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Investigating the causes of foam formation in deep pit swine manure storages

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Investigating the causes of foam formation in deep pit swine manure storages

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So he went and hired himself out to a citizen of that country,
who sent him to his fields to feed pigs…
When he came to his senses, he said, "How many of my father's
hired servants have food to spare, and here I am starving to death!
I will set out and go back to my father and say to him...
'I am no longer worth to be called your son;
make me like one of your hired servants.'"
So he got up and went to his father.
But while he was still a long way off,
His father saw him and was filled with compassion for him;
he ran to his son, threw his arms around him and kissed him.

Luke 15:15, 17-20 New International Version

ABSTRACT

The appearance of foam on the surface of deep pit swine manure storages throughout the United States and Canada is a serious concern for the pork industry. In addition to logistical issues caused by foam accumulation in deep pits, manure foam has the capacity to trap gases produced by the anaerobic decomposition of manure, leading to dangerous flammable gas concentrations upon agitation or foam disturbance. Recent flash fires and explosions at several swine facilities have created a pressing need to understand and mitigate the causes of foaming deep pits.

A number of hypotheses regarding the contributing factors of foam formation exist. These include the presence of surface active agents (proteins, volatile fatty acids, detergents, lipids, biosurfactants, etc.) that enable foam generation, the presence of hydrophobic solids that stabilize foam, the presence of certain feed components that may contribute to the foaming mechanism, the rate of biogas production from the waste, or any combination of these and other physical, chemical, and microbiological characteristics of the manure slurry. Despite a broad understanding of the aspects of foaming systems, there is a lack of knowledge of the mechanism that causes foam to stabilize in anaerobic environments. The objective of this research was to better understand foaming manure systems from a "three-phase system" approach; that is, to research the solid, liquid, and gas phases of swine manure in deep pits and how they contribute to stabilized foam. Foam mitigation strategies were considered after these aspects of foaming systems were investigated.

The two studies presented in this thesis included extensive analysis of swine manure sampled from both commercial deep pits and controlled dietary studies. The first study was a field study, with manure samples collected from over 50 swine facilities in Iowa over 13 months. These samples were analyzed for a number of parameters including temperature, pH, total and volatile solids, short-chain and long-chain fatty acid concentration, biochemical methane potential, methane production rate, surface tension, foaming capacity index, and foam stability. An extensive database was compiled so that these parameters could be compared based on the extent of foam accumulation at the sampling site. The second study involved a controlled dietary study, where the impact of carbohydrate and protein sources on foaming parameters was measured. The results allowed us to understand the direct impact of

feed components on swine manure parameters, as well as to compare the manure collected from these controlled trials to samples taken from commercial deep pits.

As a whole, these studies showed that swine manure from barns with foam accumulation exhibited significantly different trends in many biological and physicochemical parameters when compared to manure from non-foaming barns. Most notably, the rate at which biogas was generated in foaming barns was much greater than in non-foaming barns, indicating a much greater presence of the "gas phase" in foaming barns. At the same time, non-foaming barns showed a greater potential for cumulative biogas production and larger concentrations of important substrate such as short-chain fatty acids. Taken together, we see that the microbial consortium in foaming barns allows them to function as more efficient digesters, producing a greater methane flux through foaming pits, which is the driving force of foam formation. Manure collected from the surface of foaming systems was also able to produce foam at a greater capacity in laboratory tests when compared to manure from non-foaming systems, and also showed an enhanced ability to stabilize in the testing apparatus. These tests and others indicated an accumulation of a surfactant and/or stabilizing agent at the surface of foaming barns which promotes foam generation and stability. Other important differences in the temperature trends, pH, solids profiles, and surface tension measurements led to a greater understanding of the behavior of foaming swine manure deep pits. Overall, this knowledge can lead to more directed solutions, specifically in exploring sustainable ways to control the activity of the microbial community within deep pits.

CHAPTER 1

INTRODUCTION

Deep pit manure storages are an important aspect of swine production in the Midwestern United States and Canada. They are located directly beneath swine facilities, allowing manure to drop through slatted floors into the concrete storage volume. Deep pits allow producers to store manure for extended periods (6-12 months) so that it can later be pumped and applied to croplands as a fertilizer to promote crop growth (Jackson et al., 2000). Due to the prevalence of corn-soybean crop rotations in areas that use deep pits, land application is usually limited to the spring before planting or the fall after harvest. Despite the convenient long-term storage provided by these structures, they are also associated with many operational and environmental concerns for swine producers. Ammonia, hydrogen sulfide, carbon dioxide, and methane emissions can lead to health concerns within barns as well as larger scale environmental concerns with regard to greenhouse gas emissions (Jungbluth et al., 2001, Zhao et al. 2005). Other issues include the efficient pumping and safe handling of manure while applying to it to croplands, as it is a cost-intensive process with the potential for environmental spills.

In the past five years, foam accumulation on the surface of deep pit manure storages has been frequently reported, with the largest concentration of reports occurring in Midwestern states such as Iowa, Minnesota, and Illinois (Moody et al., 2009). The foam is described as a dark-brown or gray, viscous fluid with mid-sized bubbles entrained throughout (Robert et al., 2011). A picture of this foam is shown in Figure 1.

Figure 1. Foam accumulation in a deep pit as seen from a pump-out location.

Foam accumulation in deep pits has implications for logistical, environmental, and safety-related aspects of swine production. From a logistical standpoint, foam effectively reduces the storage volume available in the deep pits, forcing producers to pump and apply manure during untimely seasonal windows. Foam has also been shown to trap gaseous emissions from the manure slurry as well as affect the overall performance of the system in terms of solids settling and waste stabilization (Ganidi et al., 2009). Finally, foam accumulation has been linked to a number of flash fire and explosion incidents (Moody et al., 2009). When sudden foam agitation and collapse occurs, explosive concentrations of methane gas are achievable in the barn. Coupled with a spark source from equipment (electrical systems, space heaters, etc.) or activity (welding, grinding, manure pumping, or general maintenance) in the barn, flames can occur in the foam, spread quickly, and even cause explosions.

Unfortunately, the body of knowledge regarding foaming deep pit swine manure storages is limited. This lack of knowledge is the result of the biological and chemical complexities of anaerobic systems as well as the relatively recent nature of deep pit foaming occurrences. However, the formation of nuisance foams in anaerobic systems used in other applications is a relatively well-established body of research, particularly in municipal or industrial anaerobic digesters. In order to conceptualize foam occurrences in municipal wastewater treatment applications, Davenport et al. (2008) and other research groups have described foam formation with a "three-phase system" framework. In anaerobic systems such as digesters or manure storages, the gas phase consists of a mixture of carbon dioxide, methane, and other trace gases (or "biogas") that is produced as a result of the decomposition of organic matter by the microbial community. The bubbles of biogas travel through the liquid phase to the surface, where surface active agents (or "surfactants") increase the activity at the liquid-atmosphere interface, consequently lowering the surface tension of the waste slurry. The biogas is then encapsulated into a foam formation at the interface, which is thought to be stabilized by the presence of hydrophobic solids which prevent the drainage of liquid back into the waste slurry. In this way, stabile foams systems require critical aspects of all three phases to occur simultaneously. The systematic nature of this three-phase system approach is helpful in charactering the occurrence of foam in anaerobic systems. Thus, this framework was the foundation of the research presented within this thesis.

RESEARCH OBJECTIVES

The overall goal of this project was to gain a better understanding of foaming systems in order to prevent foam formation in deep pit swine manure storages. An investigation into the specific causes and mechanisms of foam occurrence should help to bring about the most effective, long-term solution to the problem. To this end, manure samples were collected from both commercial deep pits and controlled feeding trials to determine the impact of diet composition on the input into deep pits, as well as the physical, chemical, and biological characteristics of swine manure as it occurs in anaerobic storage systems. The objectives of both the field and diet studies are given below.

Field Study

- Create an extensive database of swine manure characteristics for samples collected from multiples depths of commercial deep pits once a month for approximately one year. Parameters were chosen based on their hypothesized importance to foam formation, including aspects from each of the three phases involved in foam formation.
- Develop a lab-scale foaming apparatus that accurately simulates the process of foam formation in deep pits by controlling the gas phase of the experimental setup. This foaming apparatus allowed for the development of two new experimental parameters: foam capacity and stability.
- Statistically evaluate the differences between foaming and non-foaming barns for each parameter in the database. This included a critical evaluation of the foaming capacity and stability test as an accurate reflection of the behavior of the manure in the field.
- Recommend potential solutions to foaming deep pits based on the understanding gained from the study.

Diet Study

- Control feed composition in a number of dietary trials to evaluate different protein and carbohydrate sources and levels, including components such as amino acids, corn-soybean meal, corn-canola meal, barley-soybean meal, and dried distillers grains with solubles (DDGS).

- Evaluate the impacts of diet on important foam-related parameters including gas production rates, foaming capacity and stability, surface tension, and solids content.

THESIS ORGANIZATION

Chapter 2 is a literature review which will highlight existing research in this area by breaking down foaming systems into the three phases discussed above. Much of the knowledge presented in the literature review is taken from research investigating biological foam formation in municipal anaerobic digesters, discussing contributing factors that translate to deep pit systems including surface active agents, hydrophobic solids, and other system parameters. The final section discusses the ability of swine manure to produce biogas in various reactor and barn-scale setups.

Chapters 3 and 4 address the research objectives outlined above, respectively. Chapter 3 is titled "An Evaluation of the Physicochemical and Biological Characteristics of Foaming Swine Manure" and will be submitted to the *Journal of Environmental Quality*. This study highlights the differences between manure from foaming and non-foaming barns, particularly in gas production trends throughout the 13-month sampling period. In addition, the performance of the bench-top foaming capacity and stability test is evaluated. Chapter 4 is titled "The Impact of Carbohydrate and Protein Source on Swine Manure Foaming Properties" and will be submitted to *Transactions of the ASABE*. This study characterized the impact of diet composition on foaming-related parameters of manure. Also, comparisons were made between foaming manures collected from commercial deep pits and those collected from the diet study. Throughout the thesis, references are included at the end of each chapter.

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CHAPTER 2

A REVIEW OF THE FACTORS CONTRIBUTING TO FOAM FORMATION IN ANAEROBIC SYSTEMS

AN OVERVIEW OF ANAEROBIC SYSTEMS AND FOAMING

Anaerobic systems are utilized in many applications of waste storage, treatment, and stabilization in various industries in the United States. In most instances, the production of biogas as a usable source of energy is exploited as a low-cost supplement to other forms of power production. In most agricultural applications, the main use of anaerobic systems is for animal waste storage during times between land applications. The emission of gases and odors from these manure storages, along with the proper disposal of the stored waste, are among the primary environmental concerns in livestock operations today. In most swine finishing facilities in the Midwestern United States, deep pit storages are located below production buildings and are used for manure storage for time periods ranging from six to twelve months.

In recent years, the accumulation of foam on top of these manure storages has been reported with increasing frequency (Moody et al., 2009). This foam accumulation poses a number of problems that must be addressed from a managerial standpoint. For example, foam can significantly reduce the amount of storage available in deep pits. As a result, the manure pumping and application cycle is stressed, forcing the producer to apply manure during untimely seasonal windows or seek other means of storage.

According to Ganidi et al. (2009), foam accumulation also has an impact on the overall characteristics and efficiency of anaerobic systems. First, the biogas (consisting mostly of methane and carbon dioxide) produced during the digestion of manure is known to be trapped within the viscous foam layer. Also, the mechanism that causes foaming in the system often leads to an inverted solids profile, creating biological “dead zones” in the manure storage and reducing the active volume. This, in turn, leads to reduced organic decomposition, negatively affecting the performance of the system. In addition, there are a number of secondary inconveniences associated with foam accumulation, such as aesthetic and odor problems, operator safety concerns, equipment damage, and the associated costs of cleanup and remediation (Ganidi et al., 2009, Boe et al., 2012).

Finally, biological foam formation on deep pit storages has implications for the gaseous emissions and overall safety at swine facilities. The flammable gases (most significantly methane) produced by the anaerobic decomposition of the manure are captured in the foam matrix. Depending on the amount of gas that is trapped in a given barn, dangerous gas concentrations are achievable as a result of a sudden breakage of foam. In some cases, foam agitation or breakage along with a spark source has caused flash fires or explosions in swine facilities (Moody et al., 2009).

Foam as a Three-Phase System

Davenport et al. (2002 & 2008) described a helpful means of characterizing foam systems in three phases. The initiation of foam production occurs as a result of both the gas and liquid phases of the system. In an anaerobic system, the gas phase is bubbles of biogas produced due to methanogenic activity. When a certain concentration of surface active agents (or "surfactants") is present in the wastewater foam production occurs. This results from a lowering of the surface tension of the solution due to increased activity at the liquid-gas interface, with surfactants literally "holding" the surface together (Bamforth, 2004) and encapsulating bubbles produced in the gas phase (Moeller et al., 2012). Finally, solids in the form of hydrophobic substances (assumed to be hydrophobic bacteria in activated sludge foams according to Davenport et al., 2002) serve to stabilize the foam that is produced by preventing drainage back into the liquid layer. The nature of foam as a three-phase system is illustrated below (Figure 1) with a wastewater sample that was mechanically aerated to induce stable foam accumulation in the Animal Waste Management Laboratory at Iowa State University.

Figure 1. A lab-scale illustration of a three-phase foam (AWML, Iowa State University).

All three phrases are a prerequisite for foaming, so a complete explanation of the presence of stable foam in deep pits must address these prerequisites accordingly. Unfortunately, the exact mechanism for foaming in deep pits is unknown due to the fact that it is a relatively recent phenomenon. Along these lines, this review will investigate foam formation within the framework of the three-phase system described above, with the most relevant research done in each phase summarized. First the liquid and solid phase contributions are addressed by reviewing research which investigates foam formation in related anaerobic systems, such as municipal anaerobic digesters. Next, the rate of biogas production from swine manure is examined by reviewing studies in that topic area. Finally, gaps in current knowledge and future research needs are discussed in light of the literature discussed in this review.

FACTORS CONTRIBUTING TO FOAMING IN ANAEROBIC SYSTEMS

Much of the current knowledge of foam formation in anaerobic systems is derived from extensive research of waste activated sludge treatment plants, where biological foam formation is a relatively common phenomenon. Many researchers attribute foaming systems to a combination of surface activated agents in the liquid phase and hydrophobic particles in the solid phase (Horozov, 2007, Ganidi et al., 2009, Dalmau et al., 2010, Di Bella et al., 2011, Hutzler et al., 2011, etc.). Essentially, foam in waste treatment plants can be described as floating biomass, as the main mechanism of biological foam stability is thought to involve filamentous bacteria with hydrophobic cell surfaces attaching to gas bubbles and rising to the surface of the system (Davenport et al., 2002). The presence of these bacteria on the gas-liquid interface of the system, as well as the presence of surface active agents, is thought to increase surface activity (consequently lowering surface tension) and stabilize foam (Ganidi et al., 2009, Boe et al., 2012). Heard et al. (2008), though, showed that bacteria cannot lead to foam formation in the absence of a surfactant, which reinforces that both of these substances are important for persisting foam systems.

Grady et al. (2011) identified a number of the specific species of microorganisms as well as chemical constituents that serve as the liquid and solid phases of foam formation in activated sludge systems. Surface active agents include detergents, fats, oils, and biosurfactants produced during metabolic processes of the biomass. Foam stabilizing bacteria include *Actinobacteria* with

hydrophobic characteristics. Both filamentous *Gordonia* spp. and *Microthrix parvicella* species have also been associated with foaming events, and are seen as primary causes of foaming in many activated sludge systems (Heard et al., 2008).

While a direct relationship between activated sludge foaming and foam production in anaerobic digesters has not been proven, it has been shown that the basic principles of foam formation as a three-phase system are helpful as a starting point in any foaming system, including deep pit manure storages. In this way, the potential causes of foaming in anaerobic digesters can be analyzed from the perspective of a general anaerobic system and compared accordingly. This will be the approach in the following sections.

Surface Active Agents (Liquid Phase)

The term "surface active agents" encompasses substances including both surfactants and "biosurfactants." Surfactants are substances that are introduced externally into the system. As previously mentioned, examples include substances such as oils, greases, detergents, volatile fatty acids, and long-chain fatty acids. On the other hand, biosurfactants include substances produced during microbial metabolism such as polysaccharides, proteins, and lipid complexes. The amphipathic nature of surfactants allows them to contain both water soluble and water insoluble components. This characteristic makes them important for foam formation and stability, specifically with respect to film generation and film thickness between bubbles in a foam matrix (Bamforth, 2004).

Davenport et al. (2008) described both the hydrophobic and hydrophilic nature of surfactants. The hydrophobic ends of surface active agents are attracted to the gas phase of the system, while the hydrophilic ends move towards the liquid phase. These properties, in turn, have a direct effect on the surface tension on the solution by increasing the amount of chemical activity at the surface. The authors continued their discussion by describing a concept called the critical micelle concentration. The critical micelle concentration (cmc) gives a measure of surface tension with respect to the concentration of surface active agents in a solution. At concentrations lower than the cmc, surface activity is low enough to prevent the formation of foam in a three-phase system. However, when the cmc threshold is surpassed, surface activity is increased and foaming may occur when the gas phase is introduced to the system. As an

illustration of this concept, Glaser et al. (2007) tested the cmc for bovine serum albumin (BSA) by varying volumes of the protein in solution and mechanically aerating the mixtures. Foam production was monitored, and an extensive characterization of the foam was performed, confirming that this protein is a viable example of a surface active agent.

There are a number of other surface active agents relevant to anaerobic digester systems, including volatile fatty acids (primarily acetic acid), lipids, and detergents (Ganidi et al., 2009, Boe et al., 2012). It is important to note, however, that the accumulation of surfactants in an anaerobic system is directly tied to the overall activity of the system. The accumulation of surfactants can occur by a number of simple or complex mechanisms. For instance, the accumulation of acetic acid in an anaerobic system would likely indicate a breakdown in the methanogenesis process, which in turn has implications for the biogas production of the system.

Along the same lines, the accumulation of chemical constituents in a specific system depends on both the degradability and availability of the substance of interest. An example of this concept is the presence of detergents in the system of interest. Detergents have relatively low degradability in anaerobic systems (Ganidi et al., 2009), which may cause one to conclude that they would be a potential cause of foaming. However, in a complete systems approach to municipal treatment plants, detergents would rarely accumulate in anaerobic reactors because of the fact that they are highly degradable in aerobic waste-activated sludge processes preceding the digester. By comparison, the direct input of cleaning chemicals into anaerobic deep pits may be a serious concern with respect to foaming potential.

Another example is the presence of building block compounds in anaerobic digesters. Proteins are less biodegradable than lipids and fibers, and in this way, are more prone to accumulation. In addition, proteins have been shown to act as foam forming and stabilizing agents (Bamforth, 2004; Glaser et al., 2007; Ganidi et al., 2009). Also, lipids have been shown to decrease the tendency for waste to foam in experimental setups (Boe et al., 2012), but have a strong impact on the organic loading, biogas production, and surface activity of anaerobic systems which could lead to a higher risk of foam formation (Chipasa and Mędrzycka, 2006). The presence of these compounds in deep pit manure storages also generates very important ties between foaming deep pits and the feed components used within the facility. For example, Kerr et al. (2006) found that varying protein and cellulose levels in the feed composition of swine

affected acetic acid concentrations in the manure collected during a controlled feeding trial. Related organic chemical concentrations and the overall pH of the manure were also shown to be affected by the varying levels of dietary cellulose and crude protein in the swine diets.

Finally, the occurrence of biosurfactants (primarily EPS, or extracellular polymeric substances) in deep pits could potentially be tied to foaming events as a critical surface active agent. According to Sheng et al. (2010), EPS are a dynamic, complex group of polymers produced by microorganisms that occur outside of cells and within microbial aggregates. The different macromolecules associated with EPS include polysaccharides, carbohydrates, proteins, humic substances, nucleic acids, and lipids. EPS play a significant role in the physicochemical properties of the biomass, including the ability for the cells to flocculate, settle, and adsorb substances. EPS bind cells together in a complex matrix which retains water and food sources, allowing cells to avoid dewatering and starvation. These functions of EPS may be particularly important in deep pits, where water use has been reduced in the past number of years (Robert et al., 2011), and access to food sources is competitive.

The loosely bound outer layer of EPS has been described as a "loose and dispersible slime layer" by Sheng et al. (2010). Öner (2013) elaborated the description of the physical characteristics of extracellular polysaccharides by describing industrial applications of EPS including use as thickeners, bioadhesives, stabilizers, and gelling agents. Both of these descriptions are consistent with biological foams, which are sticky, viscous fluids that are solids enriched (Robert et al., 2011). In addition, EPS can also play an important role in the surface characteristics of wastewater solutions, as excreted biosurfactants may lead to films at the gas-liquid interface, lowering the surface tension of the solution (Neu, 1996, Heart et al., 2008). This, of course, has implications for the foaming ability of the system. Elevated levels of biosurfactant production can occur during times of rapid biological growth, biological instability, organic overloading, or nutrient deficiency (Ganidi et al., 2009, Heard et al., 2008). The amount and quality of EPS produced in biological systems is influenced by a number of factors including the type of substrate, nutrient levels, growth phase of the bacteria, sludge age, and oxygen levels, with aerobic zones producing more EPS (Rittmann et al., 1987, Sheng et al., 2010).

Di Bella et al. (2010) studied the relationship between EPS concentrations and biological foam formation in Membrane Bioreactor (MBR) processes. As opposed to many studies linking

filamentous bacteria to foaming events in municipal treatment plants, this group found no relationship between foam-forming microorganisms and foaming reactors. Instead, the group found that foaming events were strongly correlated with high EPS concentrations in the aeration tanks at the treatment plants. In addition, this group showed several positive correlations between EPS concentration and foam indices used by the group to characterize the foam, including scum indices, foam ratings, and foam power measurements. Heard et al. (2008) also suggested the importance of biosurfactants in biological foaming systems. The groups noticed that the surface tension of waste solutions decreased during the exponential growth phase of the bacterial strains used in the study. They correlated this decrease in surface tension with biosurfactant production, and showed that samples were able to produce stable foam at this time.

The concepts discussed above illustrate the fact that the foaming potential of a system with respect to surface active agents varies substantially as the components of the system interact and as important system parameters of the slurry change in time (Bamforth, 2004). In general, the effect of different types of surface active agents on foaming events in anaerobic digesters is unknown because of the complexity of the systems, and existing literature in this area is limited for this reason. However, whereas the input of anaerobic systems in municipal settings consists of both primary (raw organics) and secondary settled waste (waste activated sludge), the input of deep pit manure storages consists entirely of animal feces and urine, wasted feed and water, and wash waters from cleaning the barn. In this way, an investigation into the specific surface active agents present within a foaming deep pit may be more focused than an investigation into a municipal anaerobic digester.

Hydrophobic Solids (Solid Phase)

The hydrophobic nature of the surfaces of certain bacteria and particles is an important aspect of foaming research in municipal wastewater treatment plants. As mentioned in the introduction of this section, *Gordonia* and *M. parvicella* have been identified as likely species that cause foaming in municipal systems. The mechanism for the production and stabilization of foam with respect to hydrophobic bacteria is relatively straight forward. As the hydrophobic properties of the cell wall drive these bacteria to the gas-liquid interface of digesters, they simultaneously lower the surface tension of the sludge and stabilize any existing foam by

preventing liquid drainage (Heard et al., 2008). To compound the foaming mechanism, any biosurfactants that the *Gordonia* and *M. parvicella* produce during metabolism create greater foaming potential. These filamentous microorganisms are introduced in municipal AD systems by surplus activated sludge (SAS) (Ganidi et al., 2011). Although these organisms are primarily obligatory aerobes, literature has shown that they are able to survive in anaerobic conditions.

Hernandez and Jenkins (1994) were able to prove this survival in a laboratory scale batch digester. In their experiment, the bacteria survived with only a 37% filament reduction. Furthermore, they were able to produce large amounts of foam at the same concentration of *Gordonia* spp. in a field-scale, municipal digester. This study establishes an important link between foaming in anaerobic digestion and foaming in different biological environments, which is important to the basis of this review. In fact, the foaming mechanism could be the same in significantly different systems if the activity of the bacteria of interest is similar in both. If this is the case, foaming in anaerobic systems as a whole may be understood more fully.

Heard et al. (2009) investigated the "hydrophobicity" of *G. amarae* and *R. erythropolis* cells. This research group had difficulty with this measurement because of the filamentous nature of the bacteria tested. However, the separation of certain bacterial species into the foaming and non-foaming partitions of the system was able to be replicated consistently, which allowed the group to measure the mass of bacteria in both the foam and liquid layers. This paper established a practical means to estimate the bacterial communities present in stable foams through partitioning, which was a strong indicator of the hydrophobic nature of certain species' cell walls. At the same time, this study illustrated the fact that testing potential foaming causes can be difficult and tedious. This is certainly another reason why the body of research with regard to foaming anaerobic systems is limited.

Davenport et al. (2008) studied a threshold concept for hydrophobic mycolata cells with respect to the critical micelle concentration concept presented for above for surface active agents. They exploited the fact that the cells were much more easily controlled than the gas and liquid phases in wastewater, which are always present. Mycolata species were controlled on a density basis and analyzed with respect to an empirical threshold established for mycolata. The group was able to show that the threshold applied for the more hydrophobic species, while the less hydrophobic mycolata did not stabilize foam in large numbers. For hydrophobic species in

general, foaming sites were typically correlated with an abundance of mycolata. The reverse was also shown to be true for non-foaming sites. These results are illustrated in Figure 2.

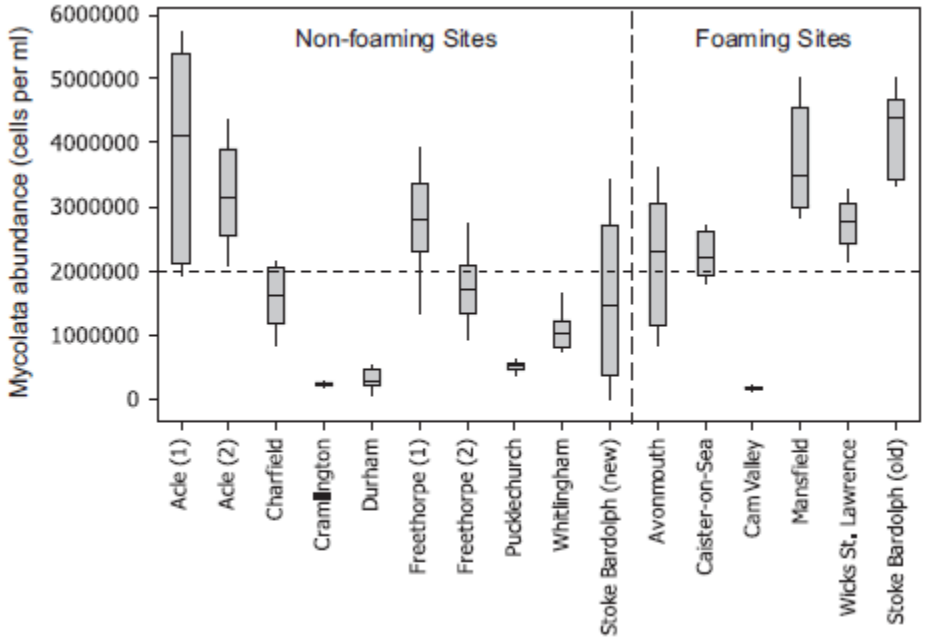

Figure 2. Mycolata concentration vs. site foaming from Davenport et al. (2008).

A number of research groups have investigated the effectiveness of small particles as foam stabilizers in lieu of conventional surfactants (Bindal et al., 2002, Blute et al., 2007, Blute et al., 2009). The effect of colloidal particles on the production of foam in the absence of surface active agents was investigated by Bindal et al. (2002). The group mechanically aerated samples with suspended silica particles in order to determine a sample's "foaminess" based on the concentration and size of particles in the sample. The experimental setup is shown in Figure 3. "Foaminess" was defined as the volume of air retained in the foam bubbles per unit volume of the suspension during steady state aeration conditions. According to Bindal et al. (2002), foam formation in the hydrophilic silica solution occurred when a particle layer formed inside the foam lamella. This provided structural reinforcement against coalescence that led to foam stability. The study showed that foaminess was directly proportional to the concentration of the suspended particles and inversely proportional to particle size. However, the introduction of different sizes of particles into the solution led to a sharp decrease in foam stability due to the rupturing of the particle layer's structure. Similar studies were conducted by Blute et al. (2007 and 2009), who found that the surface charges of particles and the degree of agglomeration had

important implications for foam generation. They also emphasized the importance of the pH of the solution (with acidic solutions showing more foaming potential), surface tension of the solution, and the concentration of silica particles.

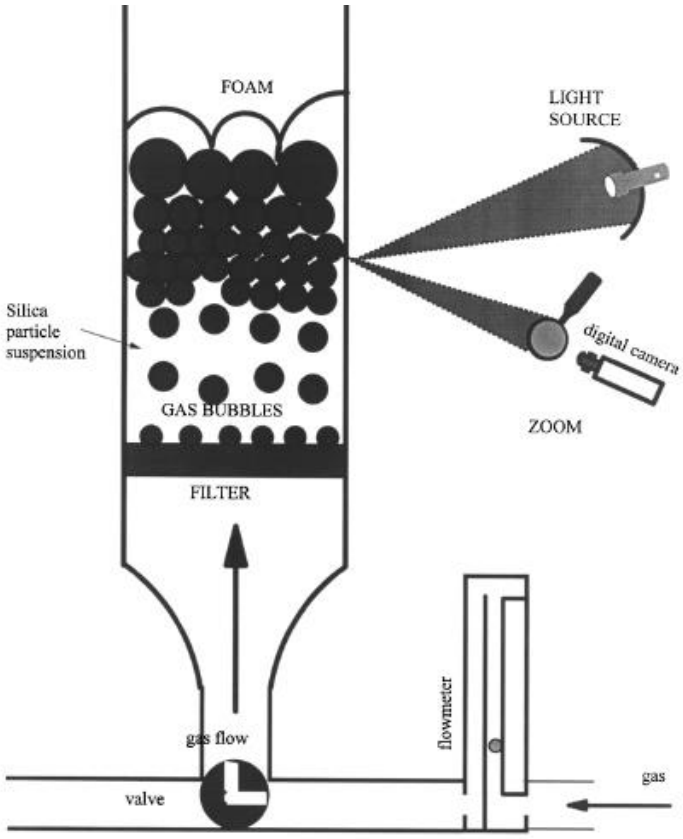

Figure 3. Foam aeration setup from Bindal et al. (2002).

Up to this point, only the *relative* concentrations of various types of hydrophobic bacteria and particles have been extensively researched with respect to the foaming potential of systems. There is still much research that is needed when considering a foaming system, especially considering the potential interaction between hydrophobic solids and surface active agents. For example, the excess production of a biosurfactant by bacteria which accumulate at the gas-liquid interface can cause a compounding effect of foam production and stability (Heard et al., 2008). In this way, biosurfactant production could be an important precursor to stable foam accumulation. Further research in this particular area will be important in order to better understand the foaming mechanism in anaerobic systems.

Organic Loading Rate and Other Operational Parameters

Mismanagement of the rate of organic loading to digesters is recognized as one of the primary causes of foaming in municipal settings. This phenomenon can also occur in a similar way in swine facilities, with a direct connection to the components and grind size of the animal feed. An organic "shock" loading leads to an accumulation of compounds that have not been degraded by the biomass. Of course, if the compounds have the ability to enhance the production or stability of foam, there is a greater chance of excess foam formation. As an illustration, the accumulation of filamentous solids and microorganisms during full-scale foaming events is represented in Table 1.

Table 1. Characteristics of foam and sludge during foaming and non-foaming events from Westlund et al. (1998).

Monitored Parameter	Foam Layer	Sludge During Foaming	Sludge (Normal Conditions)
pH	-	7.3	7.2
Total Alkalinity (g/L)	-	3.3	3.5
Total Solids (%)	6.0	2.2	2.4
Volatile Solids (%)	70	60	55
Filament Abundance	5	0-1	0-1

Ganidi et al. (2011) examined the relationship between organic loading rate and foam depth in a field-scale, mesophilic wastewater treatment facility. Also, bench-scale reactors were studied simultaneously, with a more complete analysis of substrate and microbial community involved at this level. The group's objective was to establish a critical threshold of organic loading rate that initiated foam formation and stabilization. The full scale digester was monitored over a 15 month period for foaming events. The bench-scale digesters were loaded with samples from the full scale reactor, and extensively analyzed. The main experiments measured foam production of multiple organic load rates, as well as volatile fatty acids (VFAs) and filamentous bacteria concentrations. The organic load rate of 5 kg VS m^{-3} was able to consistently produce foam, although the amount of foam produced was highly variable. The 2.5 kg VS m^{-3} sample only produced foam occasionally, while the sample of lower organic loading and the control

never produced a foaming event. Despite frequent foaming events in the bench-scale models, the full scale digester only recorded one foaming event, raising questions about the relationship of scale to municipal foaming issues. In this case, though, the group hypothesized a critical threshold of 2.5 kg VS m^{-3} for the wastewater being studied.

While the concept of shock loading to anaerobic digesters is understood from a theoretical standpoint, it is only demonstrated in the literature on a case-by-case basis. In the study above, the group concluded that a threshold was established at 2.5 kg VS m^{-3}, but they didn't prove that this threshold applied to other reactor situations with different types of inputs and waste components. One of the main difficulties in establishing a critical organic loading rate for anaerobic digesters is that the quality and characteristics of the reactor and sludge input vary in nearly every application. For this reason, the optimal operating conditions with respect to organic loading rates can vary substantially for various systems, and avoiding the formation of foam due to shock loading is essentially a case-by-case determination (Pagilla et al., 1997, Grady et al., 2011).

Mixing is another important operational parameter with regard to foam formation in AD. Mixing serves to maintain suspension of sludge particles in order to optimize contact with the biomass. Optimal mixing conditions help to avoid dead zones in the AD, increasing the active volume of the digester. Pagilla et al. (1997) studied various mixing conditions in anaerobic digesters by comparing gas-mixed and mechanically-mixed setups. When all other operating conditions were held constant, the group proved that the gas-mixed digester accumulated much more foam than the mechanically-mixed digester. The group also discussed the dangers of over-mixing and poor mixing. Poor mixing caused stratification of the solids and liquids in the digester leading to the accumulation of surface active agents at the liquid gas interface due to poor substrate degradation in that area. On the other hand, over-mixing introduced sufficient bubbles to transport hydrophobic substances to the surface which enhanced foam stabilization.

Another operating condition that is important in relation to anaerobic digester foaming is temperature. Chae et al. (2008) studied the temperature effects on biogas yields during the AD of swine manure in the mesophilic temperature range. In general, they found that gas production increased with increasing temperature and decreased with decreasing temperature. This trend is apparent in Figure 4 below.

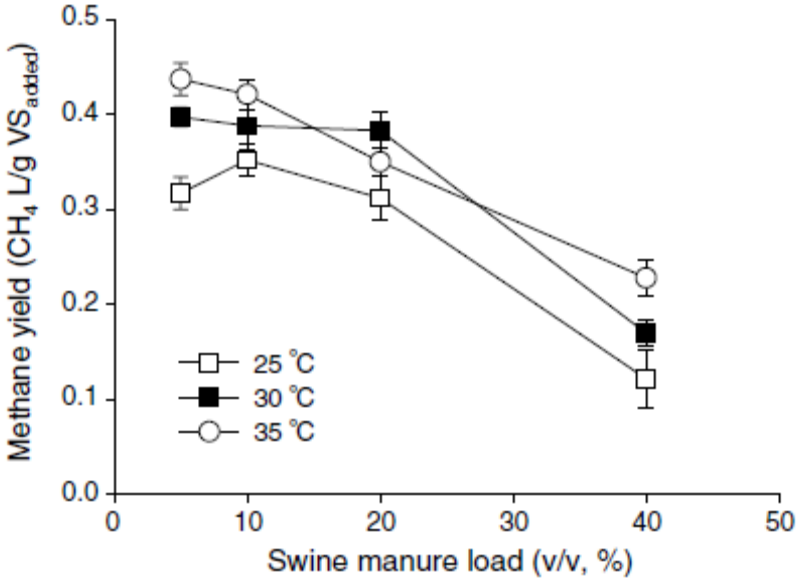

Figure 4. Gas production trends based on temperature from Chae et al. (2008).

In addition, biogas production rate was able to resume to previous rates after the temperature was dropped and then restored to the original value. With regard to system performance, an increase in biogas production could lead to increased foaming by way of increasing the presence of the gas phase of the system. Also, temperature fluctuations due to operational failures may negatively affect the activity of the microbial community, leading to a buildup of surface active agents and increased cell rupture, subsequently increasing the foaming potential of the system. Moeller et al. (2012) cited a specific example of foam formation in a biogas plant due to an increase in temperature from 35°C to 38°C, suggesting that the sudden temperature change upset the microbial community and released mucilage and storage substances from within the bacterial cells.

BIOGAS PRODUCTION OF SWINE MANURE

Up to this point in the review, literature regarding the liquid and solid portions of the three-phase system approach to foaming anaerobic systems has been covered. However, all three aspects of this framework must be present in order for stable foam to accumulate on a system. Unlike the solid and liquid phases, the source of the gas phase of foaming deep pits is relatively well understood. Biogas is produced during the methanogenesis process in anaerobic systems. During this process (Figure 5), complex organic matter is broken down to simpler compounds

and eventually converted to a mixture of methane, carbon dioxide, and other trace gases (Grady et al., 2011). This “biogas” is the driving force of foaming systems.

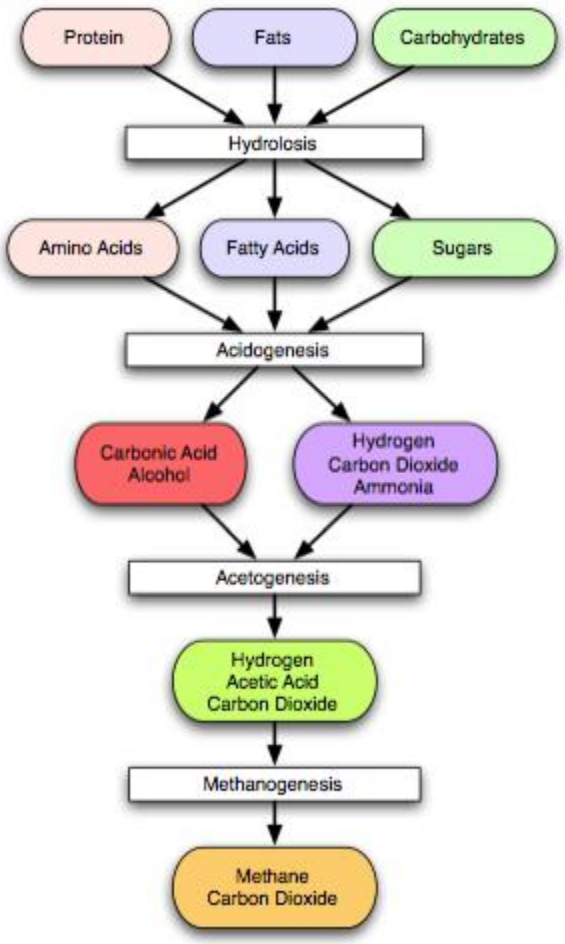

Figure 5. Methanogenesis process in an anaerobic environment from Chemistry for Clean Environment.

Methane emissions are important in many areas of study, particularly with respect to greenhouse gas concerns. While gas emission levels from swine manure storages are not directly relevant to this study, the methanogenic activity and gas production within the manure (as well as the factors affecting this activity) is particularly important for understanding the gas phase of foaming systems. Barret et al. (2012) studied two outdoor swine manure storage systems with regard to their physicochemical and microbiological characteristics over a 150-day period. They identified many of the challenges of understanding methanogenic activity in swine manure, including how characteristics such as manure composition, swine diet and antibiotic use, temperature, surface accumulation, and sampling depth influenced the microbial communities and biogas production. In addition to understanding these characteristics, differentiating between

the most abundant microorganisms and those that are the most active in methanogenesis was shown to be very difficult from an analytical standpoint. However, this group chose to monitor the temporal dynamics of both bacterial and archaeal communities, assuming that shifts in the community composition would help identify the most active groups in the methane generating processes. They found significantly higher concentrations of parameters such as total and volatile solids concentration, TCOD, ammoniacal and organic nitrogen, and alkalinity with increasing depth into the slurry, which is intuitive based on the solids profile of typical manure storages. This group also found that archaeal communities related to the genus *Methanoculleus* increased over time across all samples, varied in population based on phylotype and sampling depth, and were found to be the main contributors to methanogenesis. According to Barret et al. (2012), these results indicate that the hydrogenotrophic pathway was dominant, and that this genus may act as a potential "biomarker" for methanogenic activity. This study confirms both the complexity and importance of understanding methanogenic activity in relation to the gas phase of foaming systems.

As mentioned in previous sections of this review, components of the diets fed to animals have a strong tie to the chemical aspects of the resulting manure. These ties are also true of the biological aspects of deep pit storage systems, and the ability for the methanogens to produce biogas. One feed component of particular interest when it comes to foaming deep pits is dried distillers grains with solubles (DDGS). Jarret et al. (2011) investigated the effects of DDGS in swine diet, particularly with regard to the waste characteristics and methane production potential. This group showed that the incorporation of DDGS in swine diets increases the total amount of manure by a significant amount, as well as a corresponding increase in the organic matter excreted. The methane production potential of the manure produced from diets incorporating DDGS was reduced per unit of organic matter excreted. However, the cumulative effect of adding DDGS to pig diets actually increased the potential methane production per unit volume because of the increased organic content of the manure produced.

Other studies have established the link between the types of carbohydrates consumed by the animal and the methane production of manure (Kebreab et al., 2006), with fiber fermentation enhancing the methanogenesis process when compared to soluble carbohydrates. Hindrichsen et al. (2004) also showed that the level of lignification (related to the availability of nutrients to

methanogens) had important implications for the level and rate of methane production of manures fermented in a lab setting. The results of their fermentation studies confirmed that greater lignification reduced particle degradation (i.e. soybean hull-based manures yielded the highest fiber degradation because of its low lignification and accessible structure). The differences in methane production of the various manures produced from differing carbohydrate-based diets also showed that the interactions between the carbohydrate degrading microbes and methanogenic archaea was strongly affected by the type of carbohydrate compound that was being digested.

Biogas Production of Swine Manure in Various Reactor Setups

There are many examples of research groups that have investigated the biogas production capability of swine manure in various reactor setups (Nasir et al., 2012). A number of examples are presented below, with a summary of the reviewed data and biogas production values presented in Table 2. The magnitudes of biogas and methane production compiled in this table serve as a baseline for comparing values generated from manure collected from industrial deep pit swine manure storages.

The effect of temperature on the digestion of swine manure was studied by Chae et al. (2008). In addition to general temperature trends, the group found specific quantities of methane gas yield by the small, 1 L stirred batch reactors used in the study. For temperatures of 25°C, 30°C, and 35°C, the reactors yielded 0.33, 0.39, and 0.40 m^3 CH_4/kg of volatile solids (VS) added, respectively. Hansen et al. (1998) also investigated the effects of temperature on anaerobic digestion efficiency. As a part of the study, two different 3 L, continuous stirred tank reactors (CSTRs) were maintained at various temperatures, giving different methane yields at identical hydraulic retention times. The reactor held at 55°C produced 0.07 m^3 CH_4/kg VS added, while the reactor held at 37°C yielded 0.19 m^3 CH_4/kg VS added. These studies illustrated the importance of temperature with respect to microbial activity, substrate availability, and gas production in anaerobic systems.

The methane productivity of manures from different types of livestock was evaluated by Moller et al. (2004). A simple 1.1 L batch reactor setup was utilized and held at mesophilic temperature conditions (approximately 35°C). The swine manure slurry yielded 0.28 and 0.36

Table 2. Summary of biogas and methane production data for swine manure.

Reference	Waste Description	Reactor Type	Reactor Size (L)	T (^{0}C)	OLR (kg VS/m^3 day)	HRT (d)	VS/COD Removed (%)	Biogas	Methane	Gas Production Units
Andara et al. (1999)	Solid Fraction	Stirred Batch	245	35	1.45	60.0	65.0	-	0.17	m^3/kg VS added
Andara et al. (1999)	Solid Fraction	Non-Stirred Batch	565	35	0.80	60.0	61.0	-	0.18	m^3/kg VS added
Chae et al. (2008)	Manure Slurry	Stirred Batch	1	25	-	20.0	44.0	-	0.33	m^3/kg VS added
Chae et al. (2008)	Manure Slurry	Stirred Batch	1	30	-	20.0	55.0	-	0.39	m^3/kg VS added
Chae et al. (2008)	Manure Slurry	Stirred Batch	1	35	-	20.0	61.0	-	0.40	m^3/kg VS added
Ferrer et al. (2009)	Manure Slurry + Water	Batch	225	23	-	80.0	-	0.07	0.01	m^3/kg VS added
Ferrer et al. (2009)	Manure Slurry + Urine	Batch	225	33	-	80.0	-	0.08	0.04	m^3/kg VS added
Francese et al. (2000)	Manure Slurry + Fish Oil	CSTR	5	30	-	15.0	56.0	0.25	0.16	m^3/kg VS added
Guo et al. (2012)	Manure Slurry	CSTR	5	20	-		-	-	0.24	m^3/kg ODM
Guo et al. (2012)	Manure Slurry	CSTR	5	37	-		-	-	0.28	m^3/kg ODM
Guo et al. (2012)	Manure Slurry	Biogas Plant	800000	25	0.60	35.0	-	0.58	0.36	m^3/kg ODM
Hansen et al. (1998)	Manure Slurry	CSTR	3	55	-	15.0	-	-	0.07	m^3/kg VS added
Hansen et al. (1998)	Manure Slurry	CSTR	3	37	-	15.0	-	-	0.19	m^3/kg VS added
Hill et al. (2000)	Liquid Fraction	Dispersed Growth AF	300	35	3.02	5.0	51.6	-	0.36	m^3/kg VS added
Hill et al. (2000)	Liquid Fraction	Dispersed Growth AF	300	35	5.01	3.0	42.6	-	0.30	m^3/kg VS added
Hill et al. (2000)	Liquid Fraction	Dispersed Growth AF	300	35	7.50	2.0	34.5	-	0.22	m^3/kg VS added
Kaparaju et al. (2005)	Manure Slurry + Potato Tuber	CSTR	3.5	35	2.00	26.0	-	0.53	0.32	m^3/kg VS added
Kaparaju et al. (2005)	Manure Slurry	CSTR	3.5	35	2.00	44.0	-	0.22	0.14	m^3/kg VS added
Lansing et al. (2010)	Manure Slurry + Cooking Grease	Tubular PE Bags	250	26	0.78	40.0	95.4	0.46	0.31	m^3/kg VS added
Masse et al. (2000)	Manure Slurry	ASBR	40	17	2.30	28.0	77.0	-	0.26	m^3/kg VS added
Masse et al. (2000)	Manure Slurry	ASBR	40	17	2.30	28.0	77.0	-	0.21	m^3/kg VS added
Masse et al. (2003)	Liquid Fraction	ASBR	42	10	1.10	15.0	45.4	0.10	0.08	m^3/TCOD added
Masse et al. (2003)	Liquid Fraction	ASBR	42	20	1.20	15.0	54.2	0.35	0.27	m^3/TCOD added
Moller et al. (2004)	Manure Slurry	Batch	1.1	35	-	-	-	-	0.36	m^3/kg VS added
Moller et al. (2004)	Manure Slurry	Batch	1.1	35	-	-	-	-	0.28	m^3/kg VS added
Moller et al. (2007)	Manure Slurry	1-stage LS	130	53	4.00	23.1	52.0	-	0.32	m^3/kg VS added
Moller et al. (2007)	High Solids Mix	2-stage HS	130	53	4.00	23.3	44.0	-	0.20	m^3/kg VS added
Pagilla et al. (2000)	Manure Slurry	1-stage	10	37	-	15.0	-	-	0.25	m^3/kg VS added
Pagilla et al. (2000)	Manure Slurry	2-stage	10	37	-	15.0	-	-	0.39	m^3/kg VS added

m^3 CH_4/kg VS added for sows and pigs, respectively. Another study conducted by Moller et al. (2007) sought to enhance the methane yield of the digestion process by comparing the performance of swine manure slurry with solids enriched swine manure. The experiment was performed in pilot digesters held at temperatures in the thermophilic range. The high solids mix went through a two-stage system while the low solids mix only went through one stage. The high solids mix produced 0.20 m^3 CH_4/kg VS added compared to a much larger value of 0.32 m^3 CH_4/kg VS added for the low solids mix. Pagilla et al. (2000) also used a multistage approach for the comparison of swine manure performance in mesophilic digestion conditions. In this case, the single-stage reactor produced 0.25 m^3 CH_4/kg VS added while the two-stage system produced 0.39 m^3 CH_4/kg VS added. These research groups illustrated important differences in the digestibility of feed in different animals, as well as the resulting effects on the solids content and substrate availability in the manure. Also, the results indicated that the solids concentration of the manure slurry and the level of hydrolysis occurring in the system had important effects on biogas production.

A number of studies represented in Table 2 illustrate the importance of system operating conditions in optimizing biogas production. A novel reactor setup was explored by Hill et al. (2000) in using a dispersed growth anaerobic fermentation reactor to digest the liquid fraction of swine waste at different hydraulic retention times (HRTs). This study sought to better understand the reactor conditions that would cause a bacterial washout or organic overloading. The results showed that at HRT values of 5, 3, and 2 days, the reactor yielded 0.36, 0.30, and 0.22 m^3 CH_4/kg VS added, respectively, at 35°C. Another group (Masse et al., 2000) researched the performance of a sequencing batch reactor (40 L) at the psychrophilic temperature range while investigating the effect of antibiotics on the system. This study yielded values of 0.21 and 0.26 m^3 CH_4/kg VS added for different test runs with this setup. In a follow-up study, Masse et al. (2003) invested the effects of temperature fluctuations on the methane production of a similar reactor setup. In this study, the system produced 0.08 m^3 CH_4/TCOD added at 10°C and 0.27 m^3 CH_4/TCOD added at 20°C.

Biogas production of swine manure has also been evaluated by various groups in pilot-scale and industrial-scale applications. Andara et al. (1999) conducted a study that characterized the organic stabilization of the solid fraction of swine manure in pilot scale reactors. The temperature within the reactor was maintained at the mesophilic temperature

range, and the organic loading rates were controlled for both stirred and non-stirred reactors. The biogas production efficiency was similar for both reactors, yielding 0.17 and 0.18 m^3 methane gas per kg volatile solids (VS) added to the system for the stirred and unstirred reactors, respectively. Ferrer et al. (2009) investigated the biogas production of swine manure in low-cost setup with limited resources in order to analyze the feasibility of a pilot scale digester in a developing country. Dry manure was diluted with urine (to measure the performance of a less water-dependent process) and compared with manure diluted with water. The low-cost reactors were sized at 225 L and held at ambient temperatures within a greenhouse to save on heating costs. The cumulative gas production from the pilot scale reactor for each setup was 0.01 m^3 CH_4/kg VS added for the water dilution and 0.04 m^3 CH_4/kg VS added for the urine dilution. These values are relatively low when compared to other studies reviewed in this section. Another study conducted by Guo et al. (2012) evaluated the performance of an agricultural biogas plant in China supplied with solid pig manure collected from a farm nearby. The plant consisted of four large reactors (250 m^3) fed semi-continuously with the organic dry matter (ODM) after it was diluted slightly. The biogas plant itself produced 0.36 m^3 CH_4/kg ODM, while 5 L bench-scale reactors produced comparative gas production rates of 0.24 m^3 CH_4/kg ODM at 20°C and 0.28 m^3 CH_4/kg ODM at 37°C.

A number of other groups have attempted to mix swine manure with other organic additives, in a process termed “co-digestion.” Francese et al. (2000) co-digested swine manure with fish oil waste and bentonite from an oil filtration process. The experiment was conducted in a 5 L CSTR, yielding 0.25 m^3 biogas/kg VS added and 0.16 m^3 CH_4/kg VS added. Another study by Kaparaju et al. (2005) compared the co-digestion of swine manure, potato tuber, and associated waste with the digestion of swine manure alone. This study showed that the co-digested manure was able to produce 0.32 m^3 CH_4/kg VS added, while the isolated manure produced 0.14 m^3 CH_4/kg VS added**.** Finally, Lansing et al. (2010) studied the co-digestion of swine manure with cooking grease in tubular polyethylene (PE) bags. The reactors in this low-cost application were able to produce 0.31 m^3 CH_4/kg VS added.

Barn-scale Reduction of Methane Emissions

It is also important to mention that many research groups have investigated the reduction of methane emissions from manure storages on a barn scale. Berg et al. (2006) found that the most effective way to reduce methane emissions from swine slurry in a laboratory setting was to acidify it by adding organic acid combined with various coverings, recommending pH values below 6.0 to significantly reduce methane gas emissions. Ottosen et al. (2009) also saw a significant reduction in methane gas emissions after acidifying manure samples. Martinez et al. (2003) were able to achieve methane reductions of 20-100% by employing techniques such as mechanical separation, dilution, chemical addition, and aeration prior to storage. Other research groups have investigated ventilation strategies to maintain lower temperatures in the barn throughout the year, more frequent slurry removal and cleaning of pits to prevent formation of extensive bacterial communities, and alternative solids handling strategies (Haeussermann et al., 2006, Laguë, 2003).

One important group of chemicals that have been used to mitigate methane emissions from animal manures are ionophores, most commonly in the form of monensin. These antibiotics deplete microbial populations by attaching themselves to the lipid bilayer of the cell membranes, causing a depletion of energy production and cell death (Russell and Strobel, 1989). On a larger scale, ionophores can significantly affect the microbial community within manure storages, and disrupt methane generation. However, the effects of antibiotic addition are relatively short-lived due to the development of resistance to the antibiotics, making such a solution only temporary (Massé et al., 2000, Kebreab et al., 2006).

CONCLUSIONS AND RESEARCH IMPLICATIONS

Foaming in deep pit swine manure storages is a growing problem with serious implications for the effective storage of manure and overall safety in swine facilities. In this way, a better understanding of the exact mechanism of foam formation and the most prevalent factors that contribute to that formation is very desirable. As was mentioned throughout this review, however, the existing literature available in this topic area is limited. The complexity of anaerobic systems and the dynamic nature of their physical, chemical, and biological constituents contribute to the continued speculation with respect to foam

accumulation in various anaerobic environments. In addition, it was shown that comprehensive testing procedures are often difficult to develop and implement.

Despite a general lack of certainty regarding the mechanism of foaming in anaerobic systems, an understanding of foaming systems as three-phase systems was shown to be helpful in understanding foam theory. In this way, a more extensive database of knowledge about foam formation in municipal anaerobic digesters was able to contribute to the general knowledge of foaming anaerobic systems and how that knowledge may apply to deep pit manure storages. In order to move from the general three-phase system framework presented in this review toward a specific solution to foaming deep pits, much research is needed to address specific knowledge gaps in this area. These research needs and knowledge gaps are listed below in question form:

- What specific substances are acting as surface acting agents in deep pits? Are they derived from the feed components, other external inputs into the pit, or biological processes within the pit?
- What specific substances are serving to stabilize foam produced on the surface of the deep pit? Is it a microbiological phenomenon, or is some other hydrophobic solid stabilizing the foam?
- Is the methane production capacity of foaming pits enhanced when compared to non-foaming pits? If so, why is this the case?
- Is there a practical, cost-effective, and environmentally friendly means to remediate foam accumulation in deep pits?

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CHAPTER 3

AN EVALUATION OF THE PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERISTICS OF FOAMING SWINE MANURE

Modified from a paper to be submitted to the *Journal of Environmental Quality*

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Abstract. *Foam accumulation on deep pit manure storages is an increasing concern for swine producers because of the logistical and safety-related problems it creates. To investigate this phenomenon, samples of swine manure were collected from over 50 swine production facilities in Iowa with varying levels of foam accumulation over a 13-month period. Samples were tested for a number of physical, chemical, and biological parameters including temperature, pH, total and volatile solids, volatile fatty acid concentration, biochemical methane potential, methane production rate, surface tension, foaming capacity index, and foam stability. Statistical analysis indicated that manure collected from facilities with foam accumulation produced methane at significantly ($p < 0.05$) faster rates than non-foaming manures (0.148 ± 0.004 and 0.049 ± 0.003 L CH_4 L slurry^{-1} day^{-1} respectively, average ± standard error), and consequently had significantly ($p < 0.05$) greater fluxes of biogas moving through the manure volume. The biochemical methane production assay suggested that manure from foaming pits had less potential to generate methane (123 ± 9 mL CH_4 g VS^{-1}) than non-foaming pits (150 ± 9 mL CH_4 g VS^{-1}) while VFA concentrations were significantly lower in foaming pits (4200 ± 570 mg kg^{-1}) than non-foaming pits (9470 ± 730 mg kg^{-1}). These assays suggest enhanced anaerobic digestion efficiency from foaming barns. Other assays such as surface tension and foaming capacity indicated the accumulation of a surfactant at the manure-air interface of foaming deep pits, which may be capturing biogas bubbles generated within the manure.*

Keywords. *Anaerobic digestion, deep pit manure storage, foaming, methane production, swine manure*

INTRODUCTION

In past three to five years, there have been increased reports of foam accumulation on the surface of deep pit swine manure storages in the United States and Canada. This foam is concerning from an operational standpoint, as foam reduces useable manure storage space, forcing farm managers to pump manure out of the pits more frequently. There are also serious safety concerns, as the foam traps the gasses produced by methanogens in the manure slurry, yielding potentially explosive concentrations of methane after collapses of the foam layer (Moody et al., 2009). Examples of this foam are shown in Figure 1. In general, foam observed in the field is a dark-brown or gray, solids-rich, viscous fluid with mid-sized bubbles entrained throughout (Robert et al., 2011). This description is consistent with that of biological foams occurring in municipal wastewater treatment plants (Di Bella et al., 2011). Observation of the facilities monitored in this study indicated that the amount of foam accumulation and appearance varied by month. For example, surface accumulation in the winter months was much more condensed, i.e., more liquid with fewer gas bubbles entrained, than the foam accumulation observed in the late fall, which were often frothier, with larger bubbles.

(a) **(b)** **(c)**

Figure 1. (a) Biological foam accumulation on the surface of a deep pit storage system in Central Iowa, (b) a sample of foam taken from a swine finishing barn in Central Iowa, and (c) foam distribution of a sample after aeration.

Davenport et al. (2008) describe a useful means of characterizing the production of foam in wastewaters as a three-phase system, requiring a gas, liquid, and solid phase. In this system, the gas phase is a result of biogas production due to decomposition of organic materials, which releases methane and carbon dioxide, along with other traces gasses such as hydrogen sulfide, ammonia, and volatile organic compounds. Bubble entrainment and accumulation occurs when surface-active agents (surfactants) are present and lower the surface tension sufficiently (Glaser et al., 2007; Davenport et al., 2008), and solids in the form of hydrophobic substances stabilize the foam by preventing liquid drainage from the bubbles (Bindal et al., 2002; Horozov, 2008; Heard et al., 2009). The sustained presence of foam occurs only after all aspects of this three-phase system are present within the appropriate range for the production and stabilization of foam. This study uses this framework to conceptualize foaming of deep pit manure storages. That is, the laboratory tests selected and performed on manure samples were chosen to evaluate these aspects and identify the mechanism of foam accumulation in deep pits.

We hypothesized that samples collected from barns with existing foam layers would exhibit significantly different values for key parameters such as the rate of biogas production, the concentration of critical substrates including short-chain fatty acids, and the solids distribution within the pit. In addition, we speculated that the lab-scale foaming capacity and stability test would successfully model the foaming activity of the deep pit manure storages studied, reinforcing the trends shown by the other parameters measured in this study. Moreover, foaming capacity may provide information on which parameters my play or role in foam formation when the required gas production wasn't present within the manure during storage.

MATERIALS AND METHODS

Samples of swine manure were obtained from over 50 swine finishing facilities in Central and Southeastern Iowa. At each site, samples were taken from the same pump out location once a month for 13 months. Samples were extracted from multiple depths of the deep pit depending on the total depth of manure in the pit at the time of sampling. These depths were designated with letters A through D, as illustrated in Figure 1. The letter "A" corresponded to the foam layer itself, "B" represented the thin liquid layer at the interface of

the manure slurry and the surface, and "C" and "D" designated descending depths of the deep pit, with the "C" designation representing manure 61 cm (24 inches) below the surface and the "D" designation representing manure 122 cm (48 inches) below the surface. Throughout this paper, these various depths are referred to as "strata."

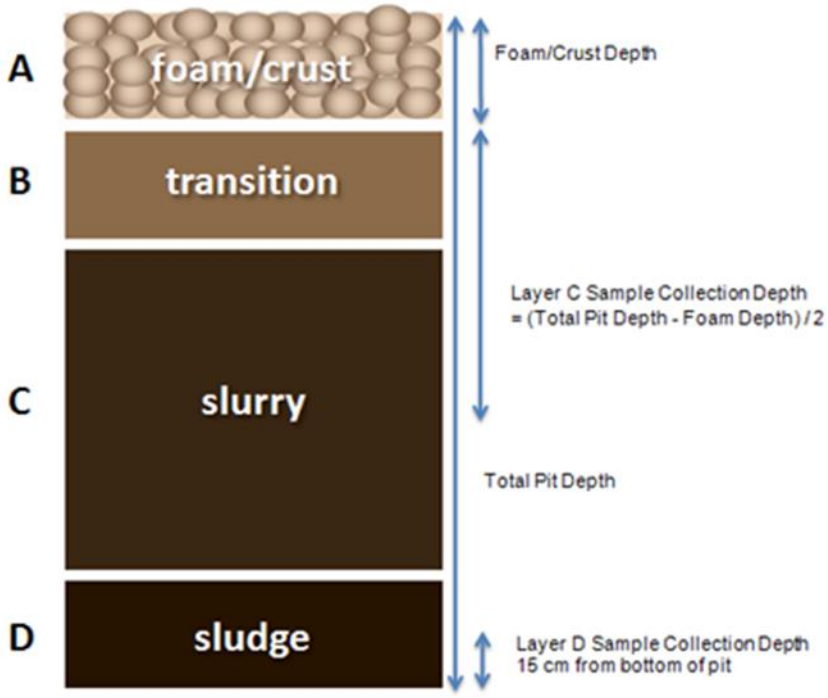

Figure 2. Schematic illustrating the sampling depth designations used during the field sampling.

The total depth of manure in the pit and the height of surface accumulation were measured on site by measuring markings on the sampling pole. In addition, the temperature of the manure at each facility was measured with a digital temperature probe from a sample collected six inches from the bottom of the pit. The pH of the manure was measured within one day of collection (EPA SW-846, Method 9040C).

Total Solids and Volatile Solids

The total and volatile solids contents of manure samples were tested according to the Standard Methods for the Examination of Water and Wastewater 2540B and 2540E (APHA, 2000). In brief, approximately 30 mL of well-mixed manure sample was poured into a pre-weighed porcelain dish and mass recorded. The crucible was oven dried at 104°C for 24 h and weighed again for dry weight of the manure, and heated in a muffle furnace at 550°C for

12 h, weighed again for ash content. Both total solids and volatile solids were reported as a percentage of sample mass.

Short-Chain Fatty Acid Analysis

The concentration of short-chain fatty acids was determined using a modified procedure reported in Webber et al. (2010). In brief, approximately 5 g of the sample was added to 15 mL centrifuge vial, centrifuged at 21,000 x *g* for 23 min at 4°C, supernatant removed, and acidified to pH 2-2.5 using 100 μL of concentrated phosphoric acid. One mL was added to a 20 mL headspace vial salted with 0.3 g of NaCl and sealed.

Samples were loaded into a GC-FID (flame ionization detector) system (Agilent 7980, Agilent Technologies, Inc., Wilmington, DE), equipped with robotic autosampler (MPS2A, Gerstel Inc., Linthicum, MD) and HP-FFAP column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies) using solid phase microextraction (SPME) headspace analysis. The samples were heated for 15 min at 70°C and extracted 5 min with SPME fiber (Carbowax/Divinylbenzene fiber, Supelco, Inc., Bellefonte, PA) prior to injection into the GC-FID system. The GC parameters were set as follows: splitless mode; inlet temperature, 230°C; inlet pressure, 169 kPa; septum purge flow, 30 mL min^{-1}; constant column flow 1 mL min^{-1} (helium); and detector temperature, 300°C. The GC oven temperature program was initial temperature, 100°C, 2 min hold; ramp of 10°C min^{-1} to the final temperature of 240°C, hold for 2 min. All calibration standards were based on external calibration.

Long-Chain Free Fatty Acid Analysis

Long chain free fatty acids were extracted from manure using stir bar sorptive extraction (SBSE) techniques. In brief, 1 g of manure sample was added to 20 mL headspace vial containing 10 mL of a pH 2 water:acetone solution (80:20 vv) containing a sorptive stir bar (Twister™ stir bar, Gerstel, Inc.). The headspace vial was placed on a heated (approximately 50°C) stir plate (Corning) set at 1 revolution s^{-1} and incubated for 2 h. Following incubation, SSB were removed, cleaned with HPLC grade water, and dried.

The SSB were analyzed by thermal desorption GS-MS analysis. In brief, SSB were placed into GC-MS system (Agilent 6890 GC with 5975N MSD, Agilent Technologies, Inc.) equipped with thermal desorption unit (Model TDU, Gerstel, Inc.), robotic autosampler

(MPS2, Gerstel, Inc.), cooled inlet (CIS4, Gerstel, Inc.), and 30 m ZB-35 column (Phenomenex, Torrance, CA). Sorptive stir bars were thermal desorbed with the following parameters: splitless mode; initial temperature, 200°C; final temperature, 350°C (hold 3 min); ramp, 220°C min^{-1}; and heated transfer line set at 320°C. The cooled inlet used glass beads and was operated under solvent vent mode with a column flow of 1 mL min^{-1} and solvent vent set at 100 mL min^{-1} for an effective split of 100:1. The inlet was operated at 450°C in for 2 min for each sample. The GC oven temperature program was the following: initial temperature, 100°C; 0.5 min hold; ramp of 25°C min^{-1}; final temperature of 360°C, hold for 2 min. All calibration standards were based on external calibration.

The MSD was operated with source set at 203°C and MS quads set at 150°C using SIM (selective ion monitoring)/Scan mode. Scan mode was set for 32-550 amu with a solvent delay of 2 min and the SIM mode was set with the following time windows, ions for select target compounds: 1) Group 1 time 2-4 min, for 60, 129. 172 (decanoic acid); 2) Group 2 time 4-5 min, for 60, 129. 186 (undecanoic acid); 3) Group 3 time 5-5.8 min, for 60, 129. 200 (dodecanoic acid); 4) Group 4 time 5.8-6.3 min, for 60, 129. 214 (tridecanoic acid); 5) Group 5 time 6.3-6.6 min, for 60, 129. 185, 228 (tetradecanoic acid); 6) Group 6 time 6.6-7.1 min, for 60, 129. 199, 242 (pentadecanoic acid); 7) Group 7 time 7.1-7.5 min, for 60, 129. 213, 256 (hexadecanoic acid); 8) Group 8 time 7.5-8 min, for 60, 129. 227, 270 (heptadecanoic acid); 9) Group 9 time 8.0-8.5 min, for 60, 129. 241, 284 (octadecanoic acid); 10) Group 10 time 8.5-8.6 min, for 60, 129. 298 (nonodecanoic acid); 11) Group 11 time 8.6-14 min, for 60, 129. 314, 326, 340, 354, 368 (eicsanoic acid).

Biochemical Methane Potential Assay

The biochemical methane potential (BMP) defines the anaerobic biodegradability of a given material (Owen et al., 1979). Specifically, the BMP test gives the total volume of methane a substrate (in this case the manure) is able to produce. Samples with higher biochemical methane potential indicate a greater ability for microbes to convert the specific substrate into biogas. The procedure in assessing the BMP of a swine manure samples collected for this study was to add 20 to 25 g of a sample to a 250 mL serum bottle (Wheaton Science Products No.:223950), with the exact mass recorded. This mass of sample was selected based on an estimated 300 mL of CH_4 produced per gram of volatile solids added as

suggested by others (Hashimoto, 1984; Burton and Turner, 2003; Vedrenne et al., 2008), who suggested a range from 244 to 480 mL CH_4 per gram volatile solids in swine manures. Next, 50 mL of inoculum was added from an active anaerobic digester maintained in the Agricultural Waste Management Laboratory (AWML) at Iowa State University. This volume of inoculum was added to achieve an approximate 2:1 mass ratio of volatile solids from the manure to inoculum, with the actual ratio varying slightly due to the volatile solids content of the manure samples. The solution are diluted to approximately 150 mL with a nutrient medium (Moody et al., 2011) and sealed with a sleeve stopper septa (Sigma-Aldrich Z564729).

Once the sample was prepared, it was incubated at 35°C while being constantly agitated. The samples were regularly checked for biogas production by inserting the needle of a gas-tight syringe (Micro-Mate interchangeable hypodermic Syringe 50cc Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) through the septa. When biogas was collected, it was injected into a non-dispersive infrared methane analyzer (NDIR-CH4 Gasanalyzer University Kiel, Germany) to obtain the percent of methane present in the gas sample. Results were evaluated based on methane produced per gram of sample as well as methane production per gram of volatile solid added (Moody et al., 2011).

Methane Production Rate Assay

The goal of the methane production rate (MPR) assay is to provide a short-term methane production measurement with a relatively simple procedure. The test indicates the rate at which indigenous bacteria produce methane, which gives a measure of current methanogenic activity. While the methane production rate test is similar to the BMP assay, it is unique in a number of ways. First, the test is conducted over a much shorter incubation time (approximately 3 d compared to over 40 d for the BMP assay) to ensure that the sample does not approach substrate limiting conditions and microbial conditions are similar to those in the pit at the time of sampling. Also, the manure sample used for the MPR assay was not inoculated or diluted; rather, the ability of bacteria present within the sample to produce biogas and methane was evaluated. Finally, the sample was incubated at room temperature rather than at 35°C, and kept stationary rather than agitated. Keeping the sample stationary

allowed the observer to record the amount of surface accumulation, foam or otherwise, that developed on the sample.

The procedure for the MPR test involved adding approximately 100 mL of well-mixed sample to a 250 mL serum bottle similar to that used for the BMP assay. Upon the sealing of the sample with a sleeve stopper septa, the exact time was recorded along with the mass of sample added to the bottle. Next, the sample was incubated at room temperature (approximately 23°C). An incubation period of approximately 3 d was selected based on preliminary trials to achieve measureable quantities of biogas and methane. Once the 3-d incubation period was over, the sample was checked for biogas production with the gas-tight syringe and analyzed for methane content using the NDIR-CH4 Gasanalyzer. During the analysis of the biogas produced, the accumulation of foam or solids on the surface of the sample was observed and recorded.

The rate of biogas production and the rate of methane production were calculated using Equations 1 and 2.

$$\mathrm{BPR}\left(\frac{\mathrm{L}}{\mathrm{L}*\mathrm{d}}\right) = \frac{\text{Biogas Produced (mL)}\times\rho_{\text{manure}}\left(\frac{\mathrm{g}}{\mathrm{mL}}\right)}{\text{Mass of sample (g)}\times\text{incubation period(min)}} \times \frac{1440\ \text{min}}{\mathrm{d}} \qquad [1]$$

$$\mathrm{MPR}\left(\frac{\mathrm{L}}{\mathrm{L}*\mathrm{d}}\right) = \frac{\left(\text{Methane }\%\frac{1}{100}\right)\left(\text{Biogas Produced (mL)}+\mathrm{V}_{\text{headspace}}\right)\times\rho_{\text{manure}}\left(\frac{\mathrm{g}}{\mathrm{mL}}\right)}{\text{Mass of sample (g)}\times\text{incubation period(min)}} \times \frac{1440\ \text{min}}{\mathrm{d}} \qquad [2]$$

Temperature Correction of the MPR and Biogas Flux Estimates

The methane production rate assay is performed at room temperature; however, a method to adjust the measured MPR to that expected at the *in situ* temperature of the manure pit was required. Batista et al. (2013) used the Arrhenius equation to model the impact of temperature on methane production rate. This equation was used to adjust the methane production rate values measured in the AWML at 23°C to the *in situ* pit temperature recorded during field sample collection. The temperature adjusted values were averaged across layers of manure collected during sampling (with the exception of the foam or crust layer) to attain an average MPR for each facility each month. In order to calculate the methane flux, the average, temperature-corrected MPR was multiplied by the recorded depth of manure for the pit. The flux was converted appropriately to give units of liters of methane per area per time in L CH_4 m^{-2} d^{-1}.

Surface Tension

Surface tension is an important parameter, which quantifies the impact of surface active agents present in solution. With respect to foaming systems, solutions with sufficient concentrations of surface active agents effectively lower the surface tension by increasing surface activity, allowing foam production (Ganidi et al. 2009). However, if the surface tension is reduced too much, the foam bubbles will pop due to the reduced strength of the bubble film. The surface tension of samples was tested using the CSC Precision Ring Tensiometer (CSC Scientific Company, Inc., Fairfax, VA). Samples were brought to room temperature and agitated gently before pouring into the sample tray. Next, the duNouy ring was placed below the surface of the liquid. The ring was slowly pulled upward through the surface of the liquid until it overcame the surface tension of the sample. The force needed to break the liquid interface was recorded directly off the circular scale of the instrument as dyne cm^{-1} (equivalent to mN m^{-1}), and reported in N m^{-1}.

Foaming Capacity and Stability Testing

The foaming capacity and stability apparatus used in this study, as well as the parameters used to evaluate the foaming characteristics of swine manure, was modified from other researchers (Ross and Ellis, 1992; Bindal et al., 2002; Hutzler et al., 2011). In brief, air was passed through an in-line gas regulator (Restek Model 21666) into a 5.1-cm diameter clear PVC column, and the flow rate of air through the column was measured and controlled with a variable area flow meter (Dwyer RMA-SSV). A sample volume of approximately 300 mL was poured into the column and the initial level was recorded. The sample was then aerated through a cylindrical air stone at 0.2 L min^{-1} until a steady state height of foam was reached or the foam layer reached the maximum height of the column (approximately 33 cm above the initial liquid level). The time of aeration was recorded along with the height of foam produced and the level of the foam-liquid interface. A foaming capacity index was calculated as the height of foam produced divided by the initial manure level and multiplied by 100 (based on our apparatus, maximum measureable foaming capacity was approximately 250).

The foam stability measurement occurred immediately after the foaming capacity was determined. Once aeration ceased, the height of foam became the initial level recorded at

time zero. Once this level was established, the descending height of the foam was recorded at expanding time intervals. Simultaneously, the ascending level of the foam-liquid interface was recorded at the same time intervals. The descending height of foam was normalized to percent of initial foam height and plotted as a function of time. A first-order exponential decay model fit the data well in most cases, and was used to estimate the half-life of the foam from the time constant as shown in Equation 3.

$$t_{\frac{1}{2}}(\text{min}) = \frac{\ln(2)}{\text{decay coefficient k}} \quad [3]$$

Statistical Analyses

Statistical analysis was performed using JMP Pro 10 (JMP Pro, Version 10. SAS Institute Inc., Cary, NC, 1989-2012). Fixed factors were established according to data collected on site, including the surface condition (foaming or non-foaming) and the stratum the manure was collected from (A, B, C, or D). The month during which samples were collected was treated as a random factor. The interaction of surface condition and sampling strata was also considered, however interactions with the random variable month were pooled to error.

RESULTS AND DISCUSSION

Monthly temperature trends of manure from both foaming and non-foaming barns are shown in Figure 3. A statistical analysis with site as a random variable, surface as a fixed effect, month as a fixed effect, and the surface x month interaction as a fixed effect indicated that month, surface status, and the surface status x month interaction were all significant ($p < 0.01$). In this case, the significant interaction indicated that pits with different surface conditions tended to warm and cool at different rates, most notably during the months of October through April. The differences during this period could have been caused by foam serving as an insulator, slowing heat loss from manure to exhaust air. Trabue and Kerr (2014) identified a similar effect in manure tanks used in feeding trials, noting that tanks with crusting and surface accumulation tended to be warmer than those without crust, due to the crust insulating the manure as colder ventilation air passed over the manure surface. Additionally, there was a strong seasonal pattern, with pits warmest in September (22.1 and 22.0°C for foaming and non-foaming pits, respectively) and coolest in February (12.7 and

10.9°C for foaming and non-foaming pits, respectively). Temperature differences within the pit influence the metabolic activities of the microbial community, the gas transfer rates, and the settling characteristics and hydrolysis of solids (Tchobanoglous et al., 2003). Increased temperatures in foaming deep pits during the winter and spring months may increase the ability of the microbes to hydrolyze and consume substrate, which may enhance the transfer of biogas and hydrophobic matter to the surface of deep pits.

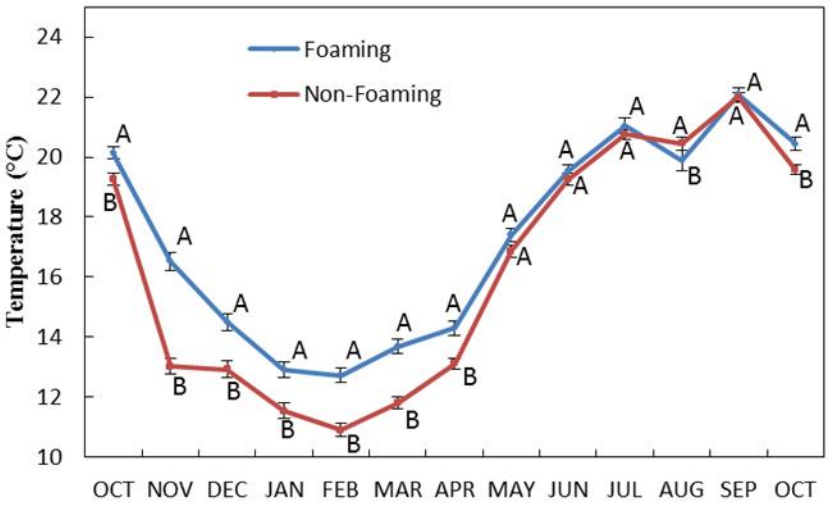

Figure 3. Manure pit temperatures taken from the bottom of the pit for foaming and non-foaming barns by month. Error bars represent the standard error of the mean for the month*surface type interaction, with differences between surface types tested at α=0.05.

An analysis of variance for pH was conducted by considering barn identification, barn surface status (foaming or non-foaming), month, sampling depth (stratum), and the interaction of surface status x stratum as fixed factors. The ANOVA indicated that there were significant effects associated with barn identification ($p < 0.01$), surface status ($p < 0.01$), month ($p < 0.01$) and the surface status x month interaction ($p < 0.05$), but no effect for stratum.. The results (Figure 4) indicated that the pH of foaming barns was more basic (pH = 7.68) than for non-foaming barns (pH = 7.51). The significance of the surface status x strata interaction was entirely due to pH differences between strata in foaming barns, specifically the A strata, while no differences by strata existed for non-foaming barns. In foaming barns, the foam accumulation itself tended to be more basic (7.74 on average) when compared to manure at lower depths (7.63, 7.66, and 7.68 for layers B, C, and D respectively). This difference is likely a result of entrapment of ammonia in foam layer which would affect pH

(Snoeyink and Jenkins, 1980). No statistical differences by strata were found for non-foaming pits.

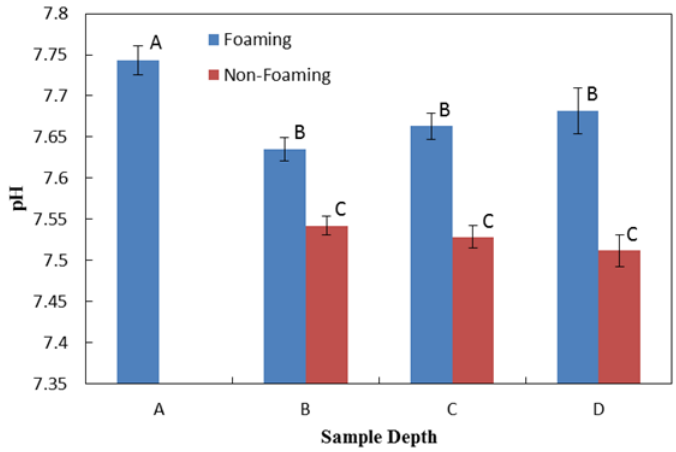

Figure 4. Manure pH by average over all month sorted by stratum and surface condition. Error bars represent the standard error of the mean for the surface status*stratum interaction. Letters are used to denote significant differences (α=0.05).

Total solids and volatile solids contents were strongly correlated to each other, with a regression of total solids and volatile solids indicating that total solids concentration explained 99% of the variation in volatile solids content. Also, a relatively consistent fraction of solids were volatile (75.0% ± 6.8%, average ± SD). A statistical model with sample month, surface condition, and stratum as factors failed to detect a significant difference in solids content with surface condition, but did find differences with strata ($p < 0.01$) and similarly with volatile solids ($p < 0.01$). Figure 5 shows total solids and volatile solids concentrations of samples collected from all facilities over 13 months based on the depth at which they were sampled. The graph shows that nearly all strata were significantly different from each other, with increasing solids concentrations as sampling depth was increased and a relatively solids-rich foam layer in sites with surface accumulation.

The average short-chain fatty acid (SCFA) concentrations for various sampling depths of selected samples are shown in Figure 6 for both foaming and non-foaming pits. Of the total SCFA concentration, acetic acid was the dominant component (52% of total on average). The total SCFA concentration in non-foaming barns was on average greater than that of foaming barns at every depth, with the average SCFA concentrations of foaming barns greatest at the surface layer and gradually decreasing at greater depths of the pit. The statistical model indicated that the impact of surface ($p < 0.01$) and month ($p < 0.01$) were

significant, and that strata the surface status x stratum interaction was not significant. The overall results show that foaming barns had significantly lower SCFA concentrations (4009 μg g^{-1}) than non-foaming barns (8301 μg g^{-1}). Also, the ratio of acetic acid to propanoic acid was significantly ($p < 0.05$) higher in the foam itself than in the slurry layer of foaming and non-foaming barns, which agrees with a study done by Sakauchi and Hoshino (1981) where this same ratio was higher in rumen fluid from bloated steers than healthy steers.

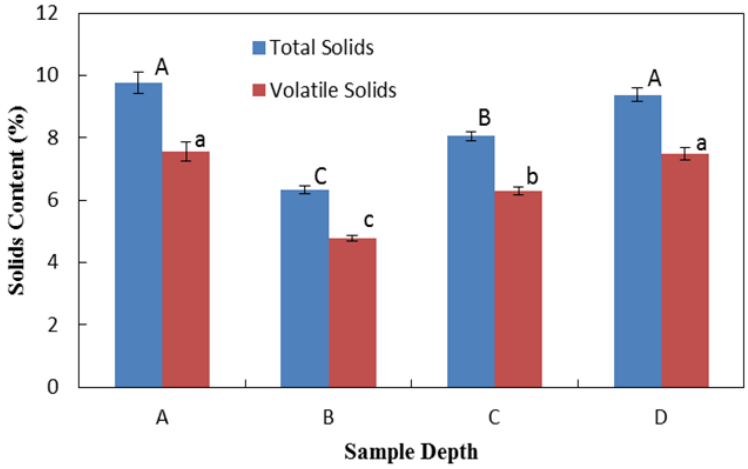

Figure 5. Total solids and volatile solids content for every sample taken over the 13-month trial period at various sampling depths (A=foam layer, B=surface interface, C=0-24" below surface, D=24-48" below surface). Differences tested at α=0.05 show differences in total solids (capital letters) and volatile solids (lower-case letters) with respect to strata.

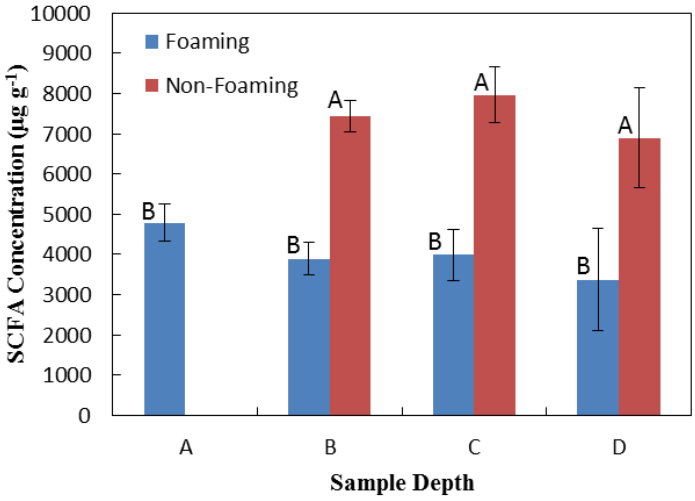

Figure 6. Average total VFA concentrations of foaming and non-foaming samples by sampling depth (A=foam layer, B=surface interface, C=0-24" below surface, D=24-48" below surface). Letters show statistical differences between foaming and non-foaming samples tested at α=0.05.

The BMP assay provided an estimate of the potential methane production a material could generate under ideal digestion conditions. Previous research by Moody et al. (2011) suggests that swine manure slurry taken from a deep pit should have an approximate methane production potential of 132 mL CH_4 g VS^{-1}. On average this group found a methane production potential of 145 mL CH_4 g VS^{-1} across all samples collected. A statistical analysis was performed to evaluate the impact of surface status, sampling month, and strata. Results from the analysis indicated that only surface was significant ($p < 0.01$). A graph comparing the biogas production potential foaming and non-foaming samples is shown in Figure 7. The significant difference in remaining biochemical methane production potential between foaming and non-foaming barns could indicate that foaming barns are operating as more effective anaerobic digesters than non-foaming barns as more of the potential for methane production has already been consumed. The difference in biochemical methane production potential between the foaming and non-foaming manures was attributed to their differences in VFA concentrations.

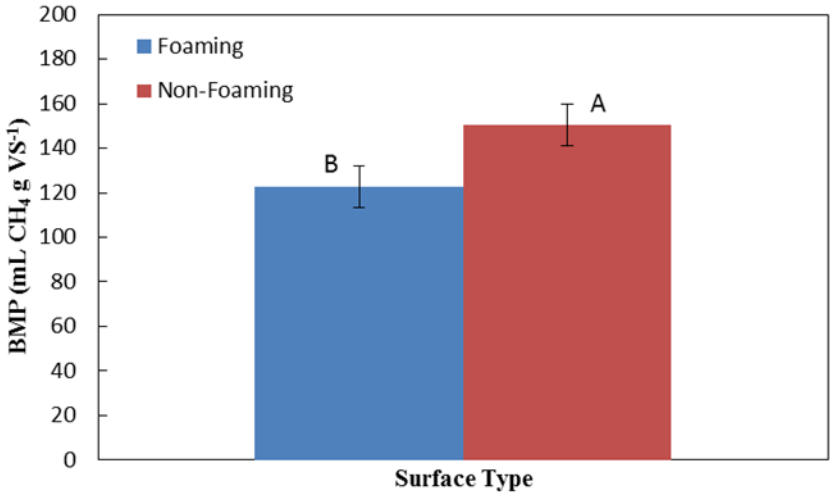

Figure 7. Average biochemical methane potential of foaming and non-foaming samples. Error bars represent the standard error of the mean. The statistical difference shown was tested at $\alpha = 0.05$.

Results for the methane production rate test showed differences between barns that exhibited foaming characteristics during sampling and those with no foam accumulation. Trends for foaming and non-foaming barns by month are shown in Figure 8. The MPR of samples from foaming barns was significantly higher ($p < 0.01$) than those from non-foaming barns for all sampling months. The statistical analysis shows that both sampling month and

surface type were significant ($p < 0.01$), while the sampling depth was not. These results provide a contrast to the cumulative gas production potential of the samples described in the previous paragraph. Taken together, the results of the BMP and MPR tests suggest foaming pits are serving as more effective anaerobic digesters than non-foaming pits. This may mean that foaming samples have a more developed microbial community, which is able to more quickly convert consumable substrate into biogas.

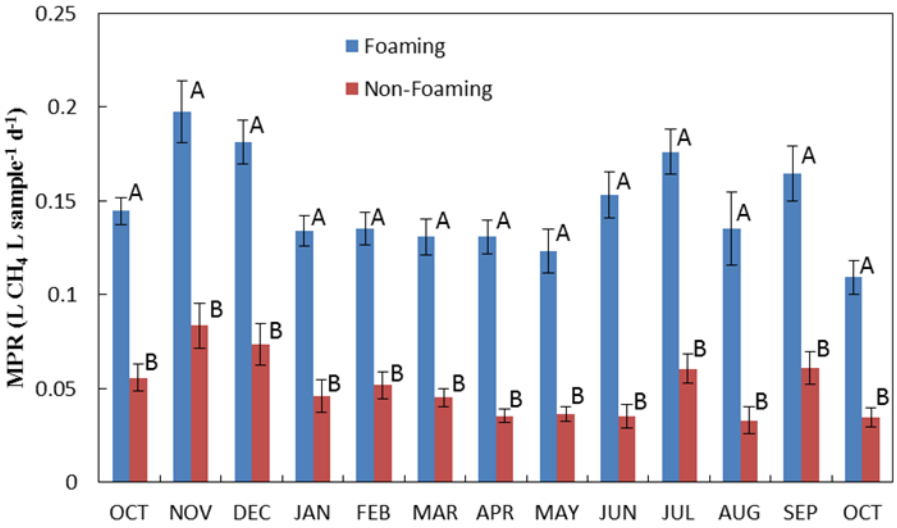

Figure 8. Average methane production rate of foaming and non-foaming samples over 13 sampling months. Error bars represent the standard error of the mean. The statistical differences shown were tested at $\alpha=0.05$.

The conversion of MPR values reported during bench top experiments to values adjusted for *in situ* temperature of deep pits made a significant impact on the monthly trends for the gas phase of studied systems. The average methane flux of foaming and non-foaming barns is shown in Figure 9. The methane flux values from each facility were derived from a temperature-corrected term averaged across sampled strata (as described in the methods section), which yielded a more intuitive line reflecting the temperature effects of the sampling month when compared to that in Figure 8. At the same time, the curves in Figure 9 continue to reflect the enhanced rate at which samples from foaming barns produced methane. Most notably, the enhanced flux of biogas occurred in pits during the summer and early fall, which mirrors temperature trends shown in Figure 3. The month of August seemingly interrupts this trend, but the number of samples collected during this month was significantly reduced due to logistical reasons, which altered the typical set of deep pits that were sampled.

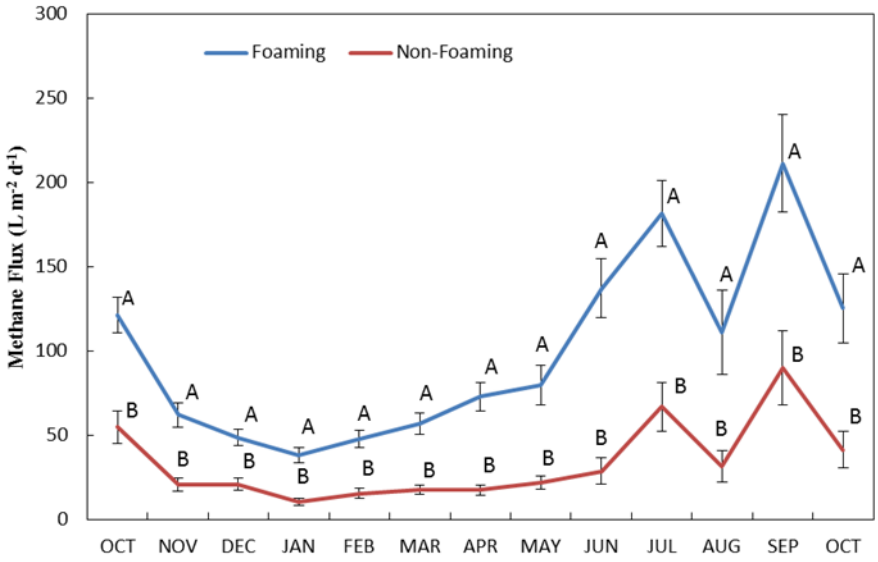

Figure 9. The average methane flux for foaming and non-foaming facilities over the 13-month sampling period. Error bars show the standard error of the mean. Letters show statistical differences between surface statuses within each month at α=0.05.

The results of the surface tension measurements are shown in Figure 10. Statistical analysis showed that both surface type and strata were significant ($p < 0.05$ and $p < 0.01$, respectively), while the surface type x strata interaction was not. As a whole, the average surface tension of non-foaming samples was lower than that of foaming samples (0.0495 ± 0.0006 N m^{-1} and 0.0515 ± 0.0006 N m^{-1}). In this case also, the surface tension was once again related to SCFA concentration, which had a significant effect ($p < 0.01$). When the SCFA concentration is included in the analysis as a covariant, no differences between the surface tension of manures from foaming and non-foaming barns were found, but the B strata still had lower surface tension than either the C or D strata. As a reference for comparative purposes, the surface tension of water at room temperature is approximately 0.073 N m^{-1} and the surface tension of human urine is 0.059 N m^{-1} (Mills et al., 1988). The surface tension of 1%, 5%, and 10% acetic acid-water solutions at 30°C are 0.068, 0.060, and 0.055 N m^{-1} (Lang and Dean, 1967) respectively. All measured values were significantly lower than pure water, with the averages within each sample depth lower for non-foaming samples than for foaming samples. This may suggest an optimal range of surface tension values that promote foaming, but the evidence is inconclusive.

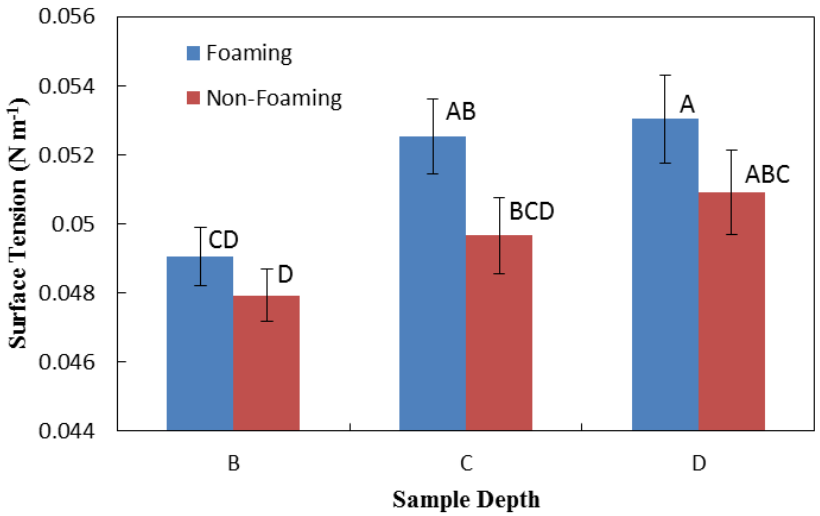

Figure 10. The average surface tension for foaming and non-foaming samples at various sampling depths (A=foam layer, B=surface interface, C=0-24" below surface, D=24-48" below surface). Error bars show the standard error of the mean. Letters are used to denote significant differences between averages (α=0.05).

For the most part, samples from foaming barns showing a significantly greater foaming capacity than those from non-foaming barns. Figure 11 suggests that there is a relative accumulation of substances that enable foam production at the surfaces of foaming barns when compared to non-foaming barns based on the differences due to the surface type x stratum interaction ($p < 0.05$), with samples from the interface of foaming sites showing a greater disparity between layers than those collected from non-foaming sites. Foam itself did not show an enhanced capacity to foam because of the fact that it was usually very solids-enriched, which minimized the ability for the foam to expand in the testing apparatus. The average foaming indices for samples collected at the interface (B layer) of foaming and non-foaming facilities are shown in Figure 12. Interestingly, some of the largest differences between foaming and non-foaming samples occur during the winter months when surface accumulation is lowest in the field. This may support the hypotheses that important surfactants and/or foam stabilizers exist in the foam itself, as these substances would have been more prevalent in the interface layer during time when foam is depressed.

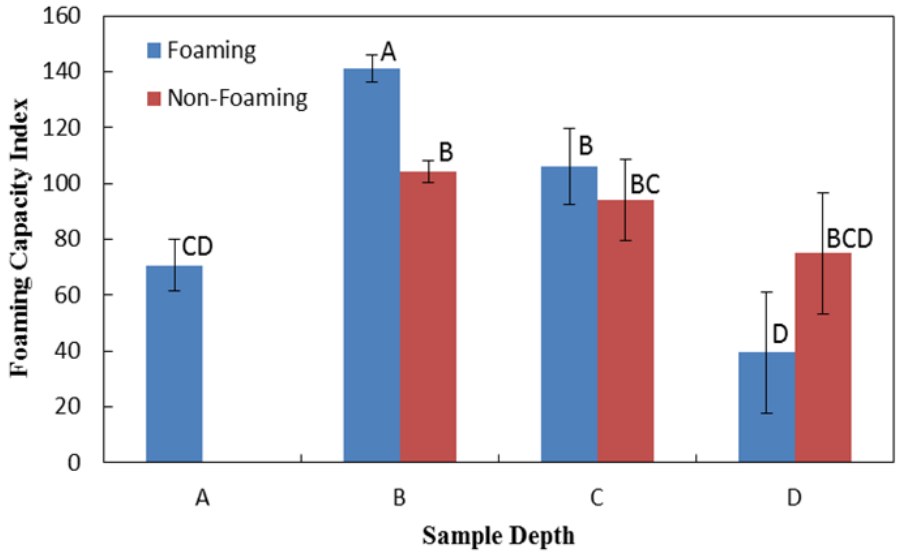

Figure 11. Average foaming capacity index of foaming and non-foaming samples by sampling depth (A=foam layer, B=surface interface, C=0-24" below surface, D=24-48" below surface). Error bars show the standard error of the mean. Letters are used to denote significant differences between averages (α=0.05).

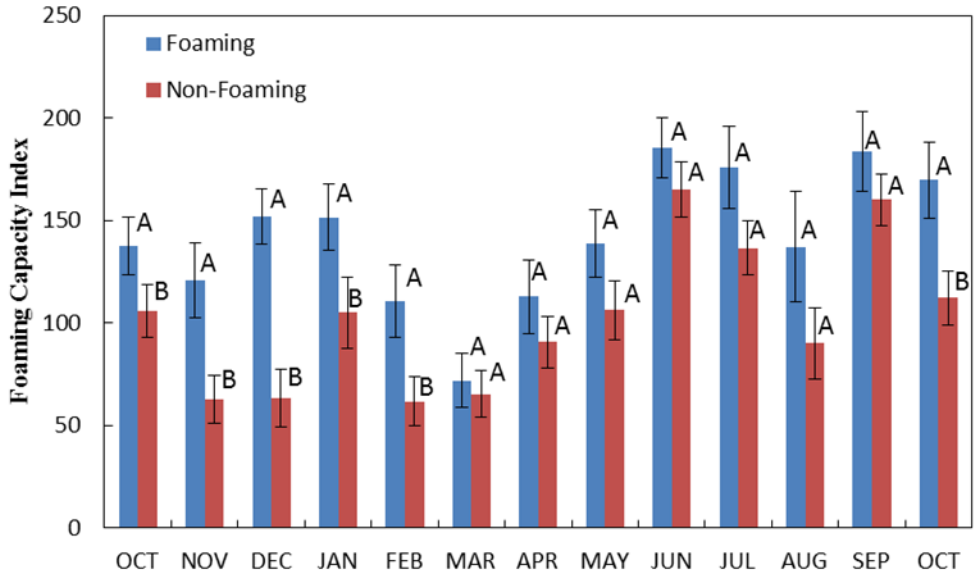

Figure 12. Average foaming capacity index of foaming and non-foaming samples by month. Error bars show the standard error of the mean. Letters show statistical differences between surface statuses within each month test at α = 0.10.

The corresponding graph representing the half-life of B-layer samples is shown in Figure 13. The results of this test showed a greater difference between foaming samples and non-foaming samples when compared to the capacity test, suggesting a greater presence of a foam stabilizing agent in foaming samples. One sampling month that didn't reflect these

differences was March, during which samples may have been diluted by heavy rainfalls during sampling.

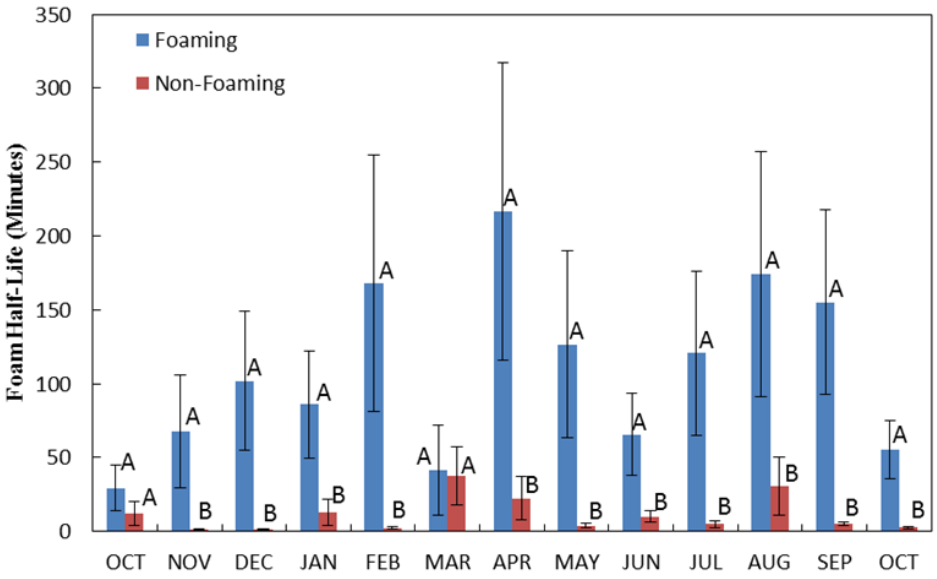

Figure 13. Average foam half-life of foaming and non-foaming samples by month. Error bars show the standard error of the mean. Letters show statistical differences between surface statuses within each month tested at α=0.10.

Another important note is that foam samples showed an outstandingly long half-life in this test (1468±18 min for foam samples compared to 105±16 min for B-layer samples collected from foaming barns). This disparity reinforces the idea that an important stabilizing agent is accumulating at the surface and in accumulation at foaming sites. One hypothesis is that LCFAs (long-chain fatty acids) are serving to stabilize biological foams (Jacobson et al. 2013). Figure 14 shows the results of preliminary LCFA testing and appears to support the stated hypothesis. However, LCFAs actual lower surface tension (Chumpitaz et al. 1999), which is the opposite of our observations (Figure 10), while LCFAs are more prevalent in the upper strata this may reflect the hydrophobicity of the LCFAs. It should be noted that fats and LCFAs are expected to accumulate at the surface. However, more work into the nature of the surface layer is needed to better understand the nature of the foam layer.

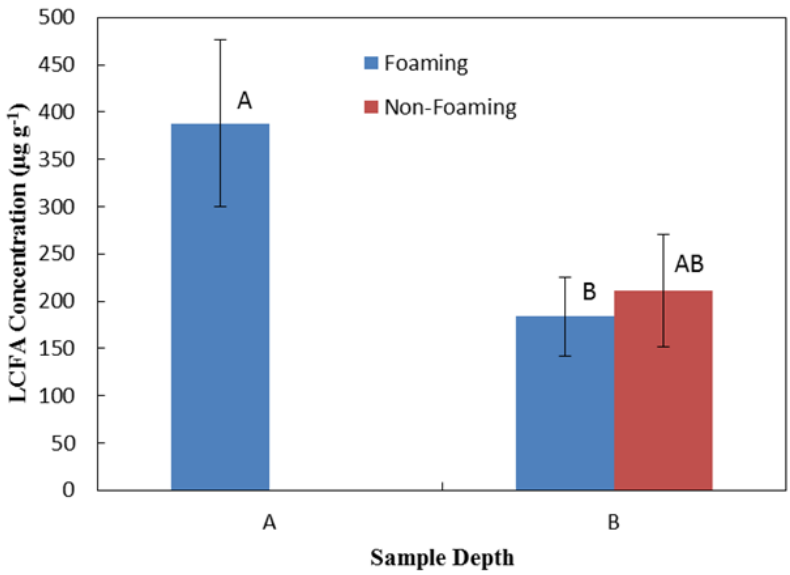

Figure 14. Average long-chain fatty acid (LCFA) concentrations in samples collected from the foam and interface layers of foaming and non-foaming sites. Error bars show the standard error of the mean. Letters show statistical differences between the different sampling depths tested at α=0.05.

CONCLUSIONS

There are several key observations that can be made with this study's data over at 13 month sampling period. First, the testing of the gas phase of foaming systems has proven a significantly enhanced rate of methane production of barns with foam accumulation in comparison to barns with no foam or crust. At the same time, the biochemical methane potential assay has indicated that samples from foaming barns have less potential to generate additional methane than those from non-foaming barns per gram of VS. Taken together, these three tests indicate that the microbial consortium within samples collected from foaming sites enable these systems to function as more efficient digesters; that is, SCFAs in foaming deep pits are processed at a greater rate leading to enhanced rates of biogas production. The results of the BMP test confirm this assertion as more consumable substrate remains in samples collected from non-foaming barns, leading to greater cumulative biogas production in a long-term incubation. It was shown to be helpful to correct MPR data to the in situ pit temperature at the time of sampling to formulate an average methane flux for each facility per month. The resulting monthly flux curve showed an intuitive monthly trend with respect to microbial response to seasonal temperature changes. This curve also reinforced the

disparity between the biogas production of foaming and non-foaming sites, especially in the late summer and early fall.

Other important physicochemical tests performed in this study show differences in the characteristics of foaming and non-foaming manures. The pH of samples collected from foaming barns was significantly higher than non-foaming samples at all depths, showing the most basic average pH in the foam layer itself which is likely a result of entrapment of ammonia. Measurements of the solids content of manure samples also revealed that the foam was solids enriched compared to samples in the slurry. Also, the surface tension of foaming manures was higher on average than non-foaming manures, with both sets of samples having an average surface tension significantly lower than that of water. Finally, data on foaming capacity and stability between foaming and non-foaming barns indicated that the surface layers of foaming barns showed an increased capacity to foam, suggesting a relative accumulation of substances important to foam formation at these sites. In addition, samples of the foam itself exhibited substantial stability, showing the presence of a stabilizing agent in the foam layer.

Acknowledgements

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CHAPTER 4

THE IMPACT OF CARBOHYDRATE AND PROTEIN SOURCE ON SWINE MANURE FOAMING PROPERTIES

Modified from a paper to be submitted to *Transactions of the ASABE*

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Abstract. *This study explored the impact of swine diet on the physicochemical properties, methane production potential, and foaming characteristics of swine manure. Manure samples were collected from controlled feeding trials with diets varying in both protein and carbohydrate level and source. Protein sources consisted of corn with amino acids, corn-soybean meal with amino acids, corn-soybean meal, corn-canola meal, corn-corn gluten meal, and corn-poultry meal. Carbohydrate sources consisted of corn-soybean meal, barley-soybean meal, corn-soybean meal-beet pulp, corn-soybean meal-distillers dried grains with solubles (DDGS), corn-soybean meal-soy hulls, and corn-soybean meal-wheat bran. Manure samples were tested for a number of parameters, including total and volatile solids, methane production rate, biochemical methane potential, surface tension, foaming capacity, and foam stability. Statistical analyses were performed to evaluate whether different carbohydrate and/or protein ingredients affected these physicochemical properties or the manure samples' ability to produce methane gas. No single diet yielded manure with all of the anticipated qualities of foaming manure (enhanced biogas production, similar surface tension to known foaming manure, and substantial foaming capacity and stability). However, the carbohydrate diet manures from the supplemental soy hulls and DDGS diets exhibited higher methane production rates (0.95±0.20 and 0.96±0.20 L CH_4/kg VS, respectively) and biochemical methane potential (322±25 and 269±22 mL CH_4/g VS, respectively) when compared to other manure types. Also, the soy hull diet yielded manure with an average surface tension that was similar to foaming manure, while also showing, along with the manure from the beet*

pulp diet, a large capacity to foam in the bench-top foaming experiments. Although the soy hull diet manure showed the most consistent foam-related characteristics, the methane production rate was comparable to manures that did not foam in commercial deep pits, and the foam generated in the bench-top experiments did not exhibit a significant ability to stabilize.

Keywords. *Anaerobic digestion, foaming, methane production, swine diet, swine manure*

INTRODUCTION

The accumulation of foam on the surface of deep pit manure storages is a serious concern for pork producers for a number of reasons. On a practical level, foam accumulation can significantly reduce the amount of space available for manure storage, which may force farm managers to apply manure during untimely seasonal windows or seek other means of manure storage. Foam accumulation also impacts safety at swine facilities. Foam has the capacity to trap gases (i.e. methane) produced by the anaerobic decomposition of swine manure, and when the foam layer is broken, release of methane is rapid enough for explosive concentrations to occur in the barn. Numerous swine production facilities have reported flash fires or explosions due to the combination of foam layer breakage and an externally provided spark or flame (Moody et al., 2009).

One conceptual framework that has been used to study the occurrence of biological foam in other industries, particularly municipal wastewater treatment, is the evaluation of foam as a "three-phase system." A similar approach will be used here to better understand the accumulation of foam in deep pits. Davenport and Curtis (2002) described the "three-phase system" framework as useful means of characterizing the production of foam in municipal anaerobic digesters. They suggested that the initiation of foam occurs as a result of both the gas and liquid phases working together to capture bubbles produced within the system. In anaerobic systems, such as deep pit manure storage systems, the gas phase is a result of biogas production from microbial activity and methanogenesis. When appropriate concentrations of surface active agents are present in the liquid layer, it facilitates foam production by lowering the surface tension of the solution with respect to water (Glaser et al., 2007; Davenport et al., 2008). Finally, hydrophobic solids are thought to stabilize the foam

by preventing or reducing liquid drainage from the foam and holding the bubbles in a stabilized structure (Bindal et al., 2002; Horozov, 2008; Heard et al., 2009).

As opposed to anaerobic systems in municipal settings, where the input consists of both primary (raw organics) and secondary settled waste (waste activated sludge), the input of deep pit manure storages consists entirely of animal feces and urine, wasted feed and water, and wash waters during times of barn cleaning. This creates a strong and well-established link between feed composition and the physical and chemical characteristics of the manure (Kerr et al., 2006; Jarret et al., 2011; Trabue and Kerr, in press). For example, Miller and Varel (2003) found that the composition of the manure and the potential release of nutrients and volatile emissions into the environment from livestock operations are partially controlled by dietary inputs. Similarly, diet can influence manure properties that lead to greater methane production potential or the stabilization of foam on the surface of the manure. The objective of this study was to analyze manures produced in controlled diet studies for parameters hypothesized to play a significant role in the overall foaming characteristics of deep pit manures. In particular, the impact of the level and source of protein and carbohydrate diets on manure properties related to gas production, manure physical properties, and foaming potential were evaluated.

MATERIALS AND METHODS

Two studies were conducted which consisted of diets formulated to vary in dietary crude protein (CP) level and source (denoted as the "protein study"), and carbohydrate level and source (denoted as the "carbohydrate study"), with the diet formulations shown in Table 1 and Table 2. In the protein study, the level and source of dietary CP was varied while holding energy, minerals, and amino acids relatively constant. Six diets were formulated. A typical industry diet (Diet B) was formulated utilizing corn and soybean meal with economically-available crystalline amino acids, while Diet A was formulated utilizing only corn and crystalline amino acids (subsequently lower in CP than Diet B). Diet C was formulated with only corn and soybean meal and no crystalline amino acids (subsequently higher in CP than Diet B). Diets D, E, and F utilized alternative protein sources (canola meal, corn gluten meal, and poultry meal, respectively) in place of soybean meal, while maintaining the CP at 17.6%, similar to Diet C. Because the protein sources were derived

from different origins, dietary fiber was allowed to vary. In the carbohydrate study, Diet A represented a diet which would typically be fed to finishing swine and was formulated similarly to Diet C in the protein study. The remaining five diets were formulated to be higher in dietary fiber (as measured by neutral detergent fiber, NDF), but differed amongst themselves relative to the source of fiber. The level of dietary CP and minerals were held constant in all diets. Because dietary fiber sources are lower in digestible and metabolizable energy for growing pigs, and because we did not elect to add supplemental lipids to equalize dietary energy, dietary amino acids levels were held in a constant relationship to dietary energy.

Table 1. Composition of experimental diets, 'Protein Source' Experiment.[1]

Ingredient, %	C/AA[2]	C-SBM/AA	C-SBM	C-CM	C-CGM	C-PM
Corn	94.83	78.47	71.29	61.13	77.72	81.53
Soybean meal	-	18.31	25.90	-	-	
Canola meal	-	-	-	36.14	-	-
Corn gluten meal	-	-	-	-	18.30	-
Poultry meal	-	-	-	-	-	17.35
Dicalcium phosphate	1.22	1.09	1.03	0.77	1.19	-
Limestone	1.28	1.14	1.07	0.95	1.28	-
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Trace mineral & vitamin mix	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine·HCl	0.83	0.24	-	0.30	0.71	0.29
L-Threonine	0.31	0.06	-	-	0.04	0.06
L-Tryptophan	0.08	-	-	-	0.07	0.03
DL-Methionine	0.17	-	-	-	-	-
L-Isoleucine	0.28	-	-	-	-	0.03
L-Valine	0.28	-	-	-	-	-
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition						
ME, kcal/kg	3,306	3,313	3,314	3,323	3,386	3,299
Crude protein, %	8.7	14.8	17.6	17.6	17.6	17.6
NDF, %	8.3	9.3	9.7	19.6	13.7	7.1

[1]All diets formulated to 0.255% standardized lysine per 1,000 kcal of metabolizable energy, 0.78% calcium, and 0.25% available phosphorus.

[2]C/AA, corn plus amino acids; C-SBM/AA, corn-soybean meal plus amino acids; C-SBM, corn-soybean meal; C-CM, corn-canola meal; C-CGM, corn-corn gluten meal; C-PM, corn-poultry meal.

Table 2. Composition of experimental diets, 'Carbohydrate Source' Experiment.[1]

Ingredient, %	C-SBM[2]	B	BP	DDGS	SH	WB
Corn	75.17	-	54.09	61.75	62.14	57.21
Barley, pearled	-	83.27	-	-	-	-
Soybean meal	22.31	14.35	21.85	7.40	20.85	17.55
Beet pulp	-	-	22.00	-	-	-
Distillers dried grains with solubles	-	-	-	28.05	-	-
Soybean hulls	-	-	-	-	14.65	-
Wheat bran	-	-	-	-	-	22.91
Dicalcium phosphate	1.06	0.79	1.07	0.24	1.06	0.69
Limestone	0.66	0.79	0.27	1.23	0.51	0.82
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Trace mineral/vitamin mix	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine·HCl	0.11	0.10	0.02	0.50	0.09	0.12
L-Threonine	-	-	-	0.09	-	0.01
L-Tryptophan	-	-	-	0.05	-	-
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition						
ME, kcal/kg	3,329	2,912	3,138	3,290	3,215	3,076
Crude protein, %	16.3	16.3	16.3	16.3	16.3	16.3
NDF, %	9.6	17.0	17.0	17.0	17.0	17.0

[1]All diets formulated to 0.255% standardized lysine per 1,000 kcal of metabolizable energy, 0.78% calcium, and 0.25% available phosphorus.

[2]C-SBM, corn-soybean meal; B, pearled barley-soybean meal; BP, corn-soybean meal-beet pulp; DDGS, corn-soybean meal-distillers dried grains with solubles; SH, corn-soybean meal-soybean hulls; WB, corn-soybean meal-wheat bran.

Figure 1a shows the facility in which the feeding trials were conducted. The facility consisted of 24 metabolism crates with corresponding manure storage tanks. Ambient temperature in the metabolism room was maintained at approximately 21°C and lighting was provided continuously. Pigs were fed 1.5 kg of the designated diet twice per day and water was supplied ad libitum through nipple drinkers. After each feeding session, feces and urine were collected and deposited in the manure storage tanks. Manure tanks were designed to have a similar surface area as used for pigs maintained in growing-finishing barns with deep pit manure storage systems. At the completion of each 40 day trial, manure within the tank was thoroughly agitated and sub-samples were collected. Samples were subsequently stored at 4°C until they were analyzed. After sample collection, tanks were aerated using a bubble diffuser to observe if the manure would generate stable foam (Figure 1b). After the first trial, the tanks were emptied and cleaned, and then the dietary trial was repeated with diets

randomized over an additional 24 pigs, thus giving 48 experimental units for each dietary study.

(a) **(b)**

Figure 1. (a) Barn with metabolism crates and manure storage tanks where the feeding trials were conducted and (b) foam accumulation in the manure storage tank after aeration to induce bubbling and foaming.

Total Solids and Volatile Solids

The total solids and volatile solids contents of manure samples were tested according to the Standard Methods for the Examination of Water and Wastewater 2540B and 2540E (APHA, 2000). After thorough mixing, approximately 30 mL of each manure sample was poured into a pre-weighed porcelain dish. After obtaining the weight of the full crucible, the sample was dried in a 104°C oven for approximately 24 hours. After drying, the sample was weighed again. After obtaining the dried weight of the sample, the crucible with the dried contents was placed in a muffle furnace at 550°C for approximately 12 hours. Once cooled, the final weight of the ash and crucible was obtained. Both total solids and volatile solids were reported in percentage of total sample mass.

Biochemical Methane Potential Assay

The biochemical methane potential (BMP) defines the anaerobic biodegradability of a given material (Owen et al., 1979). Specifically, the BMP test gives the total volume of methane a given substrate (in this case the manure) is able to produce. For this study, the BMP allowed for comparisons of methane production potential between different diet formulations, shedding light on how diet composition affects the gas phase of foaming

systems. Samples with higher BMP indicate a greater ability for microbes to convert the specific substrate into biogas, which is the driving force of foaming systems.

The procedure for assessing the BMP of swine manure samples collected for this study was to add 20 to 25 grams of a sample to a 250 mL serum bottle (Wheaton Science Products No.:223950), with the exact mass recorded. This mass of sample was selected based on an estimated 300 mL of CH_4 produced per gram of volatile solids added, as suggested by others (Hashimoto, 1984; Burton and Turner, 2003; Vedreene et al., 2008), who suggested a range of 244 to 480 mL CH_4 per g volatile solids in swine manures. Next, 50 mL of inoculum was added from an active anaerobic digester maintained in the Agricultural Waste Management Laboratory (AWML) at Iowa State University. This volume of inoculum was added to achieve an approximate 2:1 mass ratio of volatile solids from the manure to inoculum, with the actual ratio varying slightly due to the volatile solids content of the manure samples. Finally, the solution was diluted to approximately 150 mL with a nutrient medium as per Moody et al. (2011) and sealed with a sleeve stopper septa (Sigma-Aldrich Z564729).

Once the sample was prepared, it was incubated at 35°C while being constantly agitated. The samples were regularly checked for biogas production by inserting the needle of a gas-tight syringe (Micro-Mate interchangeable hypodermic Syringe 50mL Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) through the septa. When biogas was collected, it was injected into a non-dispersive infrared methane analyzer (NDIR-CH_4 Gasanalyzer University Kiel, Germany) to obtain the percent of methane present in the gas sample. Results were evaluated based on methane produced per gram of whole sample as well as methane production per gram of volatile solid added (Moody et al., 2011).

Methane Production Rate Assay

The goal of the methane production rate (MPR) assay was to provide a short term methane production measurement with a relatively simple procedure. The test indicates the rate at which endogenous bacteria produce methane, which gives a different perspective of the gas phase of foaming systems. Van Weelden et al. (2013) were able to show that manure from foaming deep pits showed significantly higher methane production rates than those from non-foaming barns (0.148±0.004 and 0.049±0.003 L CH_4/L manure per day,

respectively), making it a valuable test to include in this study. While the methane production rate test is similar to the BMP assay, it is unique in a number of ways. First, the test is conducted over a much shorter incubation time (approximately 3 to 7 days compared to over 40 days for the BMP assay) to ensure that the sample does not approach substrate limiting conditions. Also, the manure sample used for the MPR assay was not inoculated or diluted; rather, the ability of bacteria present within the sample to produce biogas and methane was evaluated. Finally, the sample was incubated at room temperature, rather than at 35°C, and kept stationary rather than agitated. Keeping the sample stationary allowed the observer to record the amount of surface accumulation, foam or otherwise, that developed on the sample.

The procedure for the MPR test involved adding approximately 100 mL of well-mixed sample to a 250 mL serum bottle similar to that used for the BMP assay. Upon the sealing of the sample with a sleeve stopper septa, the exact time was recorded along with the mass of sample added to the bottle. Next, the sample was incubated at room temperature (approximately 23°C). An incubation period of approximately seven days was selected based on preliminary trials to achieve measureable quantities of biogas and methane. Once the 7-day incubation period was over, the sample was checked for biogas production with the gas-tight syringe and analyzed for methane content using the NDIR-CH4 Gasanalyzer. During the analysis of the biogas produced, the accumulation of foam or solids on the surface of the sample was observed and recorded. Figure 2 shows a set of samples after seven days of incubation.

Figure 2. A set of samples after seven days of incubation for the methane production rate assay.

The rate of biogas production and the rate of methane production were calculated using Equations 1 and 2.

$$\mathrm{BPR}\left(\frac{\mathrm{L}}{\mathrm{L}*\mathrm{d}}\right) = \frac{\text{Biogas Produced (mL)}\times\rho_{\text{manure}}\left(\frac{\mathrm{g}}{\mathrm{mL}}\right)}{\text{Mass of sample (g)}\times\text{incubation period(min)}} \times \frac{1440\ \mathrm{min}}{\mathrm{d}} \quad [1]$$

$$\mathrm{MPR}\left(\frac{\mathrm{L}}{\mathrm{L}*\mathrm{d}}\right) = \frac{(\text{Methane }\%\frac{1}{100})(\text{Biogas Produced (mL)}+\mathrm{V}_{\text{headspace}})\times\rho_{\text{manure}}\left(\frac{\mathrm{g}}{\mathrm{mL}}\right)}{\text{Mass of sample (g)}\times\text{incubation period(min)}} \times \frac{1440\ \mathrm{min}}{\mathrm{d}} \quad [2]$$

Surface Tension

Surface tension is an important parameter which quantifies the amount of surface active agents present in solution. With respect to foaming systems, solutions with sufficient concentrations of surface active agents effectively lower the surface tension by increasing surface activity, allowing foam production (Ganidi et al. 2009). The surface tension of samples was tested using a CSC Precision Ring Tensiometer (CSC Scientific Company, Inc., Fairfax, VA). Samples were brought to room temperature and agitated gently before pouring into the sample tray. Next, the duNouy ring was placed below the surface of the liquid, and the ring was slowly pulled upward through the surface of the liquid until it overcame the surface tension of the sample. The force needed the break the liquid interface was recorded directly from the circular scale of the instrument as dyne/cm (equivalent to mN/m), and reported in N/m.

Foaming Capacity and Stability Testing

The foaming capacity and stability apparatus used in this study, as well as the parameters used to evaluate the foaming characteristics of swine manure, were adapted from a number of other studies (Ross et al., 1992; Bindal et al., 2002; Bamforth, 2004; Hutzler, 2011). These bench-top experiments gave parameters that were closely related to the foaming properties of manures, serving as additional points of comparison for the correlation analysis. Air was passed through an in-line gas regulator (Restek Model 21666) into a 5.1-cm diameter clear PVC column, and the flow rate of air through the column was measured and controlled with a variable area flow meter (Dwyer RMA-SSV). A sample volume of approximately 300 mL was poured into the column and the initial level was recorded. The sample was then aerated through a cylindrical air stone at 0.0033 L/s until a steady state height of foam was reached or the foam layer reached the maximum height of the column (approximately 33 cm above the liquid level). The time of aeration was recorded along with the height of foam

produced and the level of the foam-liquid interface. A foaming capacity index was calculated as the height of foam produced divided by the initial manure level and multiplied by 100.

The foam stability measurement occurred immediately after the foaming capacity was determined. Once aeration ceased, the height of foam became the initial level recorded at time zero. Once this level was established, the descending height of the foam was recorded at expanding time intervals. Simultaneously, the ascending level of the foam-liquid interface was recorded at the same time intervals. The descending height of foam was normalized to percent of initial foam height and plotted as a function of time. A first-order exponential decay model fit the data well in most cases, and was used to estimate the half-life of the foam from the time constant as shown in Equation 3.

$$t_{\frac{1}{2}}(\text{min}) = \frac{\ln(2)}{\text{decay coefficient k}} \quad [3]$$

Statistical Analyses

Statistical analysis was performed in JMP Pro 10 (JMP Pro, Version 10. SAS Institute Inc., Cary, NC, 1989-2012) using the Standard Least Squares procedure, with differences tested at $\alpha = 0.05$. Data were analyzed as a randomized complete block design with the individual manure storage tank as the experimental unit. In this analysis, diet was considered a fixed effect with trial as a blocking variable that accounted for differences based on when the trial was conducted. In each experiment there were eight replicates per diet.

RESULTS AND DISCUSSION

In general, the total and volatile solids contents of the manure samples were strongly correlated ($r = 0.991$) with volatile solids content increasing by approximately 0.732 g for every 1.0 g increase in total solids content. Statistical analysis of the total solids concentrations of the protein study samples indicated that the effect of diet was highly significant ($p < 0.0001$). Results showed that protein level had a significant impact on total solids content, with lower protein contents leading to lower solids contents (Figure 3a). Also, with the exception of the corn-canola meal diet, the total solids trend reflects the fiber level of the various diets. Protein source also impacted total solids content, as corn with amino acids resulted in significantly lower solids content ($\alpha = 0.05$), while corn gluten meal had significantly higher total solids content ($\alpha = 0.05$). Diet also had a significant impact ($p <$

0.01) on volatile solids content in the manure (Figure 3a). Again, the results indicated that protein level impacted volatile solids content as lower protein levels in the feed led to lower volatile solids content, with corn-corn gluten meal again resulting in higher volatile solids content than the other protein sources.

Statistical analysis of total and volatile solids concentrations in the carbohydrate diet also indicated that the effect of diet was highly significant ($p < 0.01$). Treatment effects of diet on both total and volatile solids are shown in Figure 3 (b). With respect to the diet types, the wheat bran and distillers grains diets had the highest total solids content, while the corn-soybean meal and barley diets yielded the lowest total solids content. This appears to be related to the fiber content and digestibility of the carbohydrate source (NRC, 2012). In general, the total and volatile solids contents in the carbohydrate study were substantially higher than in the protein study. However, the solids concentrations measured in both trials were lower than those found during field sampling of deep pits, which averaged around 8.3% total solids and 6.4% volatile solids.

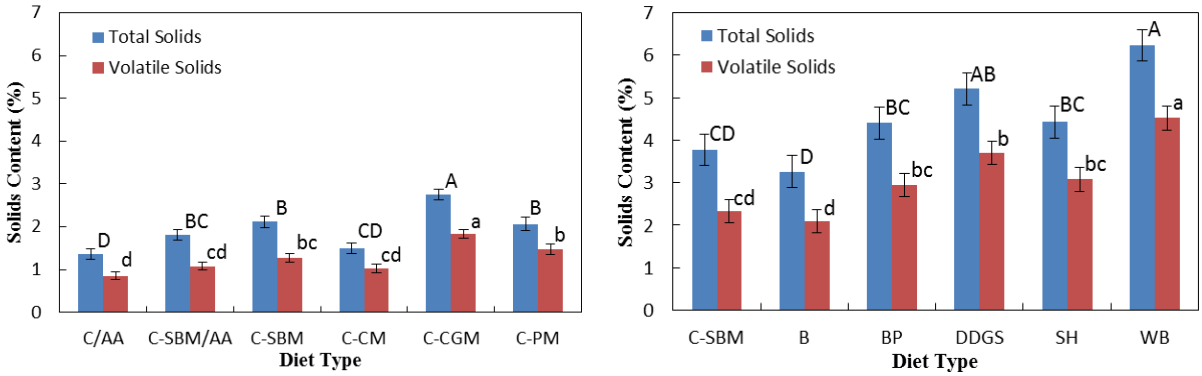

Figure 3. Average total and volatile solids concentrations for different (a) protein sources (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate sources (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent the standard error of the mean. Capital letters indicate differences (α=0.05) among total solids concentrations of the diets listed and lower case letters represent differences among volatile solids concentrations.

The average methane production rates are shown in Figure 4 (a) and (b) for the protein and carbohydrate diets, respectively. On average, the samples from the protein study had lower methane production rates than those from the carbohydrate study, which is likely related to the lower solids concentration in the protein study. In the protein study, the impact

of diet on methane production rate was not significant. However, when methane production is normalized to the mass of volatile solids to account for differences in solids concentrations, the results were different. In this case, the impact of diet was significant ($p < 0.05$) in the protein study, with the corn-soybean meal with amino acids diet having greater rates of methane production than the corn-soybean meal diet, the corn-corn gluten meal, or the corn-canola meal diet (Figure 5a). For the carbohydrate diet study, no differences were found for methane production rate on a per unit volume basis ($p = 009$) or when normalized per gram of volatile solids ($p = 0.4057$).

In general, the values for MPR found in this study were lower than those reported by Van Weelden et al. (2013) for field samples from both foaming (0.148±0.004 L CH_4/L manure per day) and non-foaming (0.049±0.003 L CH_4/L manure per day) deep pits. However, the volatile solids content of the diet study samples was also lower than manure obtained in deep pits at swine production facilities. When methane production rate was normalized to volatile solids in the manure, the field samples had methane production rates of 3.12 ± 0.11 and 1.05 ± 0.078 L CH_4/kg VS per day for foaming and non-foaming pits, respectively. In the dietary studies, methane production ranged from 0.59 ± 0.18 to 1.34 ± 0.18 L CH_4/kg VS per day, which puts the methane production rate in a similar range as reported for non-foaming manure pits in the field.

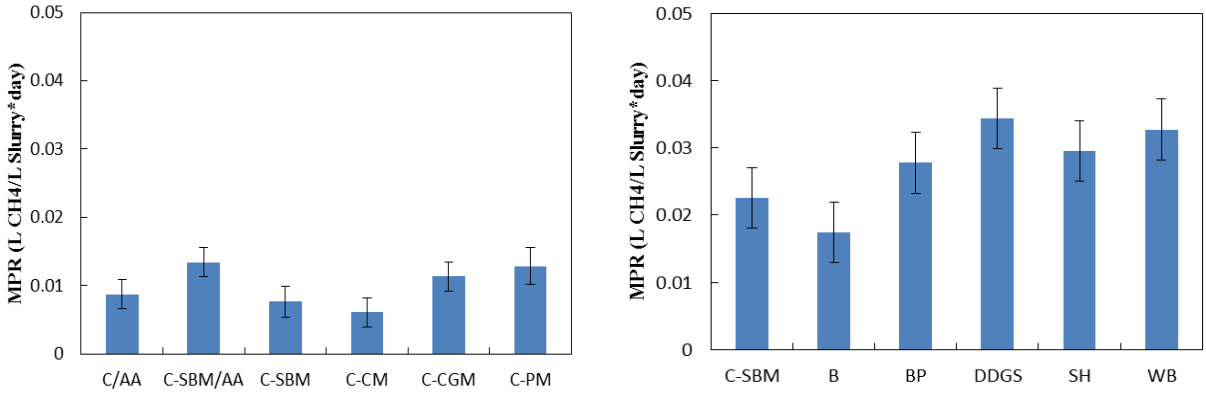

Figure 4. Average methane production rates per volume sample for different (a) protein sources (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate sources (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent the standard error of the mean.

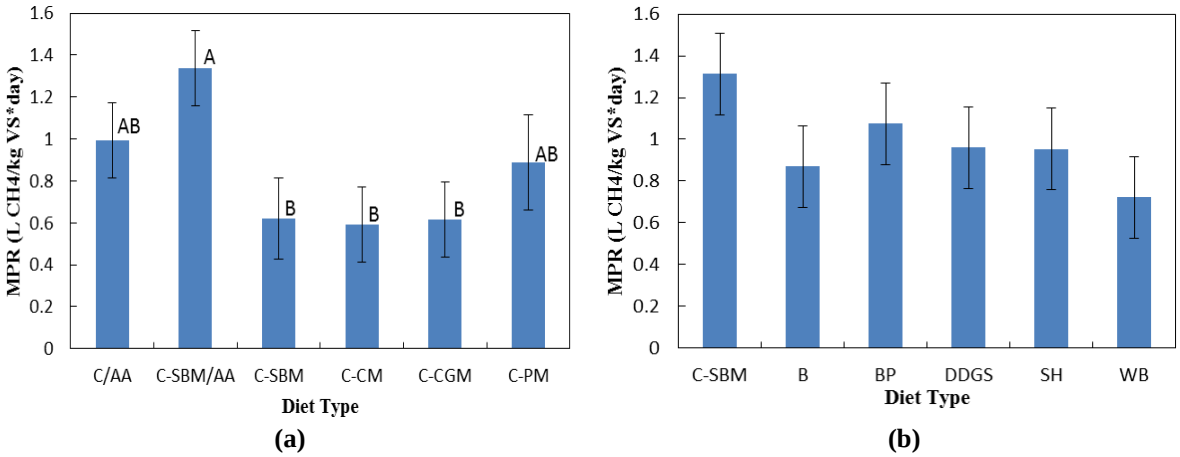

Figure 5. Average methane production rates per mass VS for different (a) protein sources (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate sources (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent the standard error of the mean. In (a), graph bars not connected by the same letter are significantly different at $\alpha = 0.05$.

The results of the BMP test give a difference measure of the gas phase of anaerobic systems in comparison to the MPR. Average values of BMP with the standard error of the mean are shown in Figure 6. The protein study did not yield any significant differences. On average the samples had a BMP of 313 ± 22 mL CH_4/g VS. The BMP results in the carbohydrate trial were highly significantly different ($p < 0.0001$). The control and corn-soybean meal diets had the highest BMP values (395 ± 23 mL CH_4/g VS), while the beet pulp (155 ± 23 mL CH_4/g VS) and wheat bran (181 ± 31 mL CH_4/g VS) diets had the lowest. Also, the barley, DDGS, and soy hull diets showed relatively high values for methane potential in comparison to the lowest values for the carbohydrate studies. In general, the BMP magnitudes reported in these studies were substantially higher than magnitudes reported for samples collected from deep pits. Van Weelden et al. (2013) reported an average value of 121 ± 86 mL CH_4/g VS for both foaming and non-foaming samples, with foaming samples showing a slightly lower average value. BMP results were comparable to others who showed fresh swine manure range between 244-480 mL CH_4 per g volatile solids (Burton and Turner, 2003; Møller et al., 2004; and King et al., 2011).

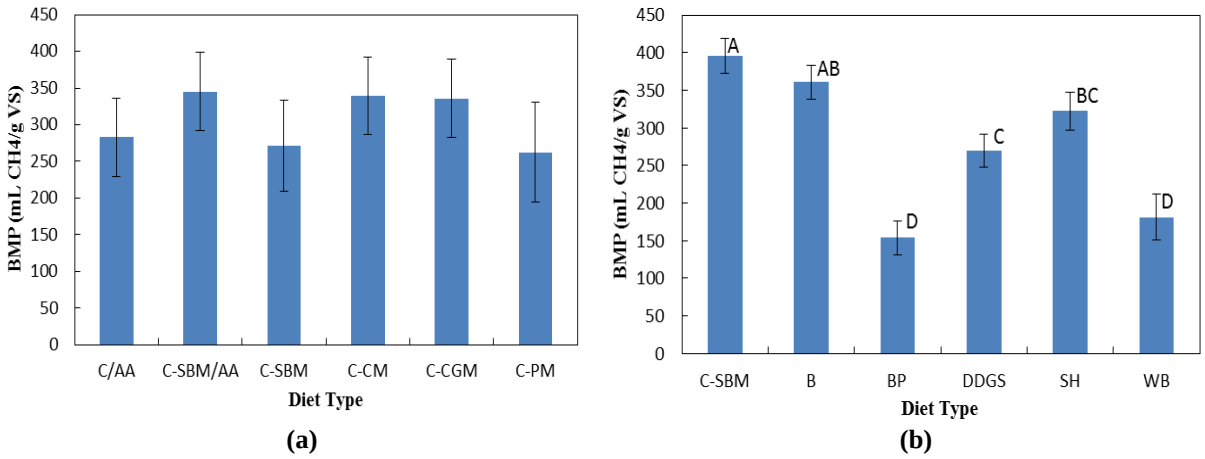

Figure 6. Average BMP per mass volatiles solids for different (a) protein diets (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate diets (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran. Error bars represent the standard error of the mean. In (b), graph bars not connected by the same letter are significantly different at $\alpha = 0.05$.

Field sampling results have indicated that increased rates of methane production are related to foam accumulation, so a diet that has both an increased MPR and an increased BMP may be indicative of a diet that has more potential to result in foaming. In this regard no single diet stood out; however, corn-soybean meal, distillers dried grains with solubles, and soy hulls tended to have high MPR combined with high BMP.

As discussed previously, biogas production (the gas phase) serves as the driving force of anaerobic foaming systems. However, a chemical means to encapsulate the bubbles produced is necessary to create a foaming system. In this regard, surface active agents increase activity at the liquid surface and lower the surface tension, which allows for bubbles to accumulate beyond this interface (Ganidi et al. 2009). This makes surface tension an important parameter in characterizing foaming systems, as it provides an indication of the level of surface active agents present in a given sample without having to identify the specific surfactant. The results of the surface tension analysis for both the protein and carbohydrate studies are summarized in Figure 8. For comparison, the surface tension of water at room temperature is approximately 0.073 N/m (Mills et al., 1988), the surface tension of human urine is 0.059 N/m (Mills et al., 1988), and the surface tensions of 1%, 5%, 10%, and 40% acetic acid-water solutions at 30°C are 0.068, 0.060, 0.055, and 0.041 N/m (Lang and Dean, 1967) respectively. Differences in surface tension were noted between diets

in both the protein and carbohydrate study ($p = 0.032$ and $p = 0.001$, respectively) as shown in Figure 8. In the protein diet study, the corn-canola meal and corn-poultry meal diets showed significantly ($p < 0.05$) lower surface tension values than the other diets. In the carbohydrate study all values were relatively similar, though manure from pigs fed the soy hull diet tended to have higher surface tension than other diets, with the wheat bran diet having the lowest surface tension. Field sampling results from Van Weelden et al. (2013) tended to indicate that manures from foaming barns had significantly ($p < 0.05$) higher surface tensions (0.0515 ± 0.0008 N/m) than manures from non-foaming barns (0.0495 ± 0.0008 N/m). In the protein study, three diets (corn with amino acids, corn-soybean meal with amino acids, and corn-corn gluten meal) had surface tensions greater those of foaming manures, one diet (corn-soybean meal control diet) had a surface tension similar to non-foaming manures, and two diets (corn-canola meal and corn-poultry meal) had surface tensions lower than the non-foaming manure. In the carbohydrate study, most diets had surface tensions similar to or lower than those found in non-foaming manures; however, the soy hull diet had a surface tension very similar to that of foaming manures. This could indicate that the use of soy hulls as a dietary fiber source may lead to a surface tension that is optimal for bubble formation.

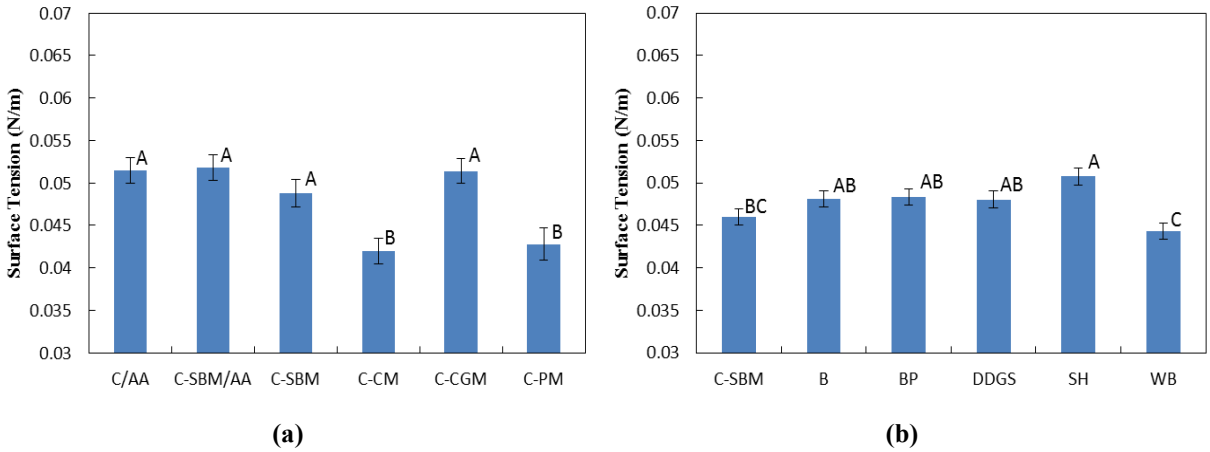

Figure 8. Average surface tension for swine feed diets of different (a) protein sources (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrates sources (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent the standard error of the mean. Capital letters indicate significant differences at $\alpha = 0.05$.

The results of the surface tension analysis are most meaningful for this study when viewed next to the results of the bench-top foaming experiments, which are shown in Figures 9 and 10 for both the protein and carbohydrate trials. There were significant differences ($p = 0.015$) in foaming capacity in the protein study where the results indicate that the corn-poultry meal diet had a significantly greater foaming capacity than the corn with amino acids, the corn-canola meal, or the corn-soybean meal diets, which all showed minimal capacity to foam. This trend, however, did not consistently match trends shown by the surface tension results for the protein study. Foaming capacity results from the carbohydrate study indicated that diet again had a significant ($p = 0.03$) impact on the foaming capacity of the manure. The beat pulp diet had the highest average foaming capacity index, which was significantly higher ($p < 0.05$) than the wheat bran and DDGS diets. In this case the beat pulp diet had a foaming capacity similar to those seen from forming manures analyzed by Van Weelden et al. (2013). Manures from the barley and soy hulls diets had foaming capacities that exceeded the foaming capacity of non-foaming manures. Manures from the other diets had lower foaming capacities than even the non-foaming manures.

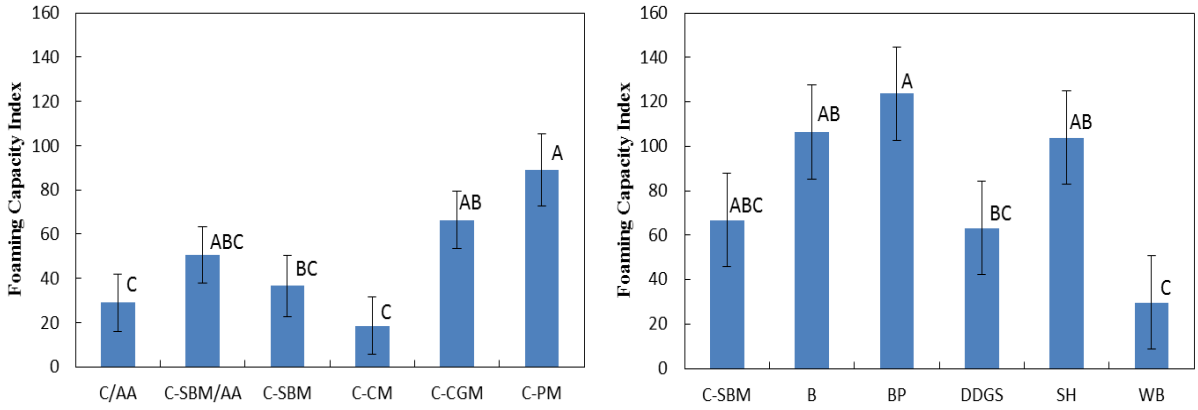

Figure 9. Average foaming capacity for different (a) protein sources (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate sources (C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent one standard error of the mean.

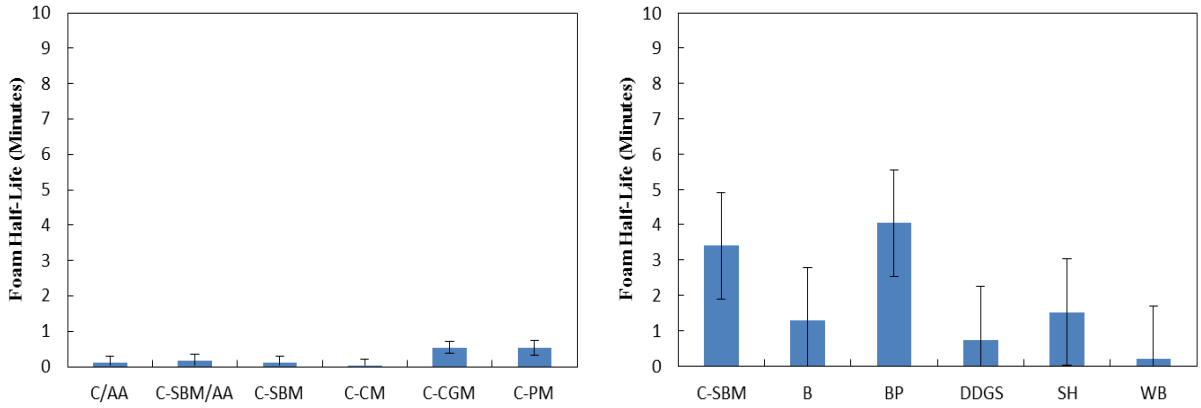

Figure 10. Average half-life of foam for different (a) protein diets (C/AA=Corn with Amino Acids, C-SBM/AA=Corn-Soybean Meal with Amino Acids, C-SBM=Corn-Soybean Meal, C-CM=Corn-Canola Meal, C-CGM=Corn-Corn Gluten Meal, and C-PM=Corn-Poultry Meal) and (b) carbohydrate diets(C-SBM=Corn-Soybean Meal, B=Barley, BP=Beat Pulp, DDGS=Distillers Dried Grains with Solubles, SH=Soy Hulls, and WB=Wheat Bran). Error bars represent one standard error of the mean.

The corresponding stability of foams is shown in Figure 10. The protein study showed no significant differences in foam half-life ($p = 0.208$), with all half-lives being very short, indicating that no mechanism was present to stabilize the bubbles, despite some capacity to foam. Similarly, samples from the carbohydrate study showed no significant differences in foam half-life, although some of the samples exhibited substantially longer foam half-lives than samples from the protein study. There was great variability among values, and in all cases foam stability was significantly lower than those found in foaming samples collected from commercial deep pits.

CONCLUSIONS

The trials conducted in this study sought to better elucidate the impact of diet composition on both the physical and biochemical characteristics of swine manures. The two studies employed various protein and carbohydrate sources in swine diets and evaluated key manure characteristics thought to be related to the formation of foam in deep pit storages. Within each study, no single diet stood out as one that exhibited enhanced methane production, physical characteristics similar to foaming manures, or enhanced foaming capacity and stability. In terms of gas production, both soy hulls and distillers dried grains with solubles tended to have both higher rates of methane production and an enhanced potential for methane production. As foaming manures tend to have higher methane production rates, this could indicate a greater risk of foaming when these dietary ingredients

are included. Similarly, soy hulls also tended to cause manure surface tension values similar to what was found in manure obtained from foaming pits. Finally, manures from the beat pulp and soy hull diets showed a relatively high foaming capacity and foam stability; however, in no cases did the generated foams exhibit notable stability, making the cause for foam stability in deep pits unclear.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

In this section, the results of the field study (Chapter 3) and the diet study (Chapter 4) are summarized in the context of the three-phase foam framework discussed in the introduction of this paper. This includes discussion of the general environmental trends of the deep pits sampled in the field (i.e. temperature and pH), the trends in solids content of samples and the solids distribution in deep pits, the concentration of important chemical constituents of the deep pits (i.e. short-chain and long-chain fatty acids), and the biogas production potential of different swine manures tested. In addition, discussion on the relationship between these isolated aspects of foaming systems will be included in order to formulate hypotheses on the mechanism of stable foam formation in deep pits.

Environmental Conditions

The average temperature of the deep pits sampled in the field study followed an intuitive trend throughout the 13-month sampling period, which is shown in Figure 3 of Chapter 3. The seasonal pattern showed that pits reached the highest temperatures in the late summer and early fall, with temperatures as high as 22.1°C recorded in September. The lowest pit temperatures occurred in the winter months, with temperatures as low as 10.9°C measured in the month of February. The broad range of temperatures in the pit has significant implications for the level of microbial activity throughout the year, which was reflected in temporal gas production results. In addition, significant differences were detected between the temperature of foaming pits and non-foaming pits during the winter and spring, with foaming pits maintaining higher temperatures during these months. These results may suggest that the presence of a stable foam layer may serve as an insulator of heat during the colder parts of the year. A similar insulating effect was noticed when crusts formed on the surface of manure storage tanks during the dietary trials. These trends may have implications for the development of the microbial community of foaming deep pits when compared to non-foaming pits.

Significantly different trends in pH were also identified when comparing manure from foaming and non-foaming barns (Figure 4 of Chapter 3). Overall, foaming barns had more basic manure at every sampling depth. Also, the actual foam layer showed a significantly higher pH (7.74 on average) than the rest of the sampling depths of foaming manure pits. These differences may be a result of ammonia capture in the foam layer; though, the overall range of average pH values measured by depth (approximately 7.51 to 7.74) did not vary greatly from neutral pH.

Solid Phase

The results of the dietary studies illustrated the link between diet composition and the solids content of manure (Figure 3 of Chapter 4). The effect of diet was highly significant with respect to both total and volatile solids content of manure samples. Diets components such as wheat bran and DDGS yielded solids rich manure when compared to soybean meal, barley, and many of the protein-based diets. These trends appeared to reflect the fiber content and the digestibility of the various diets.

The distribution of solids within deep pits reflected a relatively intuitive trend. The highest concentration of solids on average occurred at the bottom of pits, with decreasing concentrations when approaching the surface. However, the foam layer itself was very solid-enriched, with an average concentration of solids that was greater than the averages at all of the other depths of the pit. This trend may reflect the transport of fine, hydrophobic solids to the surface of foaming deep pits, which would be enhanced by the greater biogas flux through the volume of foaming manure pits.

The hypothesis regarding the role of hydrophobic solids as stabilizing agents in foaming systems is also supported by the results of the stability measurements of the foam layer, shown below in Figure 1. Samples of foam did not show a high capacity to foam in bench-top tests when compared to manure taken from other depths (Figure 11 of Chapter 3); however, foam samples showed a substantially greater ability to stabilize in the foaming apparatus used by this group. The specific identity of the stabilizing agent of interest was very difficult to identify within the scope of this study. Existing theories described in Chapter 2 involve filamentous bacteria or colloidal particles that exist in waste systems, which are both viable options in a deep pit environment.

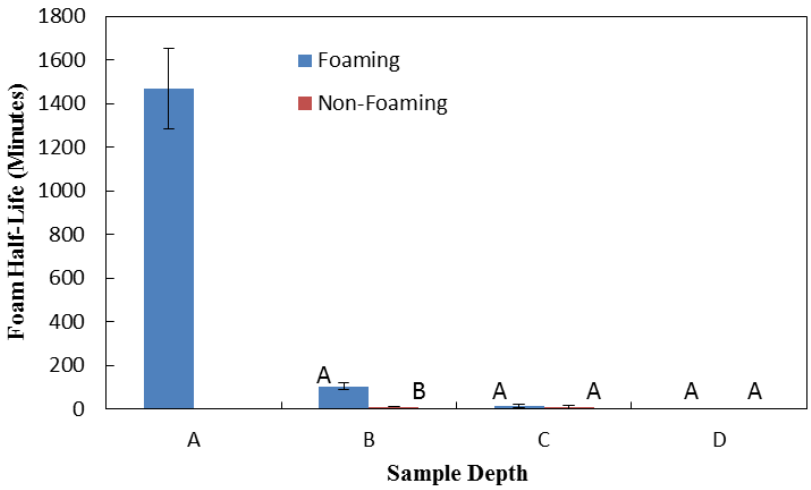

Figure 1. Average foam half-life of foaming and non-foaming samples by sampling depth. Error bars show the standard error of the mean. Letters show statistical differences between surface statuses within each stratum tested at α=0.05.

Liquid Phase

Both short-chain and long-chain fatty acid concentration measurements were taken from a number of samples from the field study. Initially, SCFAs (with acetic acid being a primary component) were hypothesized to accumulate in foaming barns while acting as a primary surfactant. The results, however, showed that SCFA concentrations were significantly higher in barns without any foam accumulation when compared to foaming barns (Figure 5 of Chapter 3). The absence of SCFAs in samples with foam accumulation was explained by their role as a key substrate in the methanogenesis process; that is, SCFAs were being more rapidly consumed by the microbial community in deep pits with foam accumulation. This concept gives a fuller picture of the performance of foaming deep pits as digesters, which will be discussed in more detail later in this chapter.

The results of the long-chain fatty acid analysis showed that in foaming barns, LCFAs were suspended in the foam layer at a higher concentration than in the corresponding interface layer. However, the concentration of LCFAs at the surface of non-foaming barns was not significantly different than those in the foam layer or interface layer of foaming barns (Figure 14 of Chapter 3). These results suggest that LCFAs are not accumulating in foaming systems in terms of overall concentration, while they may be stabilizing the foam matrix to some extent. In this way, more investigation into the role of LCFAs as surface active agents and potential foam stabilizers is needed.

Surface tension measurements were taken as an indicator of the general presence of surfactants in the liquid phase of manure samples. The results showed that the average surface tensions of all manure samples were significantly lower than the surface tension of pure water. In the field study, the initial hypothesis of our group was again proven wrong as the surface tension measurements of non-foaming manure samples as a whole were lower than those of foaming manure samples (Figure 10 of Chapter 3). For both foaming and non-foaming manures, the surface tension of the samples taken from the interface layer showed the lowest surface tension, which is intuitive as surfactants naturally accumulate at the surface of the liquid phase. These results appear to be significantly influenced by the SCFA concentration, which would explain the counterintuitive results and confirm the importance of SCFAs as local surfactants. The surface tension results may also suggest that there is an optimal range of values in which foaming is most likely to occur, with non-foaming barns showing a greater amount of surface activity which may actually prohibit the formation of foam. The impact of diet on the surface tension of manure was also illustrated in Figure 8 of Chapter 4. Various diet types yielded manures with surface tension values comparable to those found in field study samples, with the manure from the soy hull diet showing the surface tension value most similar to that of foaming manure.

The foaming capacity and stability measurements also served as viable indicators of the presence of surface active agents and foam stabilizers in the manure samples, proving to be a consistent reflection of the differences in surface accumulation of the deep pits in the field. Both the foaming capacity and stability measurements of foaming barns were consistently higher than non-foaming barns throughout the 13-month sampling period (Figures 12 and 13 of Chapter 3), though greater differences were shown in the stability results. The analysis of foaming manures collected from various depths also indicated a greater accumulation of surface active agents at the surface of foaming barns when compared to non-foaming barns (Figure 11 or Chapter 3). The foaming capacity and stability results of the dietary trials were shown in Figures 9 and 10 of Chapter 4. In this case, both the capacity and stability of manures collected from dietary trials were lower than those of field samples. This difference may be explained by the less-established microbial communities within the manure holding tanks during the diet studies, as manure was not aged to a similar extent as samples collected from deep pits. In this way, the ability of substances produced as a result of

microbial activity, or even the ability for microbial cells themselves to act as foam stabilizers, was diminished. The dietary trial samples also showed significant differences in foaming characteristics, as manure from beat pulp and soy hull diets showed a relatively high foaming capacity and stability when compared to manures from other diets. These differences suggest that dietary components may play a significant role in the ability of manures to foam, either by direct dietary impact or by the components' ability to be metabolized by the microbial community in the deep pit.

Gas Phase

The gas phase of the manures studied in Chapters 3 and 4 provided the clearest differences in the behavior of foaming pits and non-foaming pits. There were significant differences in the rate of methane production between manure from foaming and non-foaming barns (Figure 8 of Chapter 3). At the same time, the biogas production potential of non-foaming samples was significantly higher than that of foaming samples (Figure 7 of Chapter 3). Along with the significant depletion of critical substrate in foaming barns (i.e. SCFAs such as acetic acid), we see that foaming manure systems performed as more efficient digesters than non-foaming systems. In addition, we found that the methane flux through the volume of deep pits varied greatly in accord with seasonal temperature trends, while also continuing to reflect the differences between foaming and non-foaming barns (Figure 9 of Chapter 3). These differences in gas production trends could be explained in a couple of ways: a more developed population of methanogens in foaming barns, significant differences in the substrate availability in foaming barns, or the presence of some inhibiting agent in non-foaming deep pits (or some combination of these factors). These explanations will be considered further in the conclusions to follow.

No single sample of manure from the dietary trials showed the ability to produce methane at a comparable rate as samples collected from the field study, while there were some differences based on diet type (Figure 8 of Chapter 4). These differences are likely explained by the levels of natural detergent fiber and the lignin contents of the diets, which affect the availability of consumable substrate in the manures. The effect of underdeveloped microbial consortia in the manure holding tanks of the dietary studies were also reflected in

the gas production results, especially in light of the amount of biogas production potential of these samples when compared to aged manure samples in deep pits (Figure 6 of Chapter 4).

CONCLUSIONS

When knowledge of the independent aspects of three-phase foaming systems is integrated, we are able to make educated hypotheses about the overall mechanism of foam formation in swine manure deep pits. As discussed in the previous section, the most notable characteristic of foaming deep pits is their enhanced efficiency as digesters, consuming substrate such as SCFAs more rapidly than non-foaming deep pits and increasing the presence of the gas phase throughout the volume of the system. Explanations for the relatively large amount of microbial activity in foaming pits may include the following: a more developed and active microbial community in foaming pits, a significantly greater amount of consumable substrate in foaming pits, or the existence of an inhibiting agent or environment in non-foaming barns that disrupts the methanogenesis process.

Existing literature allows for a comparison of known values of methane emissions from deep pits to the methane production rates of the samples from the field study, in which foaming barns produced an average of 0.148 ± 0.004 L CH_4 L slurry^{-1} day^{-1} and non-foaming barns produced 0.049 ± 0.003 m^3 L CH_4 L slurry^{-1} day^{-1} (average ± standard error of the mean). Husted (1994) reported a range of 0.0006 to 0.052 L CH_4 L slurry^{-1} day^{-1} for stored swine manure slurry, with an estimated annual emission rate of 0.017 L CH_4 L slurry^{-1} day^{-1}. Martinez et al. (2003) reported a slightly higher range of 0.013 to 0.115 L CH_4 L slurry^{-1} day^{-1} for average daily methane emissions from raw pig slurries, and Loyon et al. (2007) reported a value of 0.075 L CH_4 L slurry^{-1} day^{-1} from stored raw slurry. The methane production rates of non-foaming manure are similar to the values reported above, while the methane production rates of foaming manure exceed all of the reported values. This comparison suggests that the disparity in gas production rate between foaming and non-foaming barns is not the result of inhibited methane production in non-foaming barns; rather, methane production in foaming barns is notably higher than typical swine manure storages.

The presence of significantly more consumable substrate in foaming barns also does not seem likely in light of the evidence provided above, especially with the differences in both SCFA concentration and biochemical methane potential between non-foaming and

foaming samples in favor of the former. Also, both foaming and non-foaming samples collected from deep pits maintained similar solids profiles and volatile solids concentrations throughout the field study. While differences in diet composition were shown to affect the biogas production characteristics of swine manure, field observations showed a wide range of gas production values in barns with the same integrator and feed source. There were also multiple observations of both foaming and non-foaming barns occurring at the same site, suggesting that similar feed characteristics and management styles can produce deep pits with very different characteristics. Higher temperatures in foaming barns during the winter and spring months may increase the hydrolysis of consumable substrate, but this effect would only compound the gas production in foaming barns rather than cause it in non-foaming barns.

For these reasons, the development of a more active microbial community seems to be the most plausible explanation of the enhanced methane production rate of foaming manure. This explanation also suggests a corresponding increase in biosurfactant levels. Heard et al. (2008) illustrated the positive relationship between microbial growth and biosurfactant production in their research of foaming systems. Di Bella et al. (2010) also endorsed the viability of biosurfactants as the primary surface active agents of anaerobic foaming systems. While it is difficult to identify the existence of biosurfactants in waste systems with laboratory techniques, the physical descriptions of extracellular polymeric substances discussed in Chapter 2 are similar to the consistency of the foam itself, suggesting that these biosurfactants may play an important role as surfactants and/or foam stabilizers in deep pit swine manure storages.

The enhanced biogas flux of foaming deep pits also has an important impact on the distribution of the solid and liquid phases of these manure storages, acting as a means to transport hydrophobic substances to the surface. Results of the foaming capacity test indicated a relative accumulation of surfactants at the surface of foaming barns when compared to non-foaming barns. The amplified transport of hydrophobic material was also supported by the unique physical and chemical qualities of the foam layer. It was solids-enriched, contained a greater concentration of long-chain fatty acids, and maintained a more basic pH than other depths of the pit. While the most important surface active agents and foam stabilizing substances were not specifically identified in these studies, we know that

their accumulation at the surface is compounded by increasing biogas flux through the deep pit.

Foam Mitigation Strategies and Future Research Needs

Based on the knowledge gained on foaming deep pits, the most effective foam mitigation strategy would be to target the microbial community in deep pits, consequently inhibiting methanogenesis, limiting the potential of biosurfactant production, and reducing the transport of surfactants and/or hydrophobic solids to the surface of the manure. There are both direct and indirect means to reduce the production of biogas in deep pits. Direct approaches would target the methanogens specifically, while indirect approaches would seek to reduce the availability of substrate to methanogens by either controlling its input into the system or affecting other microbes in the system.

The introduction of inhibitory substances is a relatively simple, low-cost approach to foam mitigation in deep pits. Concerns include the long-term environmental effects of applying treated manure to croplands, increased ammonia or hydrogen sulfide emissions, added operational costs, and the possible development of microbial resistance to the treatment. The goal of an inhibitory strategy would be to upset the balance between acid-forming and methane-forming microbes, causing an adverse shift in the microbial population or negatively affecting the growth of the microbes (Chen et al., 2008). Common inhibitors include ammonia, sulfate, antibiotics, light metal ions, heavy metals, and a variety of organic compounds including LCFAs. The addition of ammonia or sulfate into swine manure storages (which already contains high concentrations of both) would add to present concerns regarding gas emissions from swine facilities. However, future research regarding the ability for additional ammonia to prohibit methane production in swine manure may be valuable. Direct antibiotic addition to pits has also been considered for use as a pit additive in the form of monensin, an ionophore. As mentioned in Chapter 2, the ability for microbes to develop a resistance to ionophores has been documented. In this way, future research as to the long-term effectiveness of ionophores is required before this solution is recommended. Finally, the acidification of pits by the addition of organic acids may be a viable option worth researching.

The other approach to reducing methanogenic activity is indirect, with the goal of limiting substrate availability to the methanogens. This approach would involve the manipulation of swine diet, which affects the manure input into the deep pit. While preliminary research in this subject area was completed during the dietary trials in Chapter 4, continued research is necessary in order to better understand the substrate availability of manure yielded from different diet compositions, particularly in deep pit simulations with aged manure. A more novel option would be to physically capture solid manure before it fell into the pit, separating the solid and liquid portions of the manure and storing the solids in a separate location.

Other areas of future work that would be valuable in better understanding foaming swine manure and effective mitigation strategies are listed below:

- Identification of the specific surface active agent that has the largest effect on the surface tension of swine manure in deep pits as well as the optimal range of surface tension magnitudes that enable foam formation. Of particular interest are surfactants produced by the microbial consortium in deep pits, termed "biosurfactants." The development of a technique to identify the origin of these substances could be critical for understanding foam production in swine manure storages.
- Identification of the specific foam stabilizing substance, as well as techniques for preventing the accumulation of that substance at the surface of deep pits.
- Research the biological and chemical effects of annual and bi-annual pumping of manure pits, and the implications of pumping frequency on the physicochemical and biological characteristics of deep pits.

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