide emissions. Both trials included control samples which were not given any treatment. While hydrogen sulfide was the gas of concern for this study, emission levels of other gasses including carbon monoxide and oxygen were measured. While the measurements taken between the two trials differed (more measurements taken in trial two), the conditions and methods used to store, manipulate, and measure the samples were the same.

Eight manure samples were taken from the Penn State dairy barns for each trial. The manure was collected from the feeding aisle in the free stall barn that is bedded with sand. The mix of urine and feces was used as it was sampled. However, it is likely that the manure composition in a manure pit would contain more urine than the collected samples because urine had already drained from the aisle and into the storage. The samples were placed in eight separate five-gallon heavy duty plastic buckets. The samples were then randomly placed in a treatment group and prepared upon arrival at the laboratory. To prevent the manure samples from drying out during storage, a lid was placed on each bucket during storage. Due to the dry environment and lack of precipitation, this was necessary and is a source of deviation from the on-farm environment. The lid was fitted with a plastic barbed fitting which allowed gasses to escape from the buckets during storage under a fume hood. The fitting also allowed for measurement of the gasses without agitating the samples (See Figure 1 below).

Treatments

In the first trial, four treatments were tested with two samples in each treatment group. The 15 kg of manure used in each bucket represents the approximate amount of manure in the five-gallon bucket that is nearly full. All of the buckets were equalized to 15 kg before adding the treatments. The first treatment was a control group with no added materials. The second treatment was the Gypsumx1 treatment. Using the gypsum manufacturer's (USA Gypsum, Reinholds, PA) recommended level of

product usage for a bedding supplement, the inclusion rate of gypsum expected in the manure was calculated to be 0.35% of the total manure mass. This inclusion rate was used in the Gypsumx1 treatment. The gypsum was well mixed into the manure sample before storage. The third treatment was an estimated rate of gypsum inclusion based on using gypsum as the sole bedding material. This level was set at nine-fold greater (3.15%) than the first gypsum treatment, and so the treatment was labeled Gypsumx9. The last treatment contained gypsum at the bedding supplement 0.35% inclusion level and the Vital Breakdown™ additive at the manufacturer's recommended rate of two pounds per 10,000 gallons of liquid manure. This was calculated to be 0.0025% based on mass. The composition of all eight samples is presented in Table 1. Figure 1 represents the layout of the eight buckets under the fume hood in the lab. Notice the fitting in each lid for air escape. Figure 2 shows the material used for gypsum. This product was provided by USA Gypsum, and is similar to the product used in most dairies that use gypsum as a bedding amendment.

Figure 1: Bucket layout under fume hood

Figure 2: Gypsum material

The second trial was structured identically to the first trial with the exception of the treatments and the initial amount of manure used. The amount of iron oxide product available and the desired inclusion rate limited the amount of manure used in each sample. In this trial, two different iron oxide products were tested, and they were named Marchand and Brandy (names provided by the product manufacturer, Iron Oxide Recovery, Inc.). The first treatment was a control containing only 13.05 kg of manure. The second treatment contained gypsum at an inclusion rate of 0.35% once again. The third treatment contained gypsum at a 0.35% inclusion level and the Marchand product (97% FeOOH) (Hedin Environmental 2014) at a level based on the relative molecular weights of FeOOH and $CaSO_4$. To ensure one molecule of gypsum in the sample was accompanied by one molecule of iron oxide, the available iron oxide dictated the amount of gypsum used in the sample, which dictated the amount of manure used given the desired 0.35% gypsum inclusion rate (see Table 1 Notes). The fourth treatment contained gypsum and the Brandy iron oxide product. Due to the lower FeOOH content of the Brandy product (approximately 30%) (Hedin Environmental 2014), a large quantity of the product would need to be added to achieve a 1:1 molar ratio which exceeded the amount of product available for use in this study. The total amount of

product received was divided between the two samples for this treatment. This lower amount also allowed us to test whether or not a 1:1 ratio of iron to sulfur in the manure would be needed to reduce hydrogen sulfide to an acceptable level. The compositions of all eight samples can be seen in Table 1.

Table 1: Compositions of Treatment Groups

Sample	Treatment	Manure	Gypsum	Additive
Trial 1				
1	Control	15.00 kg	0.0 g	0.0 g
2	Control	15.00 kg	0.0 g	0.0 g
3	Gypsumx1*	15.00 kg	52.5 g	0.0 g
4	Gypsumx1*	15.00 kg	52.5 g	0.0 g
5	Gypsumx9**	15.00 kg	472.5 g	0.0 g
6	Gypsumx9**	15.00 kg	472.5 g	0.0 g
7	Gypsumx1 + Vital Breakdown™	15.00 kg	52.5 g	0.4 g
8	Gypsumx1 + Vital Breakdown™	15.00 kg	52.5 g	0.4 g
Trial 2				
1	Control	13.05 kg***	0.0 g	0.0 g
2	Control	13.05 kg	0.0 g	0.0 g
3	Gypsumx1	13.05 kg	45.8 g	0.0 g
4	Gypsumx1	13.05 kg	45.8 g	0.0 g
5	Gypsumx1 + Marchand	13.05 kg	45.8 g	29.9 g
6	Gypsumx1 + Marchand	13.05 kg	45.8 g	29.9 g
7	Gypsumx1 + Brandy	13.05 kg	45.8 g	53.4 g
8	Gypsumx1 + Brandy	13.05 kg	45.8 g	53.4 g

Notes:

* = Gypsumx1 = 0.0035 x Manure weight

** = Gypsumx9 = .0315 x Manure weight

*** = Manure used based on available Marchand:

Ratio of Mole weight CaSO4:FeOOH	1.532
Available FeOOH (g)	x 29.9
Gypsum used (g)	= 45.8
Desired Gypsum Inclusion Rate	÷ 0.35%
Manure in sample	≈ 13.05 kg

Measurements

In the first trial, gas (CO, H_2S, O_2) emissions and pH measurements were taken at Day 0, 6, 76, and 225. On all days except Day 0, readings were taken before and after an agitation process. The agitation process consisted of manually plunging a two-inch power drill stirrer into the manure six times, plunging the stirrer to the bottom of the sample and in a different location for each plunge. Figure 3 below shows the stirrer used for agitation. This process was intended to homogenize the sample as well as release any gasses trapped in the sample. During the agitation process, the lid was removed and the gas meter (see Equipment) was held directly above the sample, which constitutes the readings "after" agitation. The pre-agitation readings were taken via the same gas meter fitted to the barbed fitting on each lid, which measured the concentration of gasses in the head space above the manure samples.

Figure 3: Agitator

In the second trial, additional measurements were included in the protocol in addition to those measured in trial one. In addition to the gasses measured in trial one, oxidation-reduction potential (ORP) and temperature were measured. In the second trial, measurements were taken at Day 1, 7, 15, 42, 91, and 184. On days 42, 91, and 184, measurements were taken before and after the agitation process. Using the

same mixer used in trial one, the mixer was plunged into the manure ten times, thoroughly agitating the manure and releasing trapped gasses. Again, the gas meter was held directly above the sample during agitation.

After all measurements were taken, the lids were replaced on each sample and were allowed to incubate until the next measurement with no agitation.

Equipment

A multi-gas meter was used to read the levels of Carbon monoxide (CO), Hydrogen Sulfide (H_2S), and Oxygen (O_2). The gas meter used in this study is the M40 multi-gas meter from Industrial Scientific. This meter was originally launched in 2003, and has been recently retired from service (Industrial Scientific 2014). The M40 reads multiple gasses at once via the attached SP40 air pump (CO, H_2S, O_2). This meter utilizes a Lithium-ion battery and has the ability to store data for up to 75 hours. The M40 meter also has a 90 dB alarm for high readings (or low oxygen readings). The resolution of the H_2S sensor is 1 ppm and has a range of 0-500 ppm (Industrial Scientific 2014). The M40 is pictured below in Figure 4. Note the SP40 air pump is affixed to the meter and brings sampling air into the meter rather than through diffusion.

Figure 4: Industrial Scientific M40 Multi-gas meter

To measure the pH of the samples as well as temperature and oxidation-reduction potential (ORP), the SDL100 Datalogger from Extech Instruments was used. This meter was fitted with two probes for the simultaneous measurement of pH, temperature, and ORP. The Mini pH Electrode (part number 60120B) is 120mm in length and was immersed in the manure approximately 100mm during measurement (Extech Instruments 2014). This electrode has a glass sensing bulb capable of measuring pH between 0 and 14, and has an operating range of 0 to 80°C (Extech Instruments 2014). The thermistor probe used (part number 850188) has a range of 0 to 65.0°C with a resolution of 0.1°C (Extech Instruments 2014). The ORP readings have a resolution of 1 mV and the pH readings have a resolution of 0.01 (Extech Instruments 2014). Figure 5 shows the Extech SDL100 device. The accompanying thermistor probe and pH electrode is shown.

Figure 5: Extech SDL100 pH/ORP/Temperature meter

Chapter 3

Results

The results are summarized in their respective trials. Due to the difference in the days measurements were taken, it was most appropriate to keep the trials separate graphically. With respect to each other, trial two contains an additional day of measurement as well as longer period of time before the samples were agitated for measurement. This section is divided into sections for each trial, although the discussion of the results will consider the two trials together.

Trial One

Table 2 is a summary of all of the results from the first trial. These measurements include carbon monoxide (CO), Hydrogen Sulfide (H_2S), Oxygen (O_2), and pH. Measurements of pH were only taken after agitation on the days where agitation occurred. Notice some measurements of H_2S exceeded the limit of detection of the gas meter used. In this case, the maximum was reported (500 ppm). In addition, it is important to note the lid of the first control sample was left partially open between days 76 and 225 (human error). This construed the oxygen reading as oxygen was freely available to the sample head space.

To understand the relationship between storage time, agitation, and treatment group, a chart was created for each day of measurement which included both pre- and post-agitation readings. These are Day 6, 76, and 225 in the first trial (Figures 6, 7, and 8, respectively). Lastly, Figure 9 shows the differences between the treatment groups in hydrogen sulfide emissions after agitation throughout the storage period. It is important to note that in Figures 6-9, the average of the two samples in each treatment group was used as the representative for the treatment group.

Table 2: Trial One Data

	Pre-agitation				*Post-agitation*		
Sample Name	*CO* *ppm*	*O_2* *%*	*H_2S* *ppm*	*pH* *-*	*CO* *ppm*	*O_2* *%*	*H_2S* *ppm*
Day 0							
Control	0	20.6	**0**	8.35			
Control	0	20.6	**0**	8.59			
Gypsumx1	0	20.6	**0**	8.17			
Gypsumx1	0	20.5	**0**	8.31			
Gypsumx9	0	20.6	**0**	7.98			
Gypsumx9	0	20.6	**0**	7.96			
Gypsumx1 + Vital Breakdown™	0	20.6	**0**	8.21			
Gypsumx1 + Vital Breakdown™	0	20.6	**0**	8.18			
Day 6							
Control	95	14.4	**33**	6.40	2	20.5	**6**
Control	500	12.9	**352**	6.50	5	20.4	**15**
Gypsumx1	128	14.0	**31**	6.42	3	20.5	**8**
Gypsumx1	149	11.2	**31**	6.43	8	20.5	**15**
Gypsumx9	224	17.7	**28**	6.39	3	20.5	**4**
Gypsumx9	105	14.3	**36**	6.29	2	20.5	**7**
Gypsumx1 + Vital Breakdown™	970	17.0	**115**	6.48	6	20.6	**4**
Gypsumx1 + Vital Breakdown™	525	16.5	**99**	6.41	5	20.5	**6**
Day 76							
Control	0	7.9	**0**	7.08	0	20.0	**0**
Control	2	5.1	**0**	6.92	0	20.0	**0**
Gypsumx1	2	6.4	**0**	7.09	15	9.8	**500***
Gypsumx1	2	9.0	**0**	6.93	5	20.0	**128**
Gypsumx9	2	13.3	**27**	6.90	18	20.2	**500***
Gypsumx9	0	14.4	**0**	6.83	7	20.1	**182**
Gypsumx1 + Vital Breakdown™	17	14.5	**500***	6.38	6	20.1	**160**
Gypsumx1 + Vital Breakdown™	22	5.3	**500***	6.43	3	20.0	**45**
Day 225							
Control	0	20.9**	**0**	7.68	0	20.4	**74**
Control	2	11.0	**0**	7.69	0	20.2	**131**
Gypsumx1	0	14.8	**0**	7.64	2	20.2	**500***
Gypsumx1	3	10.0	**0**	7.66	2	20.6	**403**
Gypsumx9	0	15.0	**0**	7.48	4	20.9	**500***
Gypsumx9	0	18.5	**0**	7.38	0	20.9	**32**
Gypsumx1 + Vital Breakdown™	0	12.1	**5**	7.62	0	20.9	**24**
Gypsumx1 + Vital Breakdown™	2	2.0	**0**	7.75	0	20.9	**22**

Notes:

* = Above maximum gas meter reading of 500 ppm

** = Sample lid was cracked open during incubation

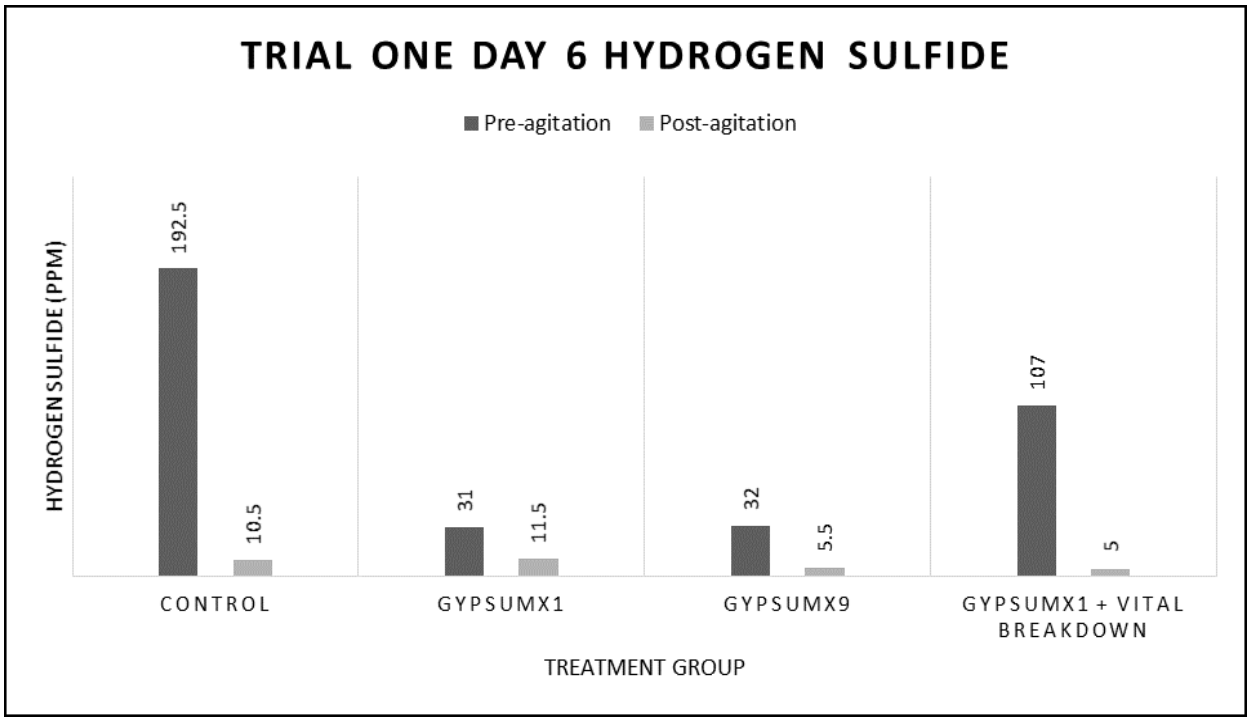

Figure 6: Trial One Day 6 Hydrogen Sulfide

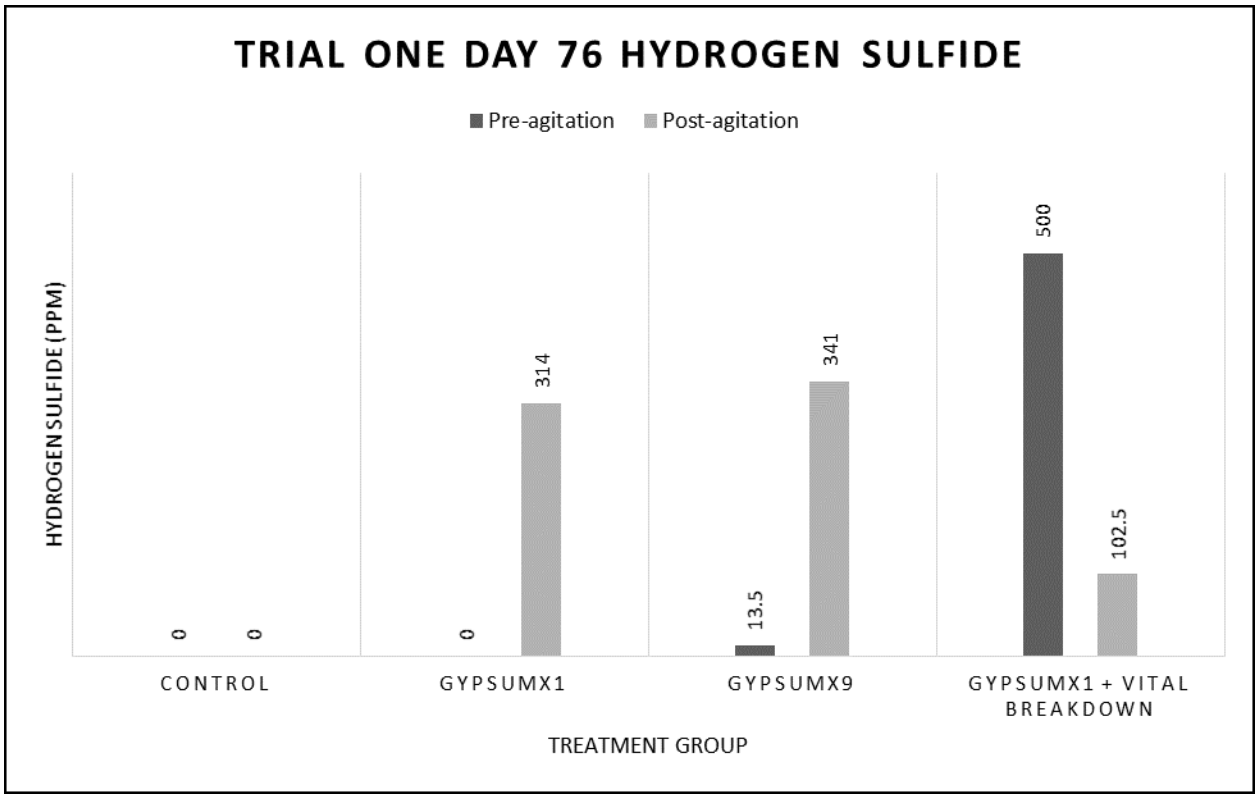

Figure 7: Trial One Day 76 Hydrogen Sulfide

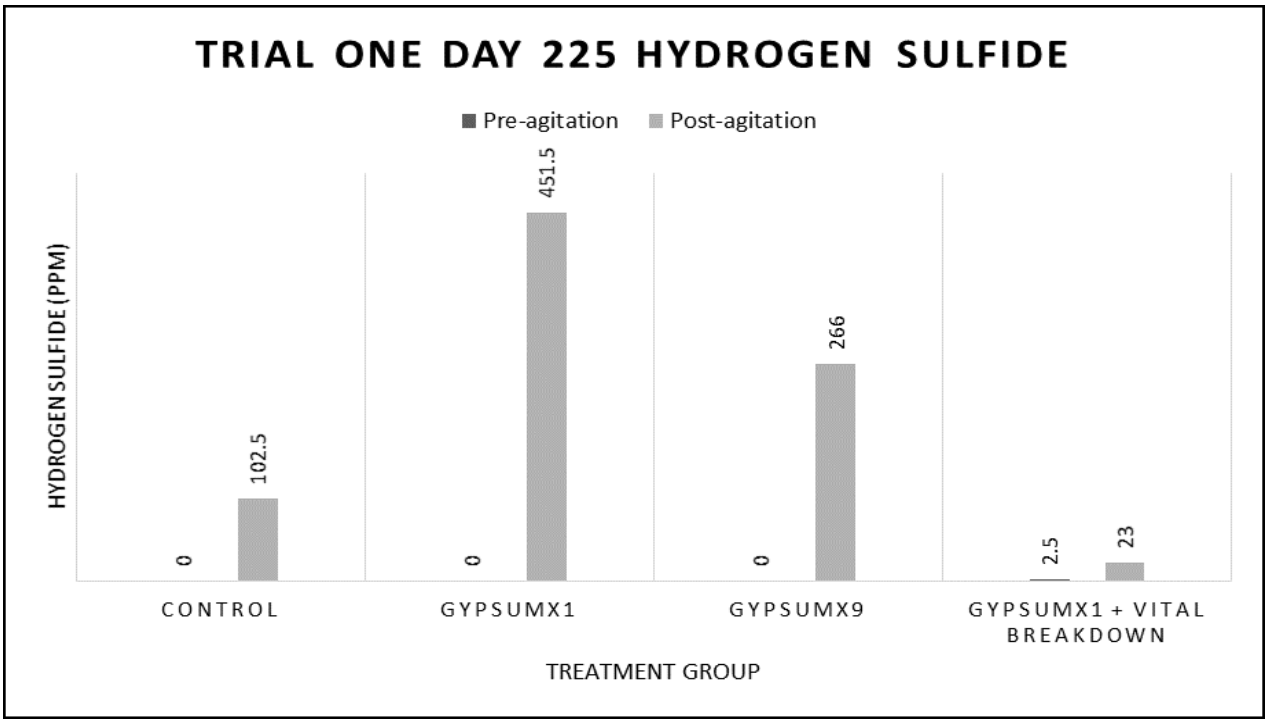

Figure 8: Trial One Day 225 Hydrogen Sulfide

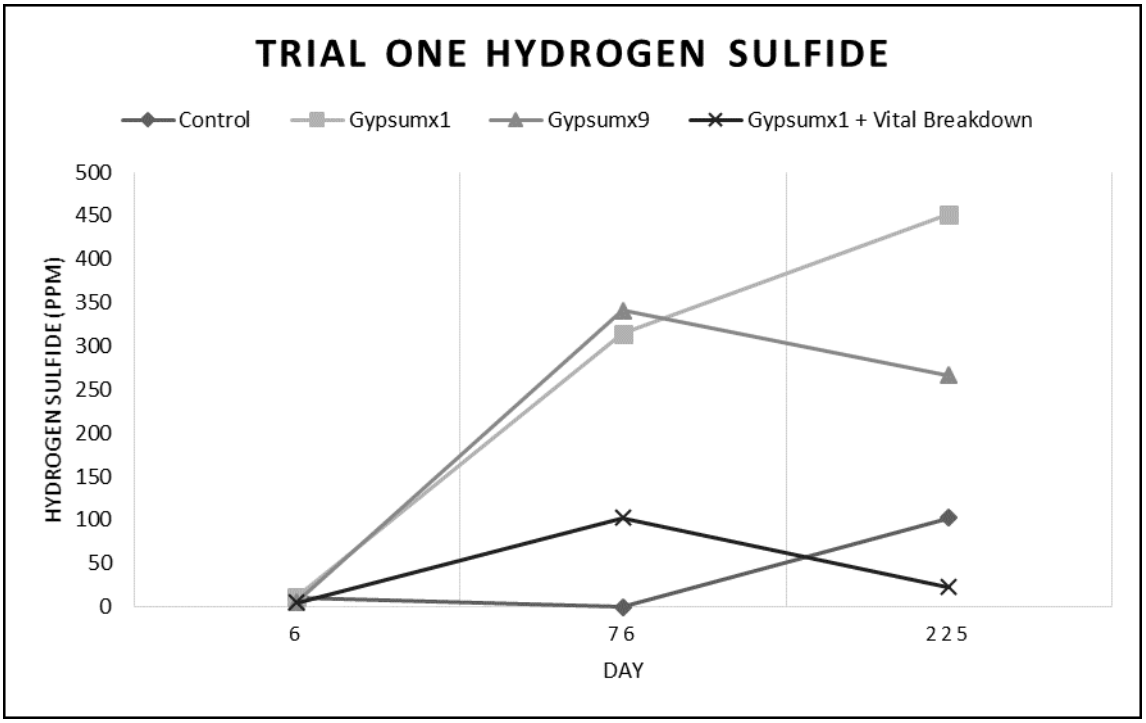

Figure 9: Trial One Hydrogen Sulfide after agitation vs. Treatment

Trial Two

The second trial spanned 184 days with six days of measurement. After collection of data from the first trial, some slight changes were made to the measurement protocol. Data collection was exactly the same with the exception of the addition of oxidation-reduction potential measurements. In the second trial, it was decided to wait until Day 42 to agitate the manure samples, to encourage the formation of an anaerobic environment.

Table 3 is a collection of all of the data collected for the second trial. These data include Carbon Monoxide (CO), Hydrogen Sulfide (H_2S), Oxygen (O_2), and pH as in the first trial, as well as oxidation-reduction potential (ORP). For formatting purposes, the table had to be split in two sections. Note the data for days 91 and 184 are below the data for days 1, 7, and 15. Agitation of the samples began on day 42. Notice there was an error in the readout of the ORP meter in the two control samples on day 1.

Once again, charts were created for each day of measurement that contained pre- and post-agitation data. These are days 42, 91, and 184 and can be seen in Figures 10, 11, and 12, respectively. Figure 13 shows the relationship between the treatment group and the hydrogen sulfide measurements across the storage period. Note that on days when pre- and post-agitation measurements were taken, the post-agitation measurement was used. As in the results of the first trial, Figures 10, 11, 12, and 13 use the average of the two samples in each treatment for the representative of the treatment group.

Table 3: Trial Two Data

	Pre-agitation					*Post-agitation*				
Sample Name	*CO*	*H_2S*	*O_2*	*pH*	*ORP*	*CO*	*H_2S*	*O_2*	*pH*	*ORP*
Day 1										
Control	5	**4**	20.9	6.80	2000*					
Control	5	**12**	20.7	7.34	1853*					
Gypsumx1	82	**102**	17.7	6.86	-2					
Gypsumx1	131	**182**	10.7	6.82	-4					
Gypsumx1 + Marchand	0	**0**	20.7	6.73	14					
Gypsumx1 + Marchand	0	**0**	20.7	6.74	11					
Gypsumx1 + Brandy	144	**13**	15.5	6.77	9					
Gypsumx1 + Brandy	4	**0**	20.4	6.79	4					
Day 7										
Control	2	**178**	12.5	6.54	20					
Control	2	**47**	10.1	6.65	14					
Gypsumx1	3	**42**	13.6	6.94	-3					
Gypsumx1	2	**7**	16.7	7.14	-15					
Gypsumx1 + Marchand	0	**8**	19.7	6.49	22					
Gypsumx1 + Marchand	0	**22**	16.8	7.14	-17					
Gypsumx1 + Brandy	2	**10**	17.4	6.70	9					
Gypsumx1 + Brandy	4	**3**	12.9	7.09	-12					
Day 15										
Control	0	**7**	16.5	7.57	-44					
Control	4	**7**	13.0	7.19	-16					
Gypsumx1	5	**4**	13.1	7.33	-27					
Gypsumx1	0	**0**	18.4	7.36	-26					
Gypsumx1 + Marchand	0	**2**	18.1	7.16	-16					
Gypsumx1 + Marchand	0	**3**	18.9	6.90	2					
Gypsumx1 + Brandy	3	**0**	14.8	7.17	-18					
Gypsumx1 + Brandy	2	**2**	20.9	7.35	-28					
Day 42										
Control	2	**0**	17.2	6.74	6	0	**33**	21.9	6.53	19
Control	0	**0**	18.0	7.01	-11	0	**28**	21.9	6.78	4
Gypsumx1	2	**0**	16.5	7.23	-22	0	**66**	21.9	6.92	-2
Gypsumx1	0	**0**	19.6	7.05	-22	0	**71**	21.9	6.97	-6
Gypsumx1 + Marchand	0	**0**	19.2	7.45	-40	0	**19**	21.9	7.03	-10
Gypsumx1 + Marchand	0	**0**	18.0	7.23	-21	0	**16**	21.9	6.91	-2
Gypsumx1 + Brandy	0	**0**	18.4	6.74	7	0	**76**	21.9	6.74	7
Gypsumx1 + Brandy	0	**0**	16.9	7.28	-25	0	**65**	21.9	6.86	1
Day 91										
Control	5	**0**	3.0	7.75	-31	0	**10**	20.9	7.01	-10
Control	0	**0**	10.7	7.90	-65	0	**11**	20.9	7.24	-25
Gypsumx1	2	**0**	10.1	7.17	-21	0	**25**	21.1	7.19	-23
Gypsumx1	3	**0**	2.9	7.36	-36	0	**68**	20.9	6.92	-5
Gypsumx1 + Marchand	2	**0**	3.1	7.49	-35	0	**0**	21.1	7.78	-56
Gypsumx1 + Marchand	2	**0**	2.9	7.47	-36	0	**0**	21.1	7.90	-61
Gypsumx1 + Brandy	4	**9**	2.4	7.48	-43	0	**21**	21.1	6.99	-8
Gypsumx1 + Brandy	0	**0**	17.7	8.11	-74	0	**137**	21.1	7.53	-44
Day 184										
Control	16	**0**	3.8	7.64	-46	0	**0**	20.7	7.69	-49
Control	0	**0**	9.5	8.32	-87	0	**37**	20.6	7.98	-69
Gypsumx1	0	**0**	7.9	7.73	-52	0	**15**	20.7	7.65	-46
Gypsumx1	0	**0**	9.8	8.26	-83	0	**78**	20.7	7.65	-47
Gypsumx1 + Marchand	0	**0**	7.0	8.11	-75	0	**0**	20.9	7.72	-52
Gypsumx1 + Marchand	0	**0**	16.6	8.33	-88	0	**0**	20.9	7.69	-52
Gypsumx1 + Brandy	0	**3**	5.5	8.02	-74	0	**8**	20.9	7.61	-44
Gypsumx1 + Brandy	0	**0**	10.1	8.32	-87	0	**130**	20.9	7.46	-35

Notes:
* = meter readout error

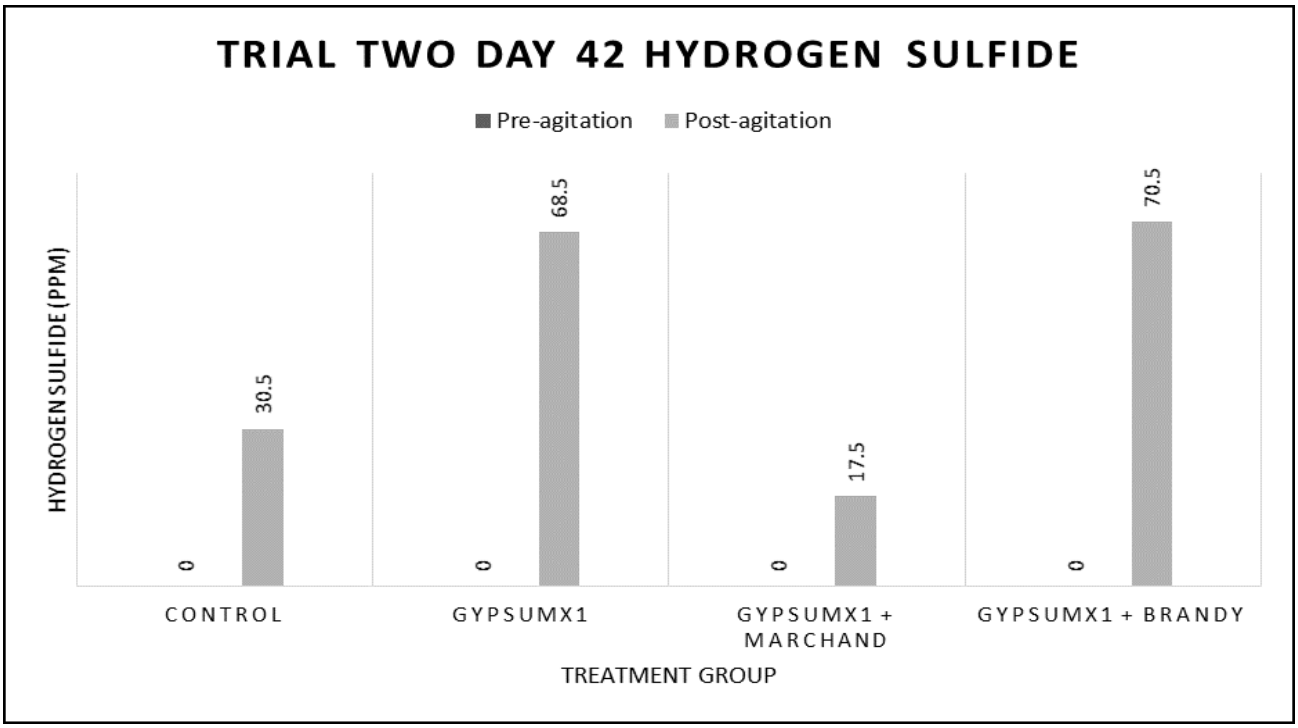

Figure 10: Trial Two Day 42

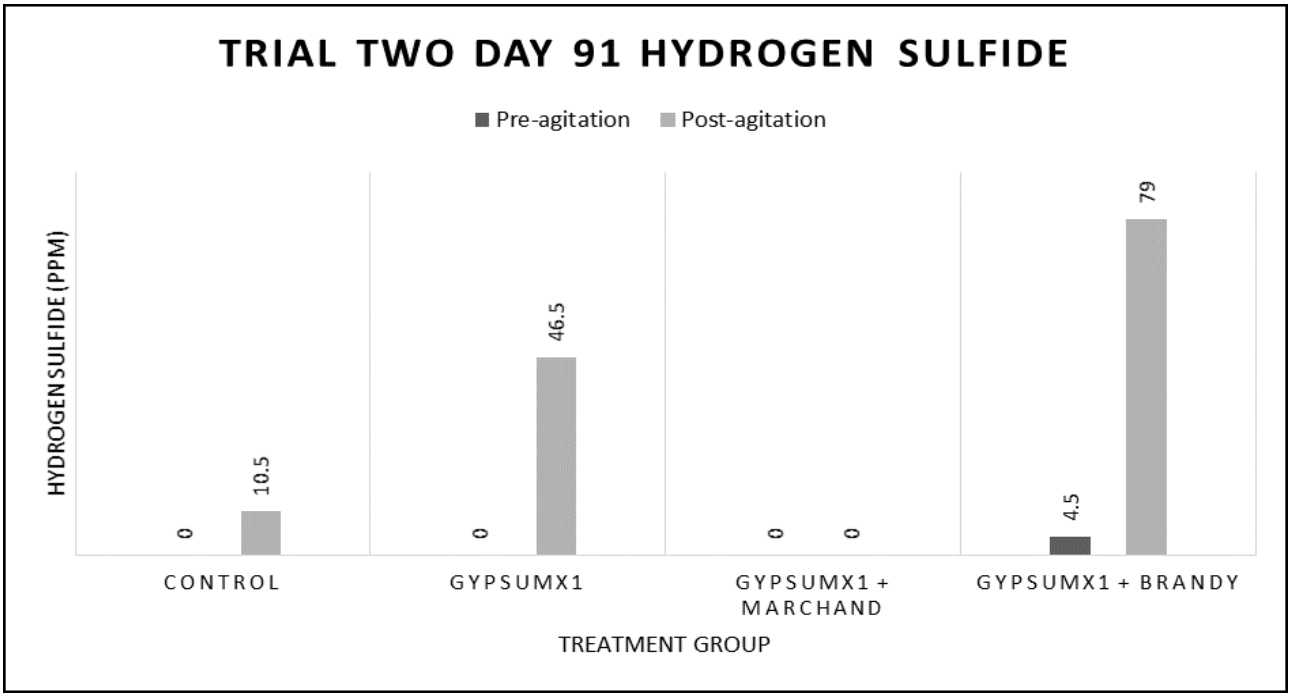

Figure 11: Trial Two Day 91

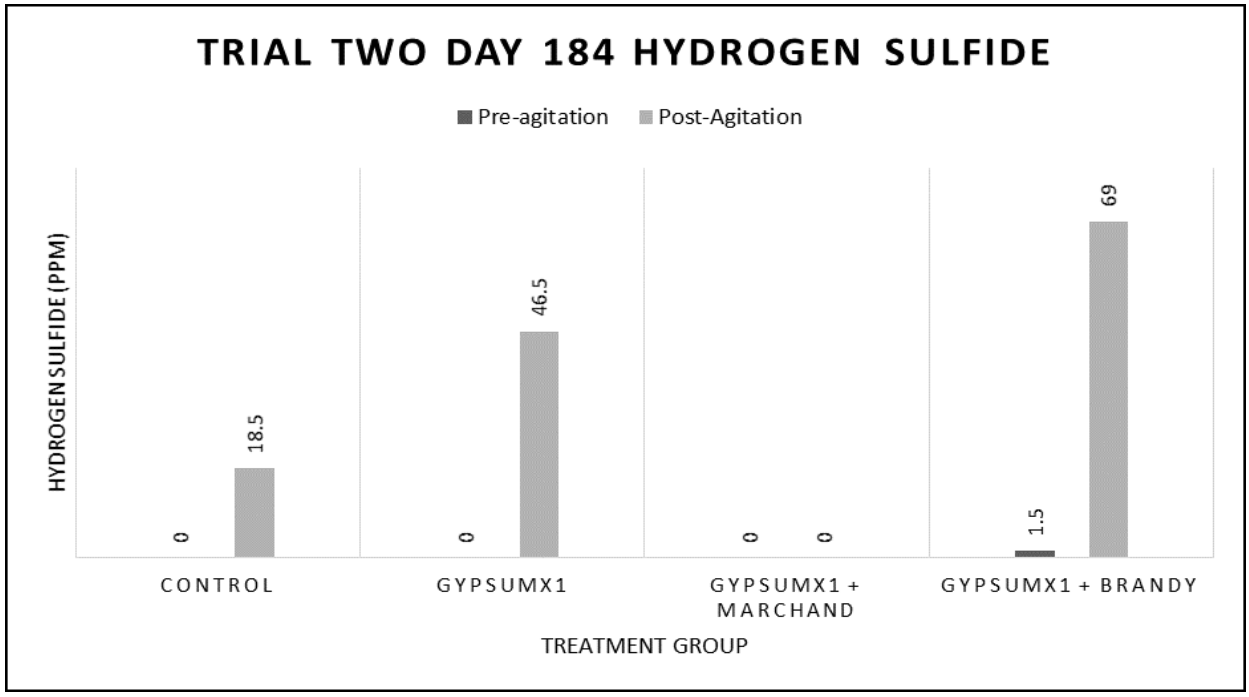

Figure 12: Trial Two Day 184

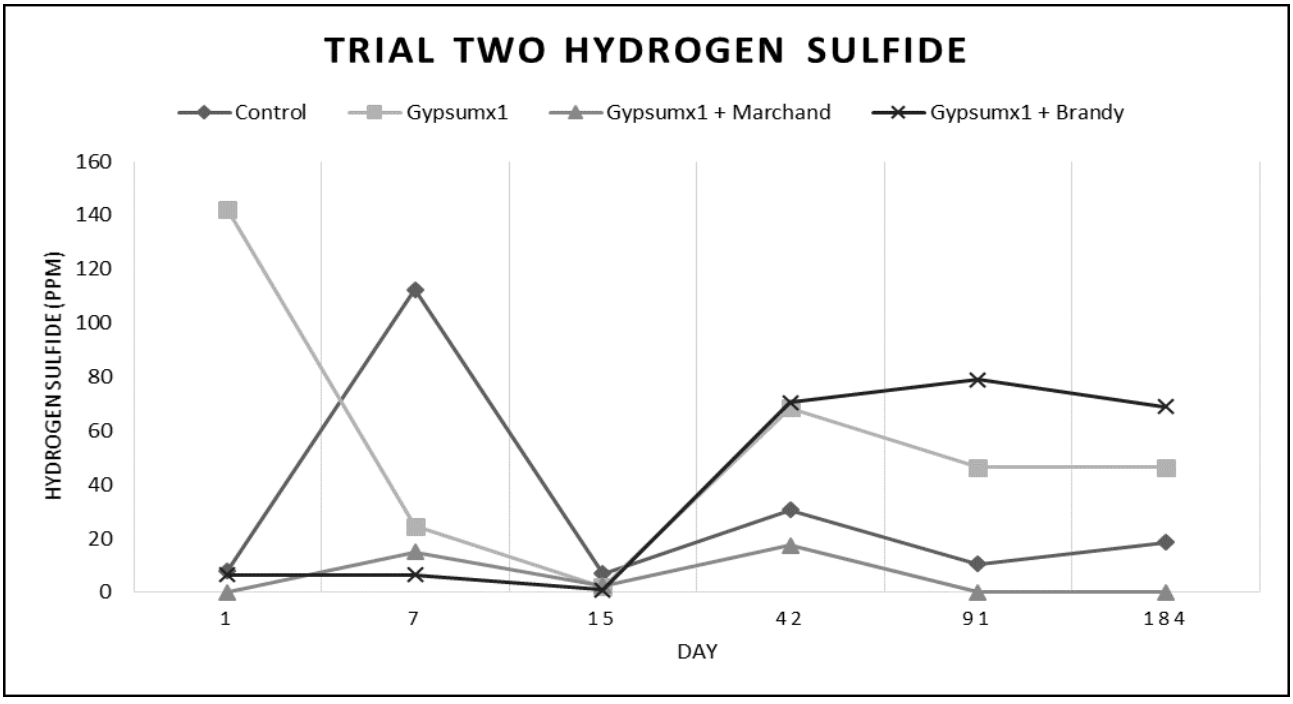

Figure 13: Trial Two Hydrogen Sulfide after agitation vs. Treatment

Chapter 4

Discussion

These two studies were conducted to evaluate the relationship between gypsum presence in dairy manure and the accumulation of hydrogen sulfide. In addition, the trials evaluated two potential solutions to mitigate hydrogen sulfide accumulation in dairy manure. Due to limitations in resources and physical laboratory space, only two samples per treatment could be tested. This likely contributed to the high degree of variation in the presented results. Regardless, trials one and two included some redundancies in treatments to strengthen the data. Both trials included control samples as well as gypsum-enriched manure. Before the results can be analyzed, it is necessary to question how well the on-farm environment was replicated in the lab.

Replicating the on-farm environment

There are some clear distinctions between what was accomplished in the laboratory and what would be observed on the farm. First of all, the controlled conditions within the lab deviate from an outside environment. The constant temperature within the lab is the most noticeable difference. Temperature readings taken throughout the course of the second trial reveal that the temperature of the manure ranged from 23.7 – 28.6 °C. This variation reflects the variation in the room temperature over the period of measurement. This minimal variation is unlike the variable temperatures that would be seen in an outdoor environment as manure is stored over the summer or over the winter months. In addition, the manure temperature remained quite high due to the elevated temperatures in the laboratory. If sulfate-reducing bacteria are indeed mesophilic and come from the living dairy animal, it could be argued their metabolic rate was increased due to the higher manure storage temperature (Madigan et al., 2012). On most dairy farms, the barns are scraped daily, and a scraper system empties manure into the pit on a regular and constant basis. This nearly continuous flow of manure into the storage introduces organic

material, microbes, and oxygen into the stagnant manure in storage. In addition, precipitation over the manure pit adds volume to the manure and alters the nutrient composition available for microbial decomposition. While it is unlikely that the anaerobic environment in a large farm storage is disrupted significantly across the entire manure storage, it is important to distinguish these differences with respect to the laboratory samples. The buckets were filled at the beginning of the trial, and allowed to incubate without any additions or disruptions, until agitation was performed. It is important to note that we agitated these samples several times over the study period. This would not be typical of a large outdoor storage that would not be fully agitated until the manure was being removed. This periodic agitation of our laboratory samples essentially cleared the manure of hydrogen sulfide each time instead of allowing it to build up. Often outdoor storages form a hard crust layer on the top of the manure, which is important for creating an anaerobic environment. Our samples accomplished the same thing, as crusts were noted only a few weeks into storage. To verify the anaerobic environment, the head space oxygen levels and oxidation-reduction potentials were measured. Results in Tables 2 and 3 show that oxygen levels are below 20% before agitation in all samples, even though oxygen was allowed to enter the head space. Table 3 includes ORP readings for all of the samples, and there is a clear decrease in oxidation reduction potential as the manure incubated. ORP readings below zero indicate an anoxic environment, while sulfate-reduction typically occurs in wastewater when the ORP is between -50 mV and -250 mV (Gerardi, 2007). The average ORP reading (excluding the two errors for the control samples on day 1) for days 1, 7, and 15 combined was -4.7 mV, while the average ORP reading for days 91 and 184 (including agitation) was -48.7 mV. While we may not have quite achieved the range of ORP readings dictated for hydrogen sulfide production, we were very close, and had clearly generated an anaerobic environment. The oxidation reduction potential went down as the manure was stored, meaning we were able to mimic the formation of an anaerobic environment in the manure samples.

Gypsum vs. Hydrogen Sulfide

The first hypothesis of this experiment was that hydrogen sulfide accumulation and emission would be greater when gypsum was present in the manure. The results certainly affirm the link between gypsum presence and hydrogen sulfide production. The first trial presents the strongest evidence for this relationship. Figure 4 shows a marked increase in hydrogen sulfide production over the control samples. Both gypsumx1 and gypsumx9 show higher hydrogen sulfide accumulation on day 76. This increased hydrogen sulfide level continued on day 225. While the explanation of hydrogen sulfide production from $CaSO_4$ in the introduction validates the relationship, these results provide evidence that those reactions do take place. Interestingly, the control samples created a bloom of hydrogen sulfide gas above the IDLH level of 100 ppm at the beginning of the study. This is likely an error, however, further investigation into the cause of this would be beneficial. For example, the manure sampled from the Penn State dairy barns may have included sulfate in some other form. Copper sulfate is a popular compound used for disinfecting foot baths, and may have been present in the manure. A nutrient analysis of the manure would have been beneficial. Regardless, hydrogen sulfide production was higher in the manure samples that contained gypsum. The second trial is less conclusive with respect to this relationship. Again, the control samples generated hydrogen sulfide on day 7, which appears to have no explanation, other than the possible presence of copper sulfate or some other sulfurous compound (such as sulfur found in the diet possibly). However, after day 7 the control samples show little hydrogen sulfide accumulation. At day 15, all of the samples contained little hydrogen sulfide. It is important to remember that up until day 42, the samples had not been agitated, and so the day 15 readings are only reflective of the head space gasses. Beginning on day 42, the results begin to be closer to what was expected. The samples containing gypsum only had increased hydrogen sulfide emissions after agitation when compared to the gypsum + Marchand and the control treatments. However, the gypsum + Brandy treatment had readings above the gypsumx1 treatment. The reasoning for the higher reading is unknown. Throughout these two trials it was

apparent that our first hypothesis was supported by the results; there is a positive relationship between the gypsum presence in the manure and hydrogen sulfide accumulation.

Gypsum Rate vs. Hydrogen Sulfide

While it was not included in the hypotheses presented in this study, the two different gypsum inclusion rates used in the first trial allowed for this comparison. The first inclusion rate was determined by the gypsum manufacturer as the rate found in manure when the gypsum product was used as a bedding additive. The second rate reflects gypsum use as the sole bedding material. This was in an attempt to encourage more hydrogen sulfide production. As can be seen in Figure 9, this was not the case. The production of hydrogen sulfide between the gypsumx1 and the gypsumx9 treatments was almost identical. It appears that the mass of gypsum present in the manure in the gypsum x1 treatment was not a limiting factor in hydrogen sulfide production. It is possible that with longer storage time a difference may have been measured between the two treatments. However, most manure will not be stored for more than 6-7 months. It appears as if simply using the gypsum as a bedding additive provides enough sulfate for the sulfate-reducing bacteria to generate deadly quantities of hydrogen sulfide, even in our small volume conditions.

Manure Agitation vs. Hydrogen Sulfide

While not explicitly stated in the first hypothesis, it was believed that agitation would be necessary to release large quantities of hydrogen sulfide gas. This is due to the anaerobic nature of hydrogen sulfide production by sulfate-reducing bacteria and the higher density of hydrogen sulfide in comparison to air. In addition, observations in the field and anecdotal evidence suggests that humans and animals are at the greatest risk for hydrogen sulfide poisoning during times of manure agitation. In both

trials, the manure was allowed to incubate without agitation for a period of time. In the first trial, this period was only six days, while in the second trial, the manure was incubated for 42 days before agitation. Evidenced by the dropping ORP readings in trial two (see Replicating the on-farm environment), we were able to generate an anaerobic environment in our manure samples. The results show wide variation early on in the two trials. In trial one (see Figure 6 and Table 2), day 6 shows considerable variation in pre-agitation hydrogen sulfide levels. Again, the controls created a large amount of hydrogen sulfide, especially the second control (352 ppm). The reason for this is unknown. However, we noted that the pre-agitation readings were higher than the post-agitation readings for all samples. This occurred only on day 6, because on day 76, the pattern began to reverse, and by 225, there was virtually no hydrogen sulfide in the head space in the buckets. In the early stages of manure storage, the hydrogen sulfide produced does not appear to be getting trapped in the manure. It is possible that small anaerobic pockets where the sulfate-reducing bacteria are producing H_2S are surrounded by more aerobic pathways to the surface of the manure. As the manure settles and closes these pathways, it forces the gas upwards and into the head space of the bucket. As the manure settles and the anaerobic portions of the manure spread and combine, the gas has little room to move and begins to accumulate. While it is not clear why so much hydrogen sulfide is produced at the very beginning of the storage period, we anticipated that as the manure sits and becomes more anaerobic, hydrogen sulfide gas would become trapped and released upon agitation. The agitation used in this study was designed to mix manure from the bottom of the bucket to the top, bringing pockets of hydrogen sulfide up through the manure and releasing them into the air. Based on the results presented in Tables 2 and 3, it is clear that hydrogen sulfide should not be a problem in stagnant manure storages until vigorous agitation is performed.

Vital Breakdown™ vs. Hydrogen Sulfide

The results of this study affirm that gypsum is the culprit in hydrogen sulfide production with the exception of our unexplained control samples early on. The most logical next step was to determine how to minimize the risk of hydrogen sulfide accumulation in the manure. Vital Breakdown™, manufactured by Homestead Nutrition, Inc., is designed primarily to minimize odors from outdoor manure storages. Its primary mode of action is to accelerate the microbial degradation process in the manure. The accelerated microbial action prevents settling and maintains a more homogenous mixture of manure. This increased activity is likely to postpone the process of developing an anoxic environment in the manure. We hypothesized that by slowing down this process, the production of hydrogen sulfide would be slowed down as well. In addition, the Vital Breakdown™ product creates a bias towards the growth of certain species of bacteria and therefore discourages the growth of other species (especially those known to produce unfavorable odors). The first trial applied a treatment of Vital Breakdown™ at a rate per manufacturer's directions. While the amount was very small on this small scale, it appeared as if the Vital Breakdown™ did reduce hydrogen sulfide emissions. Figures 6 and 7 show the emissions of each treatment at days 6 and 76, respectively. Interestingly, the pre-agitation readings for the Vital Breakdown™ supplemented samples were quite high on day 6 and day 76. A possible explanation is that the product stimulated increased fluidity and activity of the manure and maintained aerobic pathways throughout the manure which forced hydrogen sulfide gas up through the manure and into the head space. This effect, seen in other treatments, may have been magnified by the actions of Vital Breakdown™. However, on day 225, the effect of Vital Breakdown™ is reversed. The pre- and post-agitation readings were below 25 ppm. There are three explanations for these results. First, the increased microbial activity of the manure may maintain those pathways for gas escape throughout storage and the hydrogen sulfide slowly leaches out of the manure, which prevents hydrogen sulfide gas buildup. Second, it is possible that the increased microbial activity accelerates the conversion of sulfate to hydrogen sulfide to the point that the substrate runs out earlier. It is possible that by day 225, the quantity of gypsum left to react was small.

Third, it is possible that the sulfate-reducing species of bacteria were out-competed in the manure as other species promoted by the Vital Breakdown™ product predominated. In either situation, the amount of hydrogen sulfide trapped in the manure was decreased as the storage period continued. While the effect early in the incubation period may not be desirable, the product appeared effective as the storage time period was extended.

Iron Oxide vs. Hydrogen Sulfide

While the first trial showed desirable results using the Vital Breakdown™ product, the second trial investigated two additional products based on an entirely different chemical principle. Iron oxide (FeOOH) will produce ferrous iron in an anoxic environment. Ferric iron is used as an electron acceptor and is reduced to ferrous iron (Fe^{+2}). The ferrous iron will then bind to the hydrogen sulfide product of sulfate-reduction. The result is an insoluble, black iron-sulfur product. While this technique is used to isolate and identify sulfate-reducing bacteria, its effectiveness as a hydrogen sulfide remediation product is not known. Two different products, Marchand and Brandy, were used in this study. The Marchand product is almost entirely FeOOH. A chemical analysis of the Marchand provided by the product manufacturer revealed that it was 85-97% FeOOH by weight. As discussed above, the amount of Marchand available for this study dictated the amount of manure and gypsum used in all of the trial two samples. This was done to ensure an approximate 1:1 molar ratio of Fe and S in the manure environment. Sulfur is likely present in manure naturally, however only the sulfur contributed by the gypsum was accounted for in these calculations. In our calculations, a FeOOH content of 100% was used for the Marchand product. The Brandy product has a much lower iron content. According to the manufacturer, the Brandy product is approximately 66% calcite and 30% FeOOH by weight (Hedin Environmental 2014). Given the different makeup of this product, a different inclusion rate was used. As discussed previously, the amount used in the Brandy treatments was simply limited by the amount of product

available, and fell short of reaching a 1:1 iron to sulfur ratio. It is evident by the results presented in Table 3 and the trial two figures (11, 12, 13, and 14) that the two iron oxide products generated very different results.

The Brandy product did not alter hydrogen sulfide accumulation. In fact, one of the Brandy treatment samples performed worse than the gypsumx1 treatment. The reason for this is unknown. On days 91 and 184, this sample generated hydrogen sulfide emissions of over 130 ppm, well above the IDLH level of 100 ppm. It is important to note the variation between the two Brandy samples. Whether inconsistent mixing or natural variation is the cause of this discrepancy is unknown. While one of the Brandy treatments generated copious hydrogen sulfide, the other had reduced levels compared to the gypsumx1 treatment samples. Because of this, it is difficult to make a conclusion about the effectiveness of the Brandy product in reducing hydrogen sulfide accumulation. While a greater inclusion rate was used for the Brandy, the increased rate did not completely account for the lower iron content. The fact that iron was not present in the manure at a ratio of 1:1 with sulfur may have been more detrimental than predicted. While some of the hydrogen sulfide may have been bound into an insoluble iron sulfide compound, a great deal of the gas remained, and was released upon agitation. The presence of the iron sulfide product was determined by color (see Introduction). The manure in the Brandy treatments did begin to darken as the storage period lengthened, albeit at a slow rate. Figure 14 below shows the darker color of the Brandy manure after approximately 210 days of storage. For comparison, the control manure after 210 days is pictured below in Figure 15.

Figure 14: Brandy treatment manure after 210 days storage

Figure 15: Control manure treatment after 210 days storage

The Marchand product is almost entirely FeOOH. According to the results of this study, this product is effective at reducing hydrogen sulfide accumulation. The amount of Marchand mixed into the manure represented less than 0.25% of the total mass of the manure sample. From the very beginning, the

Marchand product out-performed other solutions. In the second trial, measurements were taken at day 1 instead of day 0. While these measurements were intended to be a baseline, it was surprising to see how much hydrogen sulfide had already been produced. Both of the gypsumx1 samples contained hydrogen sulfide in the head space above 100 ppm. The other treatments also contained some hydrogen sulfide. However, the two gypsumx1 + Marchand samples produced no measurable hydrogen sulfide in the headspace. By day 7, some hydrogen sulfide was present in the head space, but at a level too low to be dangerous (<25 ppm). On day 15, all of the samples were relatively devoid of hydrogen sulfide in the head space. Day 42 was the first day of agitation in the second trial. Half of the treatments generated levels of hydrogen sulfide that could be dangerous (>50 ppm). However, the average of the two gypsumx1 + marchand samples was only 17.5 ppm, even lower than the control average of 30.5 ppm. By day 91, the marchand product had apparently halted all hydrogen sulfide accumulation. Both the pre- and post-agitation hydrogen sulfide readings for days 91 and 184 were 0 ppm. Once again, this was even lower than the control. Figure 13 shows just how well this product worked in terms of lowering hydrogen sulfide accumulation. The iron-sulfide product of this reaction is insoluble and black. The presence of this product was apparent in the gypsumx1 + marchand samples. The manure in these buckets became very black throughout the storage period. In addition, no crust ever appeared on these samples. The mixture of manure in these buckets was very liquid and homogenous. The change in physical characteristics of the manure is nearly as noticeable as the reduction in hydrogen sulfide. Figure 16 below depicts the Marchand treatment manure after 210 days of storage, and once again the control manure is provided in Figure 17 as a basis of comparison.

Figure 16: Marchand manure treatment after 210 days storage

Figure 17: Control manure treatment after 210 days storage

Chapter 5

Summary and Further Work

Hydrogen sulfide is a deadly gas which can accumulate to dangerous levels in dairy manure storages and lead to severe harm or death for farm workers and animals. Over the last few years, lives have been lost due to contact with this gas in high quantities associated with manure storages (Meinen et al., 2013). Anecdotal evidence has pointed to gypsum bedding use being responsible for enhanced accumulation of hydrogen sulfide in manure storage. While other compounds may be contributing sulfur to dairy manure, it appears that gypsum has the greatest potential. Gypsum is used as a bedding amendment on some dairy farms for health benefits to the cows and economic benefits for the producer. This recycled product is absorbent, and has been claimed to lower somatic cell count by discouraging bacterial growth in the dairy bedding (Cornell PRO-DAIRY 2013). However, the unintended consequences of its use are beginning to appear. The gypsum contributes SO_4 to the manure during storage. Sulfate reduction is carried out by specific genera of bacteria including *Desulfovibrio*. As the manure storage becomes more anaerobic and a crust is formed over the manure surface, bacteria use a cascade of compounds as electron acceptors. When sulfate is used, hydrogen sulfide is often created. This gas, which is heavier than air, accumulates in the manure because it is trapped by its own density and the crust on the manure surface. Upon agitation of the manure, large quantities of hydrogen sulfide can be produced. These large quantities have resulted in human and animal death. It now has become an issue which must be solved to ensure dairy farmers, their workers, and their children, are safe while reaping the benefits of gypsum use. Several commercial products have been proposed as amendments to manure to reduce the accumulation of hydrogen sulfide gas. Vital Breakdown™ was originally designed to accelerate the microbial degradation process in manure and stimulate the growth of bacteria which do not

generate unfavorable odor. Based on the results presented here, this product shows promise for hydrogen sulfide mitigation. It is possible that the specific combination of bacteria present in the Vital Breakdown™ product discourage the growth of the sulfate-reducing bacteria. By doing this, hydrogen sulfide is produced at lower levels. In this small bench scale trial, Vital Breakdown™ did appear to reduce the amount of hydrogen sulfide produced and released upon agitation. Further research is warranted on a larger scale to investigate this as a possible solution. Another proposed solution is use of iron oxide. In the laboratory culture of sulfate-reducing bacteria, iron is used to determine the presence of the sulfate-reducing bacteria. When hydrogen sulfide is produced by the sulfate-reducing bacteria through anaerobic respiration, it binds with iron to generate an insoluble iron sulfide compound, which is black in color. The removal of the poisonous hydrogen sulfide gas allows the sulfate-reducing bacteria to reach higher populations before toxicity to this gas results. In addition, the conspicuous presence of the black precipitate affirms the presence of these bacteria. Applying these principles to hydrogen sulfide in manure, the binding of hydrogen sulfide within the manure will decrease the amount released upon manure agitation. Adding enough iron in the form of FeOOH appears to impact its effectiveness. The Marchand product used in this study was composed almost entirely of FeOOH, and was included in the manure + gypsum samples at rate to ensure an approximate 1:1 molar ratio of iron to sulfur. Iron oxide shows a great deal of promise in mitigating hydrogen sulfide risk. The Marchand product significantly reduced the amount of hydrogen sulfide released upon agitation. This product also maintained a more liquid (albeit very dark) and consistent manure. Again, further research on a larger scale is warranted.

Further work on a large scale will be necessary to validate these results. While it is clear gypsum presence in the manure increases hydrogen sulfide accumulation, it is important to consider other forms of sulfur that may be present in the manure. It is important to remember that a nutrient analysis was not done in this study due to inhibitory cost. This step would be critical for further research. Depending on the diet used on the farm, sulfur compounds may be excreted by the cows. In addition, copper sulfate baths

contribute sulfur to the stored manure. On a large scale, a farm should be closely monitored to reduce any other forms of sulfur getting into the manure with the exception of the gypsum bedding. Analyses of the water used, the diet used, and the resulting excreted manure should be done to ensure sulfur is not finding its way into the manure storage. In this setting, gypsum can be used as a bedding material, and hydrogen sulfide readings should be logged continuously over a long period of time, which includes final agitation and emptying of the pit. A similar scenario can be used investigating the two solutions studied here.

The next question becomes, if one of these solutions were to be validated, how would it be applied on the farm? Vital Breakdown™ has already been designed for on-farm use. It is being used in manure storages currently for other purposes. A simple change in loading rate of this product could implement Vital Breakdown™ as a hydrogen sulfide solution for farms using gypsum in their dairy cow bedding. The iron oxide becomes more of a challenge. An inclusion rate which generates a 1:1 or greater molar ratio of iron to sulfur is necessary. This could result in high application rates. In addition, the Marchand product used in this study was a very fine powder, and ensuring it is well mixed in a large manure storage could be a challenge. Adding the product upstream of the manure pit would ensure better mixing, but how to add the product still remains a challenge. A full economic analysis would be necessary to determine the practicality of any proposed solution. This is of course outside of the scope of this paper, and would require a greater deal of product development and farm trials.

This study has shown that large quantities of hydrogen sulfide can be produced when gypsum is present in the manure. This study has also shown that there may be some effective solutions for reducing the risk of encountering high levels of hydrogen sulfide gas.

BIBLIOGRAPHY

Cornell PRO-DAIRY. 2013. Gyspum Bedding in bedded pack barns may be dangerous. Dairy Herd Management. http://www.dairyherd.com/dairy-news/Gypsum-bedding-in-bedded-pack-barns-may-be-dangerous--229605941.html?view=all. Web. Accessed 20 September 2014.

Extech Instruments 2014. SDL100: pH/ORP/Temperature Datalogger. http://www.extech.com/instruments/product.asp?catid=66&prodid=671. Web. Accessed 11 November 2014.

Fabian, Eileen E. Personal Interview. 12 October 2014

Gerardi, Michael H. 2007. Oxidation-Reduction Potential and Wastewater Treatment. Interstate Water Report. http://www.neiwpcc.org/iwr/reductionpotential.asp. Web. Accessed 15 November 2014.

Hedin Environmental. 2014. Iron Oxide Recovery, Inc. http://www.hedinenv.com/ironoxide.html. Web Accessed 18 October 2014.

Hile, Mike. Personal Interview. 17 October 2014.

Homestead Nutrition, Inc. 2014. Bedding. http://www.homesteadnutritioninc.com/products/bedding/. Web. Accessed 18 October 2014.

Industrial Scientific. 2014. Tango TX1. http://www.indsci.com/products/single-gas-detectors/tango/. Web. Accessed 19 September 2014.

Industrial Scientific. 2014. Like all the greats, the M40 and iTX multi-gas detectors are being honorably retired. http://www.indsci.com/products/retirement/. Web. Accessed 11 November 2014.

Madigan, Michael T, J.M. Martinko, D.A. Stahl, D.P. Clark. 2012. Brock Biology of Microorganisms. 13th ed. Benjamin Cummings, San Francisco CA.

Meinen, R., D. Hill, R. Larson, A. Ozkaynak, D. Murphy, E.F. Wheeler, R. Brandt, H. Elliot.2013. Gypsum Bedding – Risks and Recommendations for Manure Handling. Extension. http://www.extension.org/pages/67660/gypsum-bedding-risks-and-recommendations-for-manure-handling#.VF_XNclLUdW. Web. Accessed 22 September 2014.

OSHA. 2014. Safety and Health Topics: Hydrogen Sulfide. https://www.osha.gov/SLTC/hydrogensulfide/index.html. Web. Accessed 16 September 2014.

Owen, J.J., W.L. Silver. 2014. Greenhouse gas emissions from dairy manure management: a review of field-based studies. Global Change Biology.

Pell, Alice N. 1997. Manure and microbes: public and animal health problem?. J. Dairy Sci. 80:2673-2681.

Reade. 2014. Ferric OxyHydroxide FeO(OH) (Granular, Powder) from READE. http://www.reade.com/products/137-hydroxide-powders/5250-ferric-oxyhydroxide-feooh-

granular-powder-feooh-ferric-oxy-hydroxide-ferric-oxide-hydroxide-metal-oxy-hydroxides-iron-oxide-hydroxide-ferric-oxide-hydroxide-cas20344-49-4-feooh-cas243-746-4-feoho-dried-ferric-oxy-hydroxide-powder-goethite. Web. Accessed 3 October 2014.

USA Gyspum. 2014. Gypsum Bedding Products and Additives. http://www.usagypsum.com/animalbedding.aspx. Web. Accessed 16 September 2014.

ACADEMIC VITA

Vance Brown
60 Municipal Lane
Port Matilda, PA 16870
vdb5024@gmail.com

Education

Pennsylvania State University — December 2010 – December 2014
Major: Animal Sciences
Major: Agricultural Systems Management
Schreyer Honors College

Scholarships, Awards, and Achievements

Schreyer Honors College Academic Scholarship — September 2010 - May 2014
College of Agricultural Sciences Academic Honors Scholarship — September 2010 - May 2014
Penn State Block and Bridle Hard-Working Senior Award — April 2014
ADSA-ASAS Academic Quadrathlon 1st place Team member — July 2013
NCBA Beef Quiz Bowl 2nd place Team member — February 2014

Work/Professional Experience

Hill View Farm: Laborer — January 2007 – Present
Assist with animal rearing (pigs and cattle), raking, baling, and general farm chores including equipment operation, maintenance, and repair

Penn State Swine Center: Student Laborer — May 2011 - Present
Assist with feeding, cleaning, breeding, farrowing, mixing feed and general farm chores including animal handling and transport

TRB Construction: Laborer — June 2009 – August 2014
Assisted with framing, roofing, wiring, plumbing, flooring, remodeling, siding, insulating

Pig Improvement Company (PIC): Genetic Services Intern — May – August 2013
Investigated issues relating to population genetics and genetic improvement using SAS software

Extra-Curricular Activities

Block and Bridle — September 2010 – December 2014
Positions Held: Webpage Chairperson, Club Marshall, Little International Swine Superintendent

Penn State Pullers — September 2010 – July 2014
Positions Held: Webpage Coordinator
Assisted with writing A-team and X-team reports for annual competition
Attended annual ASABE Quarter-Scale Competition in Peoria, IL (July 2014)