The Mechanism of Foaming in Deep-Pit Swine Manure Storage

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Mi Yan

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Dedication

I dedicate my dissertation work to my family and many friends. My loving parents, Tieren Yan and Zhiqun Zhou have never left my side. I also dedicate this dissertation to my many friends who have supported me throughout the process. I will always appreciate all they have done.

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Abstract

Pork production is one of the most important agricultural activities in the United States, accounting for about $20 billion sales in 2011. Swine farms in upper Midwestern states of Minnesota, Iowa and Illinois are primarily designed with deep pit storage of manure under the pig barns, and manure is pumped out of these barns one to two times per year for land application. In recent years, it has been observed that a layer of foam would unexpectedly develop on the manure surface that is stored in these pits. This manure foaming has becomes a growing concern in the US swine industry because it traps a significant amount of methane gas, which is explosive under relatively high concentration, causing incidents of swine worker injuries and massive loss of living pigs by barn explosions and flash fires. No specific strategy has been developed to prevent the foaming and some swine producers are adding anti-foaming agents to provide a short term solution to prevent flash fires and explosions.

Since no explanation of this manure foaming has been published, this study hypothesized several theories and then conducted related research. One hypothesis is that filamentous bacteria, which are considered the reason of foaming in municipal wastewater treatment systems, are the cause of this problem. Another hypothesis is that dried distillers grains with solubles (DDGS), an ethanol production by-product that is replacing corn and soybean in pig's diet, are the cause of this problem. To verify these two hypotheses, microbial identification and chemical property analysis were carried out on different manure samples. This study provides experimental results to test these hypotheses, and more importantly provides ideas for the mitigation of the foaming issue.

The research is expected to save labor and costs in the control of manure pit, and provide a safe environment for swine producer working and pigs living in these barns.

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Chapter 1

Introduction

Production of pork is a major agricultural enterprise in the United States, and a majority of the production occurs in the Midwest (Ohio to Nebraska and Minnesota to Missouri) and North Carolina. As an important component in U.S. agriculture, commercial pork product in the U.S. is expected to climb to 24 billion pounds (Johnson R. J., 2013). As a by-product of the pork production, a massive amount of swine manure is generated. In major pork production states such as Iowa, Illinois, and Minnesota, nearly all of the manure is utilized as fertilizer on cropland. However, since manure can only be applied to cropland either in the spring before planting or in the fall after the crop is harvested, manure needs to be stored. In the Midwest, manure storage is almost exclusively accomplished by deep pits beneath production facilities/barns.

Farms in US Midwestern region use anaerobic deep pits for storage of swine manure to conserve the valuable nutrients (especially nitrogen) instead of losing nitrogen in treatment systems, especially in lagoon systems. In this deep pits system, swine manure is collected in a deep pit beneath pigs' living area, and pumped out once or twice a year. Besides the benefits of operation simplicity, manure nutrient can be recycled back to cropland and used directly as fertilizer. One major problem of this manure management system is that pigs and manure exist together in a limited space. During the manure storage, anaerobic digestion is naturally occurring in swine manure to generate methane and carbon dioxide, with low amounts of hydrogen sulfide and ammonia (Appels et al., 2008). Under certain conditions such as during the agitation and removal of the manure

from deep pits, these gases can be exposed to the animal area (Fig. 1.1), resulting in a health risk and safety concerns.

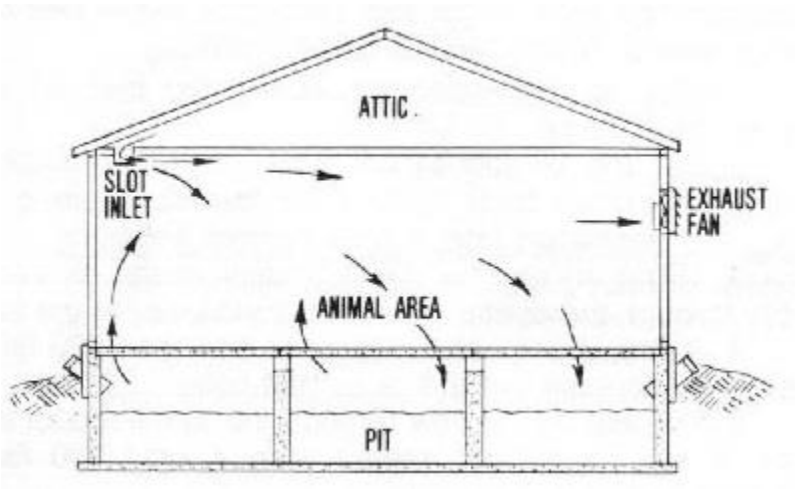

Figure 1.1. The standard structure of a pig barn (Jones et al., 2014).

Explosions in manure storage pits have a long history. Reports were existed since 1969 to noted the occurrence of "several" explosions in manure storage pits above slatted floors (Muehling, A. 1969). In 2003, two successive explosions occurred in a multi-room, mechanically ventilated, finishing pig deep pit facility near Victoriaville, Quebec, Canada. Choiniere published a paper summarizing the incident and the succeeding investigation, claimed the most possible reason as methane concentration reached the explosive level of 5 % and then ignited by barn heater (Choiniere Y. 2004). In 2009, a sharp increase in the incidence of swine barn fire and explosion accidents have been reported in Midwestern swine production facilities, and these fire have been generally considered to be caused by methane gas generated in manure pits (Schmidt and Jacobson, 2010; Burns, 2010). In all

the barns that been investigated, a thick layer of stable foam was present on top of manure. Most of these fires occurred after foam was disrupted by spraying the foam with water or agitation, and methane gas content in these foams were found between 50% and 70%, which make the foam an underlying cause of these barn explosions. Consider the lower explosion limit of methane gas is 4-6% and higher explosion limits is around 15%, the sudden disruption of these foam will easily raise the methane gas concentration in the confined barn space above the lower explosion limit.

Figure 1.2. A swine barn explosion in Emmetsburg, Iowa

Figure 1.3. Consequences of manure foaming

Unfortunately, it is not exactly known how stable foam is generated in manure pits. Foam is generally a dispersion of a gas in a liquid consisting of a large proportion of gas. The liquid phase is located in a thin film which is present between the gas bubbles. Meanwhile, surface-active compound above a certain threshold concentration is required for foam generation, and these surface-active compounds are generally considered as surfactants. Their molecules obtain both hydrophilic and hydrophobic functional groups, lower the surface tension and act as emulsifying or foaming agent. In most of the foaming systems that have been widely studied, there are gas-liquid interfaces at which the adsorbed surfactants are found on the nanometer scale (Fig. 1.4). However, surfactant is not sufficient to generate very stable foam. Without stabilizer these foams will quickly collapse by draining through liquid channels and film rupture. In order to maintain stable over a long period of time, the foam needs to be further stabilized by other components, like carbohydrates and proteins (Pelton R., 2002) or by suspended particles (Ganidi et al., 2009). Similar to the assumption, there are studies claime that formation of stable foam requires three components: (i) gas bubbles surrounded by liquid films, (ii) surfactants which reduce the surface tensions, preventing liquid drainage from gas bubble walls and (iii) stabilizer of small hydrophobic particles responsible for the long-term foam stabilization (Heard et al., 2008; Petrovski et al., 2011). In foaming manure pit, the gas bubbles is naturally provided by the generation of biogas (60% of which is methane), while surfactant and stabilizer is required to trap the gas and make the foam to persist.

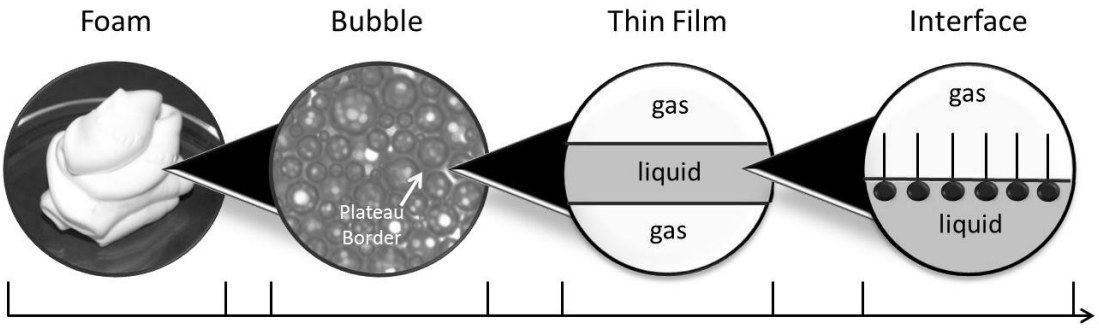

Figure 1.4. Foam structure at different length-scales, starting at the macroscopic and decreasing gradually down to the nanometric (Fameau and Salonen, 2014).

Foaming in deep manure pits is not a new problem, but until recently this issue has not been systematically studied. In contrast, similar foaming situations have been observed in the municipal wastewater treatment processes for a long time. For decades, foaming was believed to be primarily caused by massive growth of certain filamentous bacteria and mycolic acid-containing actinomycete belonging to the family *Nocardiaceae* (Shen et al., 2007; de los Reyes et al., 2002a). Researchers have reported massive growth of filamentous or actinomycete mycolata (Ganidi et al., 2009; Pagilla et al., 1997) in anaerobic digester, and some studies even proposed threshold concentrations of these filamentous bacteria cells in order to initiate the foaming (de los Reyes et al., 2002b). However, a recent opinion proposes the possibility that filamentous bacteria are not the only reason for inducing foaming. A large-scale study showed that the threshold concentration of filamentous bacteria to create foaming is very empirical and, in many cases, it only applicable to mycolata members with more hydrophobic cell wall (Davenport et al., 2008). A survey on full-scale biogas plants in Denmark also shows that no difference in bacterial communities was observed, and filamentous bacteria was not

attributed to be the main cause of foaming (Kougias et al., 2014). With these varied observations, it is reasonable to consider that filamentous bacteria may be a cause of the manure foaming, but there are probably other components that contribute to foaming issue both in wastewater treatment processes and swine manure storage pits.

In additions to the possibility that filamentous bacteria serve as biosurfactants, there are also other compounds suspected as potential surfactant in biogas plants (Moeller et al., 2012). Some of these include volatile fatty acid (VFA), lipids, detergents and proteins. It has been reported that the presence of VFAs in biogas sludge is associated with foam formation (Ross and Ellis, 1992; Westlund et al., 1998), although there is still discussion about whether VFAs are the cause or the consequence of foaming. Lipids in biogas plants are normally consist of oil or grease. Due to their hydrophobicity characteristics, they tend to diffuse to the surface and decrease the surface tension of the top layer of the waste being digested. Detergents normally enter the biogas plant as components of industrial wastewater from breweries, dairies, and paper and textile industries (Ganidi et al., 2009), and can serve as surfactants that will significantly increase foam production. Proteins in biogas plant can be a consequence of both animal excretion and microbial activity, and protein have been found to be an important compound for foam formation in wine and beer production (Goncalves et al., 2002; Blasco et al., 2011). It is unlikely to find a high content of detergent in swine manure, but other components are highly possible to exist in swine manure at certain concentrations. These are all candidates foaming agents that involved in the manure foaming process.

Results from a previous manure sample survey showed that there have been no other compositional changes in swine diet characteristics during recent years in the swine industry, except for the dramatic increase in the use of DDGS to partially replace corn and soybean meal. As a major co-product of ethanol production from corn dry-grind facilities, DDGS contains a high percentage of crude protein (~31%) and crude fat (~11%), and also a significant amount of acid detergent fiber (Kim et al., 2008). Both protein and lipids in DDGS may serve as potential surfactants, so it is doubtful if feeding swine diets containing DDGS is a major reason to enhance manure foaming. DDGS is often added to grower-finisher diets at a rate ranging from 10%-20% to reduce the feeding cost without reducing pig performance. Although there is no evidence currently, but it is worthwhile to study if there is correlation between the addition of DDGS to swine diets and this foaming phenomenon.

Compare to manure pit, industrial reactors such as anaerobic digester or aeration tank are more controlled systems, and relatively easier to develop strategy for foaming mitigation. Current solutions for mitigation of industrial (like a wastewater treatment plant) foaming include application of antifoaming agent, reduction of air supply in activated sludge, pH control, and design selection mechanism to remove specific microorganisms. Unfortunately, many of these methods are hard or impossible to apply in a swine manure pit due to the cost or the difficulty of implementation. Mitigation strategy to limit the foaming in manure pit includes addition of defoamer to break the surface foam, or the application of additives to decrease the generation of biogas. The application of monensin is by far the most effective additive on the short term control of manure

foaming (Clanton C., et al., 2012). In beef animals, monensin alters composition of the microbial population in the rumen of cattle, decreasing the amount of acetic acid and thus decreases the production of methane gas. However, this ionophore needs to be applied every year and the duration of its effects in manure pit is unknown. During the constant accumulation of manure in pits, the addition of monension application process preventive measure need to be applied periodically, and this takes extra labor and could easily be ignored. Moreover, monensin is not environmentally approved to be added to manure that is applied to cropland. Therefore, the causes of manure foaming need to be found, develop a simple and low-cost strategy to reduce this risk of manure foaming.

The main objective of the research in this dissertation is to understand the major reasons and the mechanism for foaming of the manure in a swine barn's deep pit. Since no proven cause of this manure foaming exist, this research hypothesized different theories and then conducted related studies. One hypothesis is that filamentous bacteria, which are considered the reason for foaming in municipal wastewater treatment systems, are the cause of this problem. Another hypothesis is that surfactants in swine manure, possibly protein or lipids, are the major reason for the foaming problem in swine manure stored in deep pits. If surfactants are shown to be the major cause of foaming, then DDGS could be a potential leading cause, since it is partially replacing corn as a feeding material in swine diet and increase the protein and lipid content than corn. The specific objectives of the research consist of the following items: 1) determine the compositional differences between foaming and non-foaming manure samples; 2) determine the role of filamentous bacteria in swine manure foaming process; and 3) determine the role of surfactants in swine manure foaming process. This research was expected to verify the two hypotheses,

understanding the reason for foam generation and aid in the development of specific strategies to mitigate the foaming in deep manure pits.

Chapter 2

Composition Analysis of Manure from Deep-Pit Foaming Swine Farm

Outline

Manure produced in Midwestern U.S. pig finishing facilities is usually stored in concrete deep-pits beneath the building before land application. Manure foaming in deep-pit barns has recently become a problem, causing a safety hazard. Since no obvious cause for this foaming was observed, compositional analysis was conducted to reveal differences between foaming and non-foaming manure samples to determine any specific components that can be correlated to foaming. Result shows that foaming manure samples have higher concentrations of solids, lipids, trace metals and proteins than non-foaming samples, and suggests some potential factors that may contribute in the generation of foam in manure pit.

2.1 Introduction

Pork production is one of the most important agricultural activities in the U.S. Deep-pit wean-to-finish and grow-to-finish barns with totally slatted floors are widely used on farms in the Midwestern states, including Minnesota, Iowa, and Illinois. Manure drops into the deep-pit through the slatted pen floors. Manure is removed from the pit once or twice per year and applied to cropland or pasture as fertilizer. During the storage period, anaerobic degradation occurs naturally and generates methane and carbon dioxide, as well as low amounts of hydrogen sulfide, ammonia, and many other gases (Appels et al., 2008). Under normal conditions, these gases are released into the barn's air space and exhausted by the ventilation system. However, foaming on the manure surface has frequently been observed in recent years, and roughly 25% of farms in Midwestern states have experienced foaming (Akdeniz et al., 2013). This foam is a safety concern because it traps a significant amount of methane gas. On several occasions, this layer of foam has collapsed during barn cleaning (pressure washing) or during the agitation and pumping of the manure, releasing the trapped methane and resulting in barn fires and explosions. Several injuries and even one death have been reported due to this phenomenon (Dehdashti, 2009). In addition, the foam can move up through the slats and threatens the respiratory health of the pigs. Current strategies to reduce this hazard involve limiting the foaming with anti-foaming chemicals or applying additives to decrease the generation of biogas in the manure. Unfortunately, these treatments are only temporary, as the foam may regenerate in a few months (Kryzanowski, 2012).

Formation of foam generally requires three components: (1) gas bubbles surrounded by liquid films, (2) surfactants that reduce the liquid surface tension, preventing drainage from the gas bubble walls, and (3) small hydrophobic particles, which are responsible for long-term foam stabilization (Heard et al., 2008; Petrovski et al., 2011). A surfactant is required to lower the surface tension and thereby facilitate the formation of small gas bubbles, and in some cases surfactants can also serve as stabilizers to maintain the foam. These same factors are likely required to generate foam in manure pits. The gas source for foaming in manure pits is the biogas, which is continuously generated during anaerobic digestion, while the sources of the surfactants and hydrophobic particles in foaming swine manure are still unknown.

In order to understand the foaming in manure pits, the compositions of foaming and non-foaming manure samples were analyzed to determine their differences. It was hypothesized that a compositional analysis would help identify the hydrophobic particles and surfactants involved in the formation of foam in manure pits.

2.2 Material and Methods

Sample collection

Manure samples were collected in fall 2011 from five different swine grow-to-finish farms in Minnesota and Iowa with deep-pit manure storage. The selected sites represented four farms with foaming problems and one farm without foaming at the time of sampling. All five farms fed the animals standard corn/soybean diets, with 10% to 30% dried distiller grains with solubles (DDGS) depending on the growth stage of the

pigs. At each farm, samples were collected from four barns to increase representation, for a total of 20 pits. All of these pits were emptied once per year in the fall. The initial sampling plan was to collect samples from four pit depths from top to bottom representing (1) the foam layer (taken directly from the foam, which ranged from 8 to 30 cm thick), (2) liquid top (top 10 cm layer of liquid manure under the foam), (3) liquid middle (intermediate layer of liquid manure, typically 90 cm from the top of the liquid), and (4) liquid bottom (10 to 20 cm from the pit bottom). The foam layer was not available in the non-foaming barns. The sample size was 500 mL at each site. Due to the limitations and pit depths of each barn, a total of 44 samples were collected for the compositional analysis (Table 2.1). Among the five farms selected for sampling, some manure pits had been emptied prior to sampling, leaving only 30 cm of manure and a foam layer in these pits. Therefore, for these barns, only the foam layer and/or the middle of the liquid manure were sampled. At the second barn of the third foaming farm and at the first barn of the non-foaming farm, the manure pit was relatively shallow; therefore, only the top liquid layer and bottom liquid layer were sampled.

Table 2.1. Sampling site description for the 44 samples (indicated by asterisks)

Layer	Farm 1 in Iowa, Foaming Barns				Farm 2 in Iowa, Foaming Barns				Farm 3 in Minnesota, Foaming Barns				Farm 4 in Minnesota, Foaming Barns				Farm 5 in Minnesota, Non-Foaming Barns			
1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-
2	-	-	-	-	-	-	-	-	*	*	*	*	-	-	-	-	*	*	*	*
3	-	-	*	*	-	-	-	-	*	-	*	*	*	*	*	*	-	*	*	*
4	-	-	-	-	-	-	-	-	*	*	*	*	-	-	-	-	*	*	*	*

Compositional analysis of manure samples

After collection, the manure samples were immediately chilled in a cooler with ice and sent to Midwest Laboratories, Inc., (Omaha, Neb.) to test for pH, moisture, nitrogen, sulfur, ash, trace metals, lipids, protein, and acid detergent fiber. The analysis methods for each parameter were as follows: AOAC 2001.11 (ammonia-nitrogen), AOAC 2001.11 (total nitrogen), AOAC 985.01 (micronutrients), SM 2540G (moisture), EPA 9045C (pH), AOAC 990.03 (crude protein), AOAC 954.02 (acid hydrolysis), ANKOM Technology method (acid detergent fiber), and AOAC 942.05 (ash). The values of organic nitrogen, energies, and of each parameter on a dry matter basis were calculated.

Long-chain fatty acids (LCFAs) were extracted from the manure and measured by gas chromatography. To prepare the GC samples, the manure was freeze-dried and ground into powder. Approximately 0.3 g of dry manure powder was added to 10 mL of chloroform and methanol solvent (chloroform: methanol = 2:1) and shaken at 150 rpm for 16 h. Next, 2.5 mL of water was added to the mixture, vortexed for 1 min, and centrifuged at 7000 rpm for 7 min. The bottom layer (chloroform layer) was then filtered through 0.45um filter to remove solid particles (Folch et al., 1957). LCFAs in the chloroform layer were analyzed with a gas chromatograph (7820A, Agilent Technologies, Santa Clara, Cal.) equipped with a flame ionization detector and a DB-FFAP capillary column using hydrogen as carrier gas at a flow rate of 0.75 mL min-1. Injector temperature was set at 250 ℃, and detector temperature was set at 300 ℃. Oven temperature was initiated at 100 ℃ and held for 5 min, raised to 240 ℃ at a rate of 10 ℃ min-1, and held at 240 ℃ for 20 min. Specific LCFAs in the samples were identified and

quantified by comparing the peak area with standard chemicals (palmitic acid, stearic acid, oleic acid, and linoleic acid).

Statistical analysis

A sample description in terms of means and standard deviations was first determined for an intuitionistic evaluation of the differences between samples for each parameter. To evaluate each tested parameter and its importance in the foaming process, inferential statistical tests and t-tests were applied to determine the significance of differences between samples using SPSS 13.0 (IBM, Armonk, N.Y.). In the inferential statistical tests, homogeneity of the variance was checked first to help choose the correct method. For sample groups that lacked equal population variance, specific analysis methods (Kruskal-Wallis H-tests and independent sample t-tests) were conducted instead of standard analysis of variance (ANOVA). In both analyses, differences between samples were considered significant when $p < 0.05$. This research primarily conducted comparisons on samples taken from different layers and on same-layer samples collected from different farms, with the purpose of identifying possible correlations between foaming status and manure composition. The concentrations of each parameter in different layers were also expected to reveal a correlation between foaming status and chemical distribution. In addition to the analysis described above, ordinal logistic regression was applied using foaming or non-foaming as a response. This analysis used all the test parameters as independent variables and determined whether any parameter could be correlated with

the foaming of manure samples. All these analyses were conducted on two aspects, evaluating the difference in each parameter on a sample basis and on a dry matter basis.

2.3 Results

Description and layer comparison of foaming and non-foaming samples

The 44 samples were categorized into seven groups. Group F1 included samples collected directly from the foam layer, defined as foam layer samples. Groups F2, F3, and F4 were samples collected from the liquid below the foam in foaming barns and represented the top, middle, and bottom layers, respectively. Samples in groups F2, F3, and F4 were defined as foaming liquid samples. Similarly, groups NF2, NF3, and NF4 were samples collected from the three liquid layers in non-foaming barns (top, middle, and bottom, respectively) and were defined as non-foaming liquid samples.

The means and standard deviations of all tested parameters for the seven groups are presented in table 2.2. Compared with the liquid layers, most parameters have higher values in the foam layer, especially total solids, calcium, acid hydrolysis, and total digestible nutrients. For these four parameters, values for the foam layer samples are almost 50% higher than the means for all 44 samples. Because of the 0.5% detection limit, only four samples had accurate readings of acid detergent fiber; therefore, acid detergent fiber was not included in further statistical analysis. Acid detergent fiber is the portion of fiber that composed of cellulose and lignin, and the low readings indicate low fiber content in the manure samples. Protein, lipids, and total digestible nutrients also had

higher values in the foam layer, indicating higher organic components. The higher protein content may represent a higher amount of microorganism biomass in this sample group. Using the data in table 2.2, inferential statistical tests were conducted to determine if the differences between foaming and non-foaming manure for each parameter can be considered statistically significant. Comparisons were conducted between groups F2 and NF2, between groups F3 and NF3, and between groups F4 and NF4, which are the same-layer samples from foaming and non-foaming barns. The same comparison between groups F1 and NF2 was added to the analysis because both of these sample groups are from the top layers of manure pits. The homogeneity of variance was checked first; equal population variance was not observed in all parameters, so independent sample t-tests were conducted to evaluate the difference in means. For parameters for which group variances could not be treated as equal, the p-value for the situation in which equal variance was not assumed was used in the evaluation. Major differences were found between groups F1 and NF2. Nearly all parameters had p-values less than 0.05 except ammonium-nitrogen, total nitrogen, potassium, and sodium. Significant differences between foaming (group F2) and non-foaming (group NF2) liquid top samples were found only for sulfur, copper, iron, manganese, zinc, and LCFAs, while a similar trend was observed for liquid middle samples (groups F3 and NF3) and liquid bottom samples (groups F4 and NF4).

Table 2.2. Means and standard deviations of tested parameters in the seven sample groups.[a]

	Sample Group						
Tested Parameter	F1 Foam Layer	F2 Foaming Top	NF2 Non-Foaming Top	F3 Foaming Middle	NF3 Non-Foaming Middle	F4 Foaming Bottom	NF4 Non-Foaming Bottom
Number of samples	16	4	4	9	3	4	4
pH value	8.1 ±0.2	8.4 ±0.1	8.2 ±0.1	8.3 ±0.1	8.2 ±0.2	8.3 ±0.1	8.1 ±0.2
Total solids (%)	9.01 ±1.96**	4.63 ±0.39	4.08 ±0.70	5.18 ±1.06*	3.67 ±0.21	5.75 ±0.87	7.68 ±4.71
Ammonium-nitrogen (%)	0.46 ±0.06	0.47 ±0.05	0.50 ±0.05	0.45 ±0.05	0.52 ±0.04	0.44 ±0.04*	0.51 ±0.04
Organic nitrogen (%)	0.42 ±0.09**	0.23 ±0.06	0.21 ±0.16	0.25 ±0.06*	0.17 ±0.04	0.29 ±0.05	0.29 ±0.08
Total nitrogen (%)	0.88 ±0.13	0.70 ±0.06	0.71 ±0.20	0.70 ±0.10	0.69 ±0.08	0.73 ±0.08	0.80 ±0.11
Phosphorus (%)	0.44 ±0.12**	0.23 ±0.01	0.23 ±0.06	0.26 ±0.07*	0.20 ±0.01	0.28 ±0.05	0.38 ±0.18
Potassium (%)	0.38 ±0.10	0.40 ±0.05	0.36 ±0.04	0.38 ±0.05	0.36 ±0.03	0.41 ±0.04	0.37 ±0.02
Sulfur (%)	0.12 ±0.05*	0.08 ±0.00**	0.06 ±0.01	0.07 ±0.01*	0.06 ±0.01	0.08 ±0.01	0.07 ±0.02
Calcium (%)	0.31 ±0.11**	0.09 ±0.01	0.08 ±0.02	0.10 ±0.03	0.07 ±0.01	0.09 ±0.02	0.12 ±0.05
Magnesium (%)	0.11 ±0.04**	0.07 ±0.01	0.06 ±0.02	0.07 ±0.02*	0.04 ±0.01	0.09 ±0.02	0.11 ±0.06
Sodium (%)	0.08 ±0.02	0.08 ±0.00	0.07 ±0.01	0.08 ±0.01	0.07 ±0.01	0.09 ±0.01*	0.07 ±0.01
Copper (ppm)	47 ±26**	48 ±14**	4 ±0.6	28 ±19*	3 ±1	49 ±21*	5 ±2
Iron (ppm)	158 ±57**	113 ±6**	58 ±12	104 ±14**	53 ±8	114 ±13	95 ±50
Manganese (ppm)	31 ±13**	20 ±1**	9 ±2	18 ±3**	8 ±1	22 ±3	14 ±7
Zinc (ppm)	118 ±54**	86 ±26*	38 ±6	80 ±22**	38 ±8	86 ±32	58 ±30
Ash (%)	2.10 ±0.23**	1.22 ±0.44	0.80 ±0.13	1.24 ±0.36	0.84 ±0.09	1.30 ±0.38	1.10 ±0.37
Crude protein (%)	2.69 ±0.56**	1.02 ±0.60	1.3 ±0.32	1.48 ±0.56	1.23 ±0.15	1.44 ±0.41	1.24 ±0.41
Acid hydrolysis (%)	3.01 ±1.18**	0.95 ±0.35	1.01 ±0.26	1.02 ±0.55	0.94 ±0.22	1.34 ±0.13	1.19 ±0.62
Long-chain fatty acids (g L^{-1})	4.51 ±2.31*	1.21 ±0.48**	2.77 ±0.55	0.84 ±0.73**	3.27 ±0.65	1.33 ±1.09*	3.46 ±1.29
Acid detergent fiber (%)	0.68	N/A	N/A	0.61±0.01	N/A	N/A	1.18
Total digestible nutrients (%)	9.20 ±2.90*	3.49 ±2.71	5.21 ±2.35	4.40 ±2.57	4.72 ±1.36	4.87 ±2.48	5.62 ±2.38
Digestible energy (%)	0.18 ±0.06*	0.07 ±0.05	0.10 ±0.05	0.09 ±0.05	0.09 ±0.03	0.10 ±0.05	0.11 ±0.05
Metabolizable energy (%)	0.17 ±0.05*	0.06 ±0.05	0.10 ±0.04	0.08 ±0.05	0.08 ±0.03	0.09 ±0.05	0.11 ±0.04

[a] Asterisks indicate p-values: ** indicates comparison results with $p < 0.01$, and * indicates comparison results with $0.01 < p < 0.05$.

Effects of Sample Location and Foaming Status

One more test was conducted to evaluate whether the manure analysis data were affected by farm location and to determine the interaction of the foaming status of samples and their inhomogeneity (foam layer). The homogeneity of variance was checked first, and equal population variance was not observed in all parameters. Instead of using the standard two-way ANOVA, the Kruskal-Wallis H-test was conducted to

evaluate the impact of these three factors (farm location, foaming status, and inhomogeneity).

The first test was to determine the effects of farm location. Samples from non-foaming pits (groups NF2, NF3, and NF4) were excluded, and only foaming layer samples from foaming pits (groups F1) were included in the test of the farm location factor to avoid interference from the foaming status. The second test was to determine the effects of foaming status. Only the foaming samples have a foam layer (group F1), and the foam layer seems dramatically differently from the liquid manure samples. Therefore, group F1 samples were excluded in the test of the foaming status factor to avoid the influence of sample layers. The results of Kruskal-Wallis H-test are shown in table 2.3.

Table 2.3. Evaluation of the impact by farm location, foaming status, and inhomogeneity of the sample (p-values).

	Groups F1, F2, F3, F4		Groups F2, F3, F4, NF2, NF3, NF4	
	(Group F1)			
	Farm	Sample	Foaming	Sample
Original Manure	Location	Layer	Status	Layer
Total solids (%)	0.038	0.000	0.024	0.179
Ammonium-nitrogen (%)	0.084	0.715	0.007	0.851
Organic nitrogen (%)	0.256	0.000	0.358	0.139
Total nitrogen (%)	0.099	0.004	0.540	0.264
Phosphorus (%)	0.055	0.001	0.156	0.078
Potassium (%)	0.216	0.822	0.097	0.840
Sulfur (%)	0.056	0.000	0.001	0.450
Calcium (%)	0.059	0.000	0.092	0.363
Magnesium (%)	0.096	0.004	0.039	0.130
Sodium (%)	0.339	0.868	0.002	1.000
Copper (ppm)	0.033	0.153	0.000	0.998
Iron (ppm)	0.042	0.001	0.001	0.458
Manganese (ppm)	0.011	0.017	0.000	0.368
Zinc (ppm)	0.049	0.071	0.000	0.626
Ash (%)	0.569	0.000	0.015	0.470
Crude protein (%)	0.068	0.000	0.290	0.386
Acid hydrolysis (%)	0.006	0.000	0.921	0.383
Long-chain fatty acids (g L^{-1})	0.080	0.001	0.000	0.355
Total digestible nutrients (%)	0.005	0.001	0.359	0.716
Digestible energy (%)	0.004	0.001	0.408	0.669
Metabolizable energy (%)	0.006	0.001	0.367	0.621

Based on the results in table 2.3, the p-values of farm location are <0.05 for many parameters, indicating the impact of different individual farms on the manure composition. Each farm may have different practices (e.g., water, diet, genetics, manure management, etc.), which were not included in this study. The analysis of foaming status on liquid samples found ammonium-nitrogen, trace metals, ash content, and LCFAs as significant parameters that have different concentrations between foaming manure and non-foaming manure. Other significant parameters in the foaming status analysis were sulfur, magnesium, and sodium, of which sulfur and magnesium were considered to be affected by solids content. Thus, using this analysis, sodium, ammonium-nitrogen, trace metals, ash content, and LCFAs are the five factors considered to be affected by sample foaming status.

Comparison among foam layer, foaming liquid, and non-foaming liquid samples

The results in table 2.2 show detailed comparisons between each sample layer and also show that the liquid samples in each layer are similar. Based on sample composition, the samples can be classified into just three categories: foam layer samples (group F1), foaming liquid samples (groups F2, F3, and F4), and non-foaming liquid samples (groups NF2, NF3, and NF4). Comparisons were conducted among these three categories (table 4). Independent sample t-tests were applied to evaluate the differences in means. For parameters for which group variances could not be treated as equal, the p-value for the situation in which equal variance was not assumed was used in the evaluation. To verify

if a difference among categories was caused by sample solids contents, a similar analysis was conducted on a dry matter basis. The data used in the analysis on a dry matter basis were the values divided by the sample's solids content.

The comparisons in table 2.4 verify the previous statistical analysis results, showing that the most significant differences are between foam layer samples and liquid manure samples, while the differences between foaming liquid manure and non-foaming liquid manure are very small. Meanwhile, significance levels by sample and on a dry matter basis are different for many parameters. For example, the comparison between foam layer samples (group F1) and all liquid manure samples (groups F2, F3, F4, NF2, NF3, and NF4) lists ammonium-nitrogen, potassium, and sodium as the three most non-significant parameters; however, their p-values all become <0.001 when expressed on a dry matter basis. In contrast, many parameters become non-significant when expressed on a dry matter basis. This indicates that parameters such as organic nitrogen and crude protein are affected by solids content. Their high concentration in the foam layer results from the high solids content in the foam layer. For some water-soluble parameters, such as sodium, comparison on a dry matter basis does not contribute any meaningful conclusions or significance.

In addition to these analyses, ordinal logistic regression was conducted using foaming or non-foaming as a response, but it did not result in a regression model with any significant factors. The similarity of composition between foaming liquid manure and non-foaming liquid manure could be the main reason for the failure in model construction,

and thus no statistic relationship can be concluded between foaming status and the parameters discussed above.

Table 2.4. Comparison of p-values between three sample categories.

Original Manure	Group F1 Mean	Groups F2, F3, F4, NF2, NF3, NF4 Mean	Groups F2, F3, F4 Mean	Groups NF2, NF3, NF4 Mean	F1 vs. F2, F3, F4, NF2, NF3, NF4		F1 vs. F2, F3, F4		F1 vs. NF2, NF3, NF4		F2, F3, F4 vs. NF2, NF3, NF4	
Number of samples	16	28	17	11	16 vs. 28		16 vs. 17		16 vs. 11		17 vs. 11	
Significance (d.b. = dry basis)					(as is)	(d.b.)	(as is)	(d.b.)	(as is)	(d.b.)	(as is)	(d.b.)
Total solids (%)	9.01	5.22	5.18	5.27	0.000	N/A	0.000	N/A	0.001	N/A	0.930	N/A
Ammonium-nitrogen (%)	0.46	0.48	0.45	0.51	0.387	0.000	0.694	0.000	0.025	0.000	0.002	0.047
Organic nitrogen (%)	0.42	0.24	0.25	0.23	0.000	0.873	0.000	0.726	0.000	0.966	0.516	0.796
Total nitrogen (%)	0.88	0.72	0.71	0.74	0.000	0.000	0.000	0.000	0.011	0.003	0.474	0.175
Phosphorus (%)	0.44	0.26	0.26	0.28	0.000	0.557	0.000	0.969	0.002	0.214	0.667	0.006
Potassium (%)	0.38	0.38	0.39	0.36	0.884	0.000	0.750	0.000	0.381	0.001	0.054	0.539
Sulfur (%)	0.12	0.07	0.08	0.06	0.001	0.292	0.003	0.114	0.000	0.975	0.008	0.077
Calcium (%)	0.31	0.09	0.10	0.09	0.000	0.000	0.000	0.000	0.000	0.000	0.568	0.755
Magnesium (%)	0.11	0.07	0.07	0.07	0.000	0.076	0.001	0.052	0.015	0.570	0.780	0.068
Sodium (%)	0.08	0.08	0.08	0.07	0.760	0.000	0.653	0.000	0.218	0.000	0.006	0.888
Copper (ppm)	47	24	38	4	0.005	0.608	0.257	0.120	0.000	0.000	0.000	0.000
Iron (ppm)	158	93	108	70	0.000	0.675	0.003	0.052	0.000	0.027	0.004	0.000
Manganese (ppm)	31	16	20	10	0.000	0.467	0.004	0.265	0.000	0.001	0.000	0.000
Zinc (ppm)	118	68	83	45	0.000	0.743	0.022	0.095	0.000	0.059	0.000	0.000
Ash (%)	2.10	1.12	1.25	0.92	0.000	0.550	0.000	0.112	0.000	0.014	0.016	0.006
Crude protein (%)	2.69	1.32	1.36	1.26	0.000	0.357	0.000	0.981	0.000	0.023	0.514	0.072
Acid hydrolysis (%)	3.01	1.06	1.06	1.06	0.000	0.003	0.000	0.065	0.000	0.000	0.970	0.099
Long-chain fatty acids (g L^{-1})	4.51	1.91	1.05	3.16	0.002	0.196	0.000	0.004	0.069	0.209	0.000	0.000
Total digestible nutrients (%)	9.20	4.66	4.30	5.23	0.000	0.004	0.000	0.004	0.001	0.063	0.304	0.038
Digestible energy (%)	0.18	0.09	0.09	0.10	0.000	0.004	0.000	0.004	0.001	0.065	0.331	0.037
Metabolizable energy (%)	0.17	0.09	0.08	0.10	0.000	0.008	0.000	0.005	0.001	0.130	0.300	0.027

2.4 Discussion

Although newly reported in deep-pit manure storage facilities, foaming has been a major issue in wastewater treatment processes, especially in activated sludge systems. For decades, biological foaming was believed to be primarily caused by massive growth of certain filamentous bacteria and actinomycete-containing mycolic acid (de los Reyes

and Raskin, 2002; Ganidi et al., 2009; Pagilla et al., 1997; Shen et al., 2007). However, studies also have shown evidence that undue emphasis has been given to filamentous mycolata as a cause of foaming (Davenport and Curtis, 2002) and that the threshold concentration of mycolata cells may only be applicable to mycolata members whose cell walls are more hydrophobic (Davenport et al., 2008). Therefore, this research did not focus on any specific filamentous bacteria as a cause of foaming but instead investigated the physical and chemical differences between foaming and non-foaming samples. The samples used in this analysis were collected right before or after manure was emptied from the pits and represented the later stage of foaming when collected from foaming barns. One drawback of the dataset is that all non-foaming samples came from the same farm. Compared to the foaming samples, which had farm-to-farm variation, the non-foaming samples were limited to barn-to-barn variation. This could lead to an underestimation of the variation in the non-foaming samples. A larger amount of sampling activity may change the analysis results, especially for non-foaming manure. With the current dataset, some parameters that indicate significant differences between samples are further discussed below.

Categories of parameters

Solids content was used to evaluate the differences between sample groups. Based on the mean solids content and p-value between groups, foaming liquid manure and non-foaming liquid manure had similar solids contents, and the solid components in foaming manure were highly accumulated in the foam layer. However, it is hard to draw a

conclusion that the manure in the foaming barns had significantly higher total solids content than the manure in the non-foaming barns. This is mainly because the depth of each layer is unknown, which makes it difficult to evaluate the overall solids content in each barn.

Based on the results shown in tables 2.2 and 2.4, the parameters included in the analysis can be classified into four categories. The first category includes parameters that showed no significant difference by sample but became significantly different on a dry matter basis. The most probable reason for this result is that the chemicals were dissolved in liquid and did not have a high concentration of solid components. In this case, the data on a dry matter basis misrepresent the concentrations in the manure. Parameters in this category include potassium and sodium.

The second category includes parameters that showed significant sample differences, but the difference became insignificant on a dry matter basis. A possible reason for this finding is that the chemicals existed mainly in solid form, and their concentration was highly impacted by the solids content in the manure samples. Parameters in this category include organic nitrogen, phosphorus, sulfur, magnesium, LCFAs, and crude protein.

The third category includes parameters that showed a significant difference between the foam layer samples and all other samples, but the difference became insignificant between foaming liquid samples and non-foaming liquid samples. Their distribution was highly concentrated in the foam layer, and it was hard to distinguish whether this phenomenon was a cause or an effect of foaming. Parameters in this category include

calcium, acid hydrolysis, total digestible nutrients, digestible energy, and metabolizable energy.

The fourth category includes parameters that showed a significant difference between foaming samples and non-foaming samples, including ammonium-nitrogen, trace metals (copper, iron, manganese, and zinc), and ash content. Total nitrogen was not included in this categorization because the values of organic nitrogen in this analysis were calculated as the difference between ammonium-nitrogen and total nitrogen.

Trace metals

Significant differences in trace metal content were found between foaming and non-foaming manure samples (Fig. 2.1). Trace metals are considered essential feed ingredients to meet the nutrient requirements of farm animals, and the trace metal content in swine manure can be correlated with both intake and absorption. In practice, only 5% to 15% of dietary copper, iron, and zinc are apparently absorbed by the animal's digestive system, and the absorption of manganese is even less (Ma et al., 2012). Animal diets are formulated by nutritionists based on least costs, with trace metals provided premixed from feed suppliers. The exact compositions of animal diets are normally difficult to obtain, and the diets change depending on cost and availability of ingredients and animal growth stage. High inclusion of corn ethanol co-products such as DDGS in animal feed has become a common practice in the U.S. pork industry primarily because of the low cost of DDGS compared to other ingredients. The link between the DDGS in animal feed and the high concentration of trace metals in manure is unknown.

Trace metals are usually added to animal diets to promote growth (Ma et al., 2012), but they have not been directly connected to foaming. High doses of copper were reported to have an inhibitive effect on the anaerobic digestion of swine manure and may decrease biogas production, and similar effects can also be found for zinc. However, as their concentrations in both foaming and non-foaming manure samples were far less than the inhibition level, the effects of trace metals on anaerobic degradation and biogas production should not be a concern. It is more reasonable that the high concentrations of trace metals in foaming manure samples were the result of the foaming process, in which these trace metals resulted from the degradation of organic compounds and the mass generation of biogas. An interesting fact is that bile excreted from the liver serves as the major pathway of copper excretion, while bile salts have long been identified as biological surfactants (Hofmann and Mysels, 1987). It is worthwhile to investigate if a high concentration of bile can be detected in foaming manure and whether it stabilizes the foam.

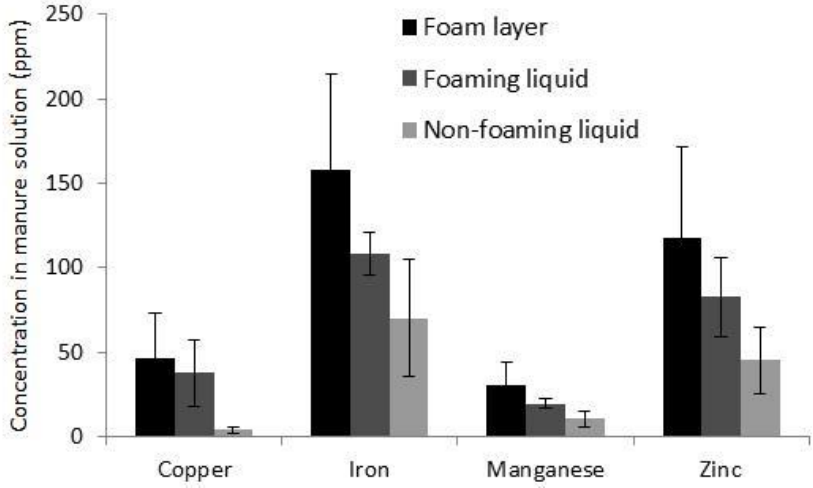

Figure 2.1. Concentrations of trace metals in manure sample

Lipids and long-chain fatty acids

Acid hydrolysis in this analysis refers to the lipids in the manure samples, and it is possibly natural to detect the highest lipid content in foam layer samples, as lipids may easily float onto the surface layer of a water solution. Lipid is not soluble in water, so the lipid in manure samples must be attached with solid particles and suspended in solution. Despite the high lipid content in the foam layer (3.0% ± 1.2%), the lipid contents in foaming and non-foaming liquid samples were similar (1.1% ± 0.5% in foaming liquid and 1.1% ± 0.4% in non-foaming liquid), and no significant difference was observed ($p = 0.970$). However, when expressed on a dry matter basis, the difference in lipid content became more evident (24.8% ± 12.3% in foaming liquid and 19.0% ± 4.5% in non-foaming liquid, $p = 0.099$, Fig. 2.2). The depth of each layer is unknown, so the distribution of total lipids in the manure pits is hard to estimate. However, based on the dry matter basis data, it is reasonable to conclude that higher lipid content is expected in foaming manure samples.

Lipids, especially triglycerides, have a negative effect on foaming, and high lipid contents are considered to be responsible for the lower foaming properties of whey protein (Karleskind et al., 1995). However, foaming in industrial wastewater that was high in grease, fat, and oil was primarily blamed on the massive growth of filamentous bacteria (Davenport and Curtis, 2002; de los Reyes and Raskin, 2002; Heard et al., 2008). If a similar situation happened in the manure pits, then the increased lipids in the foam layer would provide a good environment for the growth of certain bacteria species.

Coincidently, the comparison of protein content between the foaming and non-foaming liquid samples showed a trend similar to that of lipid content, and the difference become more evident on a dry matter basis ($p = 0.514$ for sample basis, and $p = 0.072$ for dry matter basis). More research is needed to determine if the increased protein content derives from bacteria or directly from swine excretion.

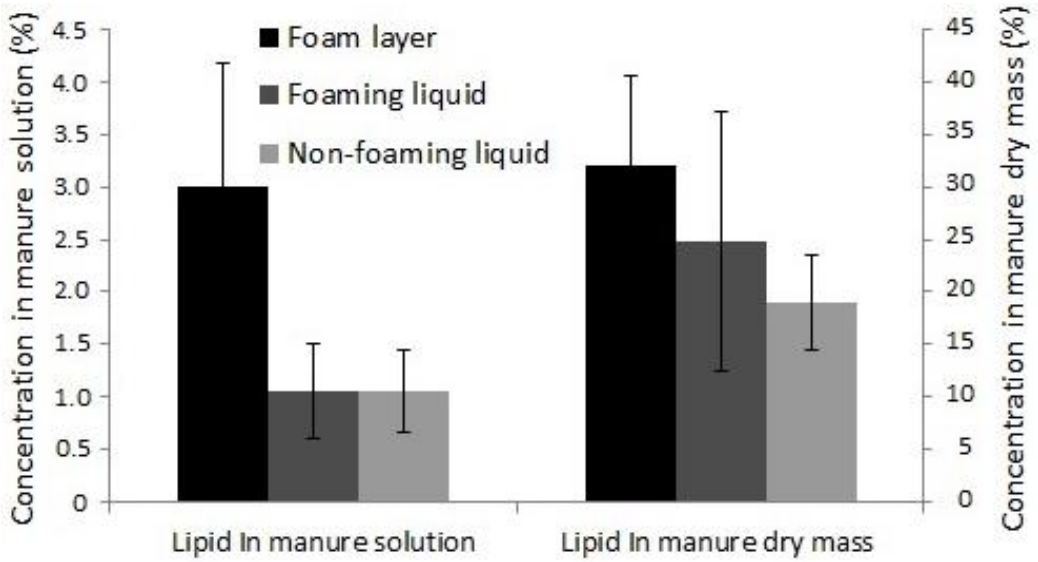

Figure 2.2. Concentrations of lipids in manure samples.

LCFAs are part of the lipids that can be extracted from the manure sample. They are dissociative fatty acids that are released from triglycerides and contain a hydrophobic carbon chain with a carboxyl group at one end. In the non-foaming manure samples, they were homogeneously distributed in different layers, so there is high possibility that they were bonded with particles and remained suspended in the manure. Similar to other types of lipids, an inhomogeneous distribution of LCFAs was found in the foaming manure samples, and LCFAs were present in the foam layer during foaming (Fig. 2.3). Previous

research on manure foaming has shown that LCFAs can significantly improve manure's foaming ability (Yan et al., 2014), and the concentration of LCFAs in the foam layer further indicates that LCFAs could be important in the foaming process. Meanwhile, LCFAs did not exactly follow the distribution trend of lipids and had a higher percentage in non-foaming manure when normalized by solids content. One possibility is that LCFAs do not serve as a sufficient parameter in manure foaming, and other chemicals in manure have similar a function. Further research on this aspect is expected to explain the function of LCFAs in the foaming process.

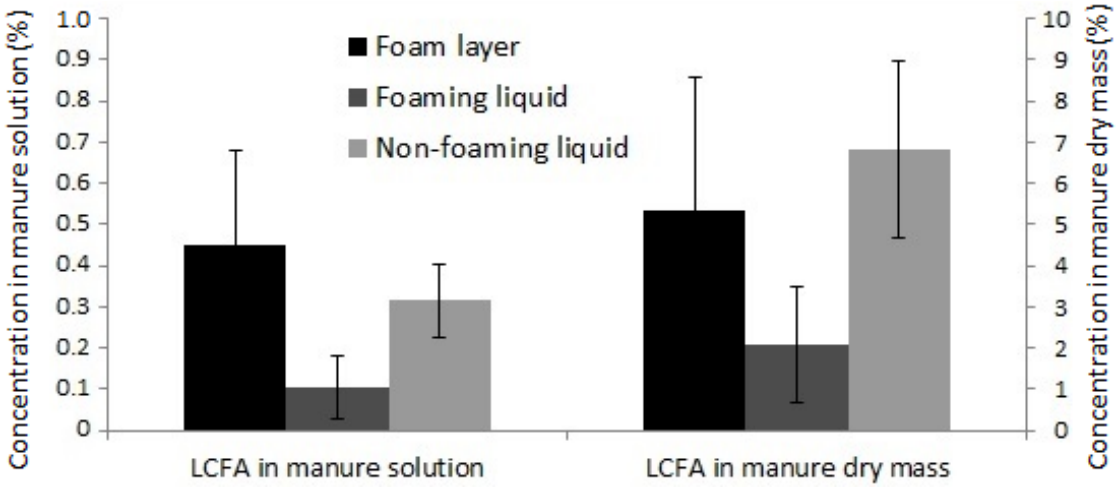

Figure 2.3. Concentrations of long-chain fatty acids in manure samples

Total solids

The statistical analysis showed that foaming liquid manure and non-foaming liquid manure had similar solids contents (Fig. 2.4), while foaming liquid manure was more homogeneous. The abnormally high solids content in the foam layer was probably due to the biogas trapped in bubbles surrounded by solid particles. The foam layer was

composed of gas bubbles, liquid, and solids, which naturally contained increased total solids content and reduced liquid content. Compared with non-foaming manure, foaming manure was more likely to be unique in terms of solids content characteristics, which stabilized the gas bubbles.

The absorption of particles at bubble interfaces is the basic driving mechanism in many dynamic foaming processes, and different types of particles are known to cause foaming in rivers, dispersed sludge, and other water environments without the presence of a surfactant (Hunter et al., 2008). Solid particles are observed to strongly influence the stability of foam, and this influence strongly depends on the particle shape, size, wettability, and concentration. Colloidal hydrophilic particles or nanoparticles were proven to stabilize foam by a self-layering phenomenon in the confined space of foam lamella, and the degree of foaming created in this situation was a strong function of particle size (Bindal et al., 2002; Vijayaraghavan et al., 2006). The wettability of solid particles refers to their hydrophobicity, and amphiphilic particles were found to be the primary cause for an increase in solution foaminess, while hydrophobic particles can be used as foam breakers (Dippenaar, 1982; Hunter et al., 2008). The effect of amphiphilic particles to increase solution foaminess is diminished at high particle concentrations due to flocculation (Vijayaraghavan et al., 2006). The difference in solids composition between foaming and non-foaming manures should lie in these aspects. Because swine pit manure is a liquid system with many suspensions and sediments, there is undoubtedly a complex mixture of solid components in suspension. Analysis of the solid particles in manure could be important for understanding the foaming mechanism.

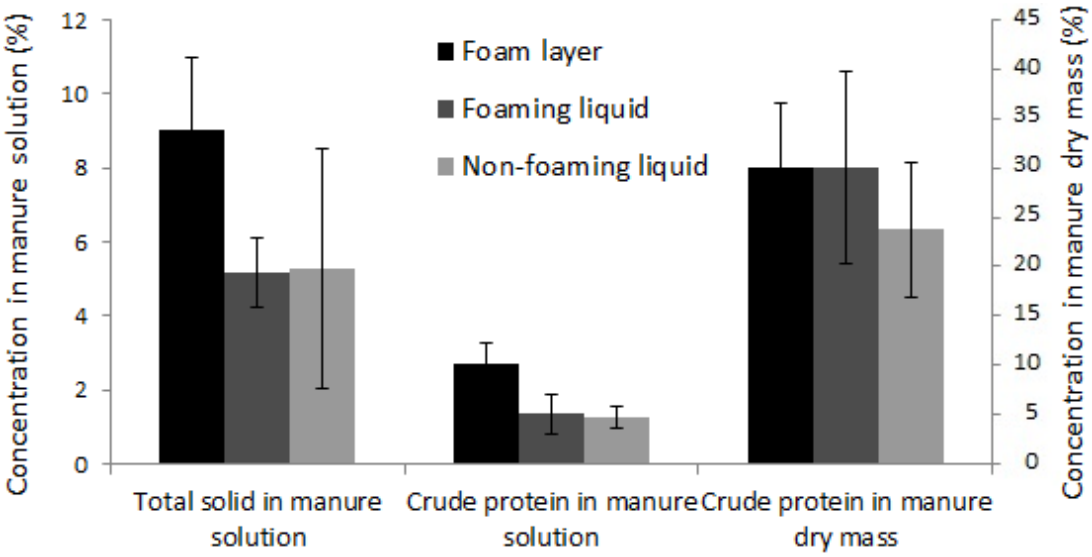

Figure 2.4. Concentrations of total solids and crude protein in manure samples.

Protein

From the results of the composition analysis, protein was a significant parameter in the manure dry matter. It accounted for an average of 30.0% ± 8.2% of the dry matter in foaming manure and 23.7% ± 6.8% of the dry matter in non-foaming manure. The difference in protein concentration between foaming liquid manure and non-foaming liquid manure was statistically insignificant ($p = 0.514$ on sample basis and $p = 0.072$ on dry matter basis, Table 2.4). When considering the foam layer samples, the percentage of protein was obviously higher in foaming manure on a dry matter basis ($p = 0.027$, Fig. 2.4). Protein has been found to be significant in food foaming processes. For foams in milk and other foods, the surfactants are the proteins present in the system (Huppertz, 2010). Foam formation and stability are also considered to be important functional properties of food proteins and have a widespread applicability in many food products (Kinsella, 1981).

Foam formation with the involvement of protein occurs in three steps: (1) soluble globular proteins diffuse to the air/water interface to reduce surface tension, (2) proteins unfold at the interface and orient the hydrophilic and hydrophobic groups, and (3) polypeptides interact with one another via non-covalent interactions and potentially covalent bonds to form a stabilizing film (Yin et al., 2014). In wine and sparkling wine products, glycoprotein invertase from grapes has been shown to be involved in foaming, as significant decreases in the invertase content correlated with decreases in foam quality (Dambrouck et al., 2005). Mannoproteins released from yeast cell walls during fermentation have also been identified as a foaming glycoprotein in wine (Goncalves et al., 2002). Proteins with similar foaming characteristics are also found in beer production, with protein LTP1 and protein Z identified as the most important in foam formation (Blasco et al., 2011). Glycoprotein is considered to retard film drainage and increase the air/water interface stability, and foam stability can be strongly increased by the existence of protein-polysaccharide interactions (Schmidt et al., 2010). If the foaming process in manure is similar to the foaming processes that occur in beer or wine, then a group of proteins must be involved in manure foaming. Verification of this group of proteins will help solve the manure foaming problem.

Other parameters

Some other parameters also showed higher concentrations in either the foam layer or the foaming liquid manure samples, such as ammonium-nitrogen, organic nitrogen, calcium, phosphorus, digestible nutrients, digestible energy, and metabolizable energy.

The digestible nutrients, digestible energy, and metabolizable energy were based on calculation and indicate the organic components in the manure samples. Organic nitrogen indicates the partial nitrogen concentration in the manure, which probably correlates to the protein content. No information was found in the literature on the impact of these parameters on foaming in liquid systems. The most probable way in which they affect foaming in swine pits is through the promotion or inhibition of microorganisms.

2.5 Conclusion

Of all the parameters analyzed, pH remained at a similar level among the manure samples, and potassium and sodium were found to have similar concentrations between foaming and non-foaming manure. The foam layer, due to its high solids content, concentrates the following components: organic nitrogen, phosphorus, sulfur, calcium, magnesium, LCFAs, acid hydrolysis, and crude protein. Ammonium-nitrogen and trace metals were found to have higher concentrations in foaming manure and higher percentages in foaming manure solids.

Chapter 3

Bacteria Community Analysis of Foaming Swine Manure in Minnesota's Swine Deep-Pit

Outline

Over the past years, hazardous foaming occurred frequently in Midwestern swine production facilities that store manure in deep pits. In order to reveal the mechanism of manure foaming, analysis on microbial community was conducted to identify if filamentous bacteria is the key inducing factor in this process. Results indicated that filamentous bacteria did not constitute a significant portion of the bacteria community in manure samples collected, even though significant differences in microbial community were observed between foaming and non-foaming manure samples. This result indicated that filamentous bacteria might not be the main reason for foaming in deep pit, and it is more possible that foaming is induced by the change of manure composition rather than the impact of microorganisms.

3.1 Introduction

Over the past years, hazardous foaming has occurred frequently in some Midwestern swine production facilities. The persistent foaming accumulated in the deep manure storage pits and can grow to depths over 1.2 m, trapping large amount of biogas generate from the anaerobic degradation of the manure in the pit. It creates difficulty in management by reducing the storage capacity of the manure pit, and in some extreme cases foam came up through the slats to threaten the respiratory health of animals. Considering the high methane percentage in biogas, the sudden release of trapped biogas during the removal of manure also created a major safety hazard of either explosion or flash fires. Initial research clearly showed that the risk of foaming increased dramatically over the two year period of 2009 and 2010, but there is no statistical evidence that management, diet, genetics, building age or design features can be correlated to this foaming issue (Jacobson et al., 2011).

Extensive foaming in manure pits is a new topic, but foaming occurs in various industrial processes and their mechanisms have been widely studied. From the introduction of continuous-flow reactors, sludge bulking and foaming has been one of the major problems affecting the biological wastewater treatment process (Peng Y. et al., 2003). Besides wastewater treatment plants, a survey of biogas plant operators also revealed a high percentage of foam forming problems (Moeller et al., 2012). Current understanding of foaming are mostly based from a microbiological point of view, with Norcadia and Microthrix sp. the most frequent filamentous bacteria observed during the foaming process. There are studies claim that place undue emphasis to filamentous

bacteria (Davenport & Curtis, 2002; Schilling & Zessner, 2011), and foaming examples have also been reported without the involvement of filamentous bacteria (Peng Y. et al., 2003). But in general, the formation of foam in wastewater treatment plants (WWTPs) is considered to be caused by filamentous bacteria (Blackall et al., 1988; de los Reyes et al., 2002; Martins et al., 2004; Rossetti et al., 2005). Nocardia, often referred to as "Mycolata", are a group of filamentous bacteria that contain mycolic acids in their cell walls. They were found to uptake a wide range of organic compounds and present high cell surface hydrophobicity (Kragelund C, et al., 2007). *Microthrix parvicellatha* is considered the most commonly seen bacteria in foaming, and high concentrations of ammonia and low temperature are the main factors to trigger the fast growth of *M. parvicellatha* (Rossetti S., et al., 2005). It is normally believed that filamentous bacteria will form a hydrophobic membrane on the surface, capture gas generated inside the waste, and create a dark and brown foam with small and stable gas bubbles.

To generate stable foam, there are three components that are required to exist in the system: gas bubbles, surfactants and foam stabilizer (Heard et al., 2008; Petrovski et al., 2011). Gas bubbles are the major components of foam, while surfactant is needed to reduce surface tension and prevent liquid drainage from liquid films. Foam stabilizers are responsible for the long-term foam stabilization, and cannot be clearly distinguished from surfactants in many situations. While biogas generated from anaerobic digestion provides enough gas generation and bubbles, surfactant and hydrophobic particles stabilizer are unidentified in manure pit foaming. For filamentous bacteria induced foaming, it could be difficult to diminish or control the foam because these bacteria were involved in solid-

stabilized foam and some chemical antifoamer may not be effective. Traditional foaming control methods for municipal wastewater treatment plants include reduces aeration, enlarged surface scum traps, chlorination and forceful water sprays, basically aimed on reducing the amount of filamentous bacteria or the amount of gas bubbles generated. But compare to the highly controlled systems like activated sludge, a manure storage pit is a more natural system where many of these same methods are hard or impossible to apply.

Based on the research of foaming in wastewater treatment plants, it is hypothesized that certain filamentous/actinomycete species are the cause of foaming of swine manure stored in deep storage pits. The major objectives of this research is to identify the possible microbial species that result in swine manure foaming by studying the diversities of microbial communities in the foaming manure and non-foaming manure, and determine whether filamentous/actinomycete species are accumulated in foaming manure. If so, it will be possible to directly decrease the risk of foaming by eliminating their population or their favorable growing environments.

3.2 Material and Methods

Sample collection

Two rounds of sampling were conducted to collect sample used in this study. In the first sampling, manure samples were categorized into two groups: foaming manure sample, and non-foaming sample. A total of four samples were collected from two farms in Minnesota, each had both foaming and non-foaming barns. In these four samples, sample 1 (foaming manure) and sample 2 (non-foaming) are from the same farm, while

another set of foaming liquid and non-foaming manure samples (sample 3 and 4, respectively) came from the other farm.

Field manure samples for sequencing were collected on the second round sampling from five farms in Iowa/Minnesota, in which four of the farms were reported to have foaming and the fifth farm was free of foaming. From the highest to the lowest level, samples were taken from four pit depths in each barn and marked as (1) foaming layer (taken directly from the foam), (2) liquid top (the top layer of liquid manure), (3) liquid middle (the intermediate layer of liquid manure), and (4) liquid bottom (the bottom layer of liquid manure). Based on inquiries with the farm managers, no anti-foaming or defoaming agents were applied at these five sampling sites. Altogether, 41 samples were collected, chilled immediately after collection, and stored at -20 ℃ until analyzed. The samples can be categorized into three groups: foam layer sample, foaming liquid sample and non-foaming sample.

Microscopy examination and fluorescent in situ hybridization (FISH)

The microscopy examination was applied to the first round of manure samples with an optical microscope and crystal violet staining, so a visual observation of the manure sample content could be done. Four samples were also sent to Vermicon AG, a commercial company in Germany, uses specific gene probe targeting to test the existence of 9 filamentous bacteria commonly seen in foaming wastewater.

Measurement of Foaming Capability

Foaming capability of manure samples was measured on the 41 samples to evaluate the differences between samples. 50 mL of manure sample was added to a transparent pipet with an inner diameter of 2.54 cm. Nitrogen gas was pumped into the bottom of the cylinder through a plastic pipette (1 mm tip diameter) at a constant flow rate of 100 ml/min to generate bubbles. Instead of air, nitrogen gas was used to prevent potential oxidation during these tests, and the density of nitrogen gas is approximately the same as biogas or methane. The volume that stable bubbles reached in the pipet was recorded as the sample's foaming capability. Sine a one meter length pipet was used in this study, a maximum detection limit was 450 ml. The foaming capability of each manure sample was measured in triplicate.

Sequencing analysis

Second generation sequencing was conducted to get detail microbial community information for the 41 samples collected in the second round by illumina sequencing. An equimolar mix of 5 forward primers (5'-CNACGCGAAGAACCTTANC, 5'-CAACGCGAAAAACCTTA CC, 5'-CAACGCGCAGAACCTTACC, 5'-ATACGCGARGAACCTTACC, 5'-CTAACCGAN GAACCTYACC) and reverse primer (5'-CGACRRCCATGCANCACCT) were used in the amplification process target at V6 region. In both sequencing process, each sample attached specific barcodes on primers for differentiation after sequencing.

Total genomic DNA was extracted from samples by the MoBio PowerSoil DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA) according to instructions, followed by PCR reaction with specific primer on each sample. After amplification, PCR products went through purification by Gel and PCR clean-up system (Promega, Cat # A9281, Madison, WI), and purified PCR products were sent to BioMedical Genomics Center in University of Minnesota for sequencing.

Sequence data were processed and analyzed using MOTHUR (Schloss P.D., et al., 2009). Sequencing readings with the correct barcode and forward primer sequences were included in subsequent analyses, and data was then filtered to exclude sequences containing any ambiguous bases, homopolymers larger than 7bp, or a quality score that averaged below 35 in any 50bp window (Schloss P.D., et al., 2011). Remaining sequences were aligned to RDP7 16s database (Cole J.R., et al., 2009) and any sequence which did not align well was removed. Potential chimeras were identified and excluded from analysis using UCHIME (Edgar R.C., et al., 2011). Finally, remaining sequences were classified. A random subset of sequences was chosen from each sample to match the lowest number among all samples in order to balance sampling effort and ensure comparable diversity measures.

3.3 Result

Filamentous bacteria in manure samples

From the first round sampling, filamentous bacteria were observed in manure sample. Among 4 samples for the FISH analyzed, samples 1 and 2, which came from the

same farm, had respectively 18% and 14% of bacteria identified as *Nacardioform actinomycetes*. However, samples 3 and 4 from another farm did not find any filamentous bacteria (Table 3.1). The abundance of filamentous bacteria probably is more related to sampling location instead of foaming situation, since a high percentage of filamentous bacteria can also be found in non-foaming samples.

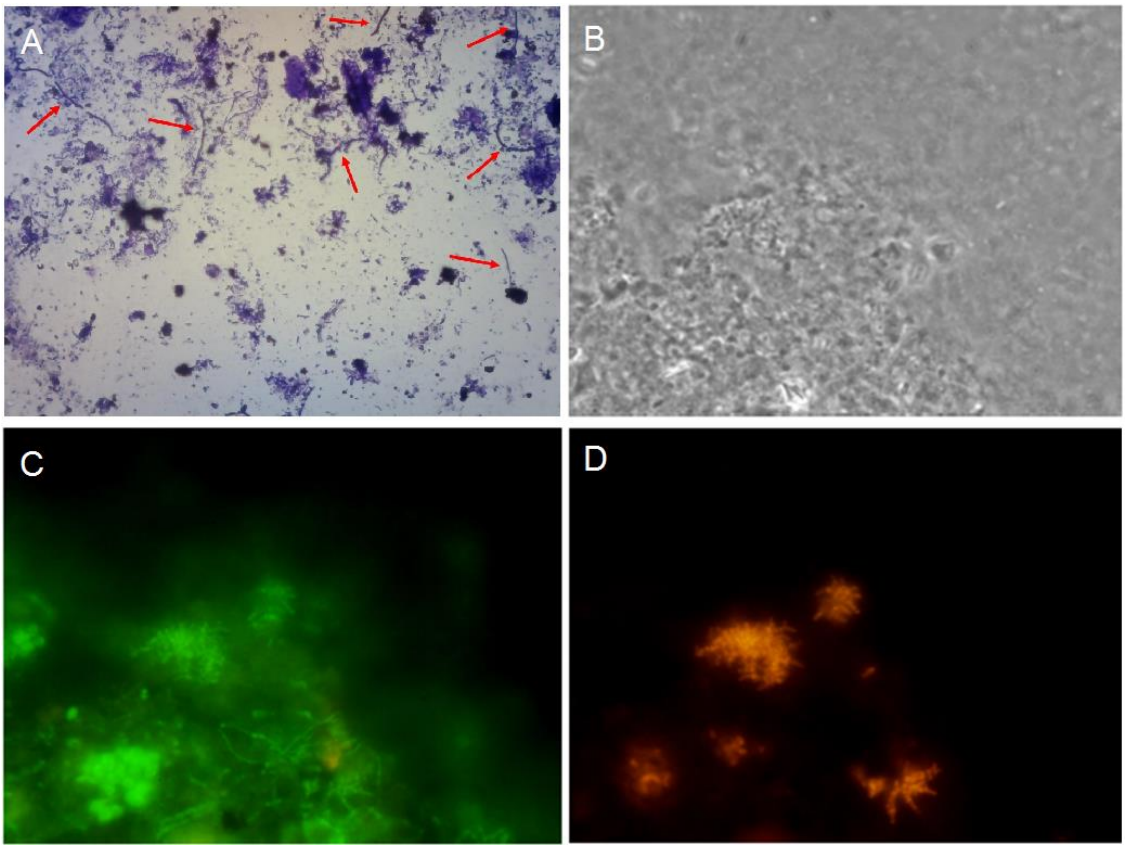

Figure 3.1. Microscopic examination and FISH analysis of foaming manure samples. A: Microscopic examination of foaming manure sample. Red arrows points to small filamentous discovered. B, C and D: filamentous bacteria *Nocardioform actinomycetes* that observed by FISH analysis of manure samples.

Table 3.1. Filamentous bacteria percentage among samples in FISH

Filamentous Bacteria analyzed	Sample 1 (foaming, farm 1)	Sample 2 (non-foaming, farm 1)	Sample 3 (foaming, farm 2)	Sample 4 (non-foaming, farm 2)
Norcardioform actinomycetes	18%	14%	<0.1%	<0.1%
Chloroflexi	<1%	<1%	<0.1%	<0.1%
Eikelboom Type 1851	<0.1%	<0.1%	<0.1%	<0.1%
Haliscomenobacter hydrossis	<0.1%	<0.1%	<0.1%	<0.1%
Microthrix parvicella	<0.1%	<0.1%	<0.1%	<0.1%
Thiothrix	<0.1%	<0.1%	<0.1%	<0.1%
Eikelboom Type 021N	<0.1%	<0.1%	<0.1%	<0.1%
Alysiosphaera	<0.1%	<0.1%	<0.1%	<0.1%
Nostocoida limicola II	<0.1%	<0.1%	<0.1%	<0.1%

Sequencing result

Foaming capability analysis Indicated that only samples from the foam layers have the capability to generate stable foam. Meanwhile, a large variation on this foaming capability existed which is mostly related to the low foaming capability of samples taken from farm 3 (Table 3.2). For other samples, they do not have the capability to stabilize the foam generated during the analysis.

Table 3.2. Foaming capability of samples.

Foaming Capability (ml)	Foam (foam layers in farm 1,2,3,5)	Foaming liquid (other layers in farm 1,2,3,5)	Non-foaming liquid (farm 4)	Total
Mean	246.85	4.44	1.18	82.33
N	16	14	11	41
Std. Deviation	214.24	3.44	1.54	165.82

Illumina sequencing conducted on these 41 samples shows that the bacterial community structure of the top, middle, and bottom manure samples from all farms

differed only by weighted Unifrac analysis ($P < 0.001$), and not by unweighted Unifrac or analysis of similarity (ANOSIM) analysis. This suggests that the core bacterial community of these samples is similar but the relative abundance of different species varies. The similarity analysis on sequencing result shows that sample similarity is impacted by their sampling location (Fig. 3.2). Nearly all samples from the same farm have shorter distance than other samples in this figure, even though a large difference on their foaming capabilities existed. But in general, there is a clear distinction between samples collected from the foaming farm and non-foaming farm, and foaming samples are more closely correlated. Sequencing result shows that the most frequent phylum detected in these samples are *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. *Firmicutes* (predominantly gram-positive) populations are significantly higher in non-foaming manure than in foaming manure and the other two groups (predominantly gram-negative) are correspondingly lower. *Clostridia* are the predominant groups of bacteria in *Firmicutes*, and also the major hydrolysis bacteria during anaerobic digestion. The major decline of *Firmicutes* occurs not only in the surface layer of pit manure but also in the pit's deeper layers beneath the foam (Fig. 3.3), so it is less possible that the decrease of *Clostridia* related to the change of anaerobic environment.

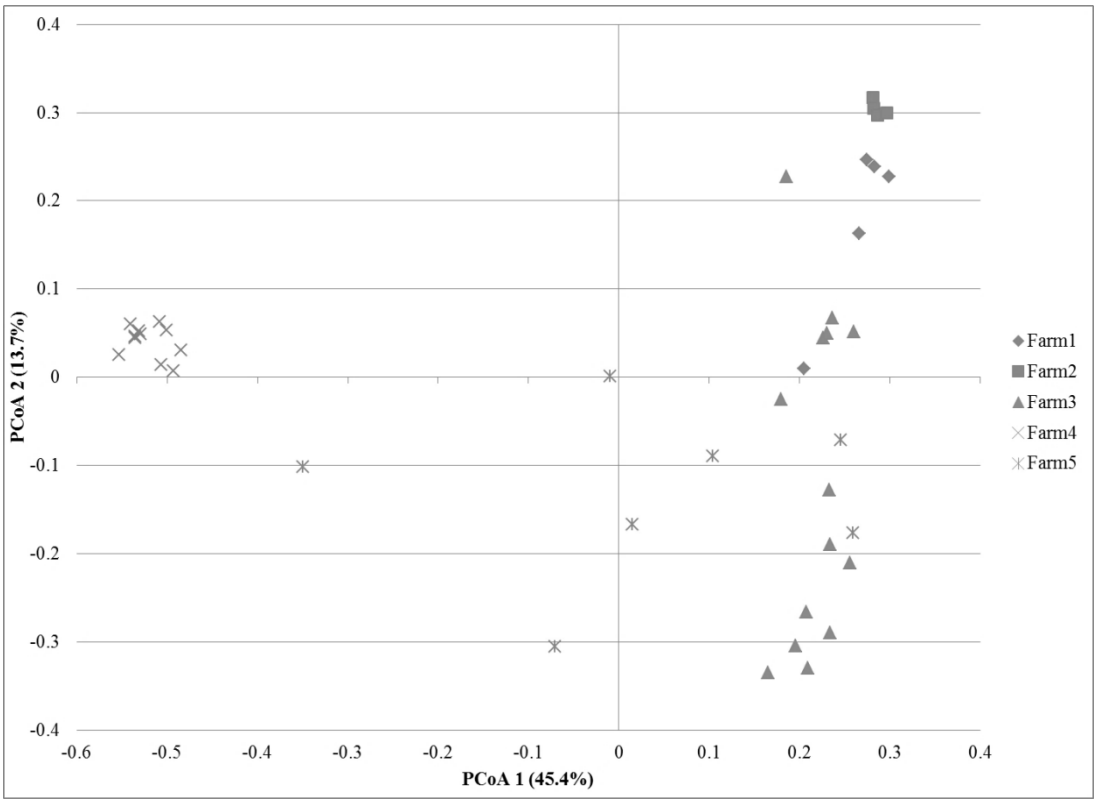

Figure 3.2. Principal coordinate analysis of samples grouped by farm. Percentages indicate the amount of variability explained by each axis. Clustering by farm was significant by analysis of molecular variance (ANOVA; $P < 0.001$).

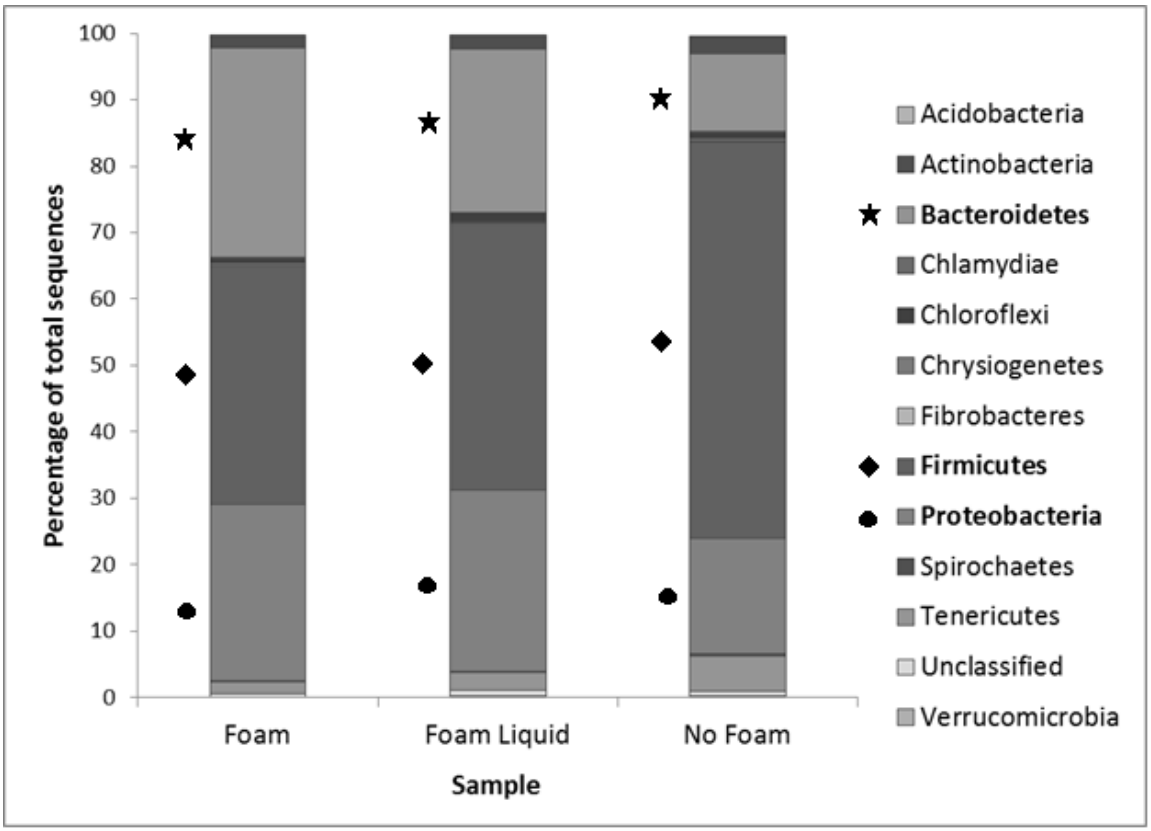

Figure 3.3. Distribution of bacteria phylum in three sample types (in average). Phylum percentage in each sample group is listed alphabetically from top to bottom.

Detail bacterial composition in samples

Both the foaming manure and non-foaming manure contains *Bacteroidetes*, *Firmicutes* and *Proteobacteria* as the most abundant phylum. *Bacteroidetes*, *Porphyromonadaceae* is the most abundant family that was observed in the three sample groups. The most significant shift is the much higher percentage of *Flammeovirgaceae* observed in non-foaming sample (2.28% in non-foaming sample compare to 0.38% in foam layer and 0.48% in foaming liquid). Compare to *Bacteroidetes*, *Firmicutes* had a more complex community consisting of *Carnobacteriaceae*, *Clostridiaceae 1*, *Clostridiales_Incertae_Sedis_XI*, *Peptostreptococcaceae* and *Ruminococcaceae* which

are the most abundant family that was observed in Firmicutes. They constitute the largest portions of the community and have a significant difference percentage between foaming and non-foaming samples. *Carnobacteriaceae*, *Clostridiales_Incertae_Sedis_XI* and *Ruminococcaceae* are found to be more frequent in foaming samples, while *Clostridiaceae 1* and *Peptostreptococcaceae* have higher abundance in non-foaming samples. Besides these high frequent families, the difference in microbial community was also discovered on some low abundant families, like *Halobacteroidaceae* (0.36% in non-foaming sample compare to 1.03% in foam layer and 1.26% in foaming liquid). Similar to *Firmicutes*, *proteobacteria* has a complex community with some major components. *Acetobacteraceae*, *Anaplasmataceae* and *Methylocystaceae* are the most major families observed, in which *Acetobacteraceae* is enriched in the non-foaming samples, *Anaplasmataceae* is highly enriched in foam layer samples and *Methylocystaceae* is highly enriched in foaming liquid samples. Besides these three families, the change of microbial community was also discovered on some low abundant families, like *Alteromonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae* enriched in foam layer samples and *Desulfomicrobiaceae*, *Helicobacteraceae*, *Syntrophorhabdaceae* enriched in non-foaming liquid samples. Detail compositions are shown in Figure 3.4, 3.5 and 3.6.

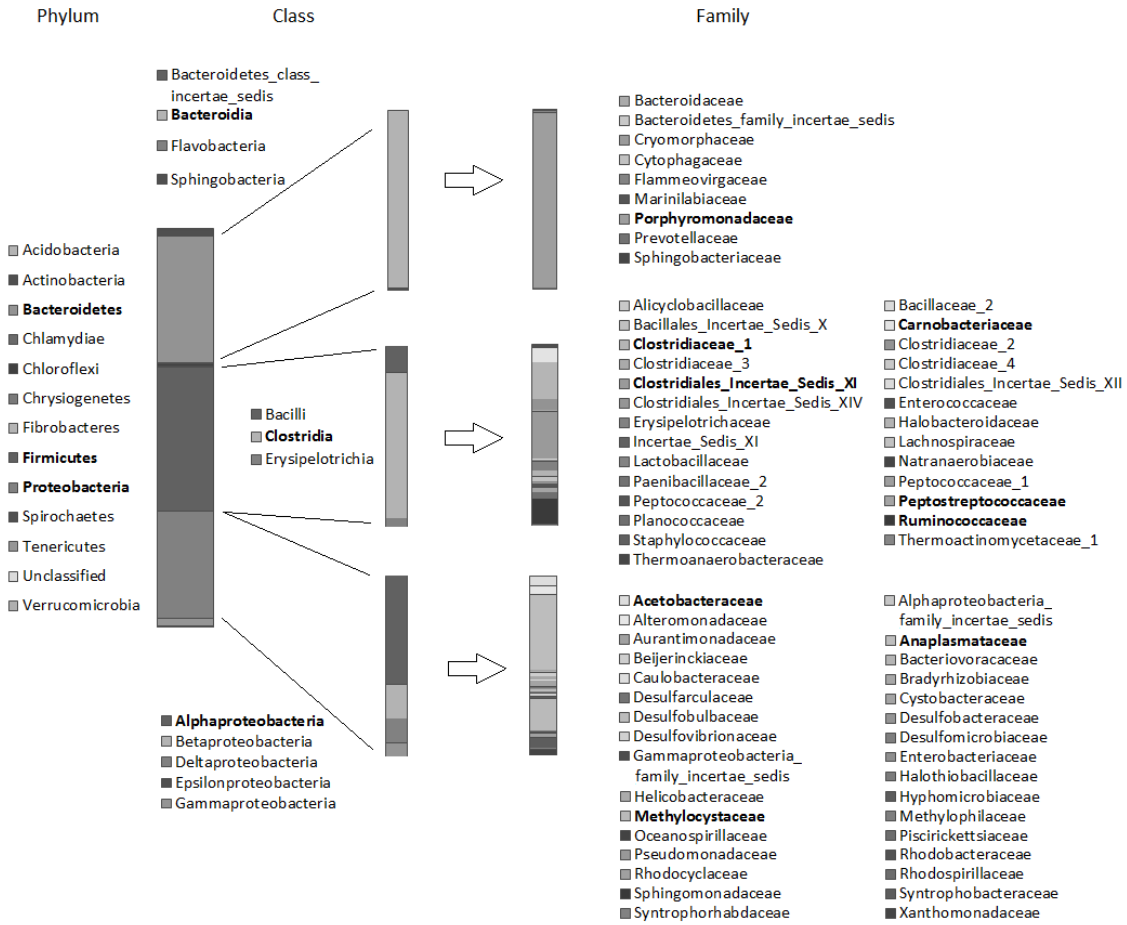

Figure 3.4. Bacterial communities of 16 foam layer samples (on average). Words in bold type indicate bacteria groups that take the most major portion at their level.

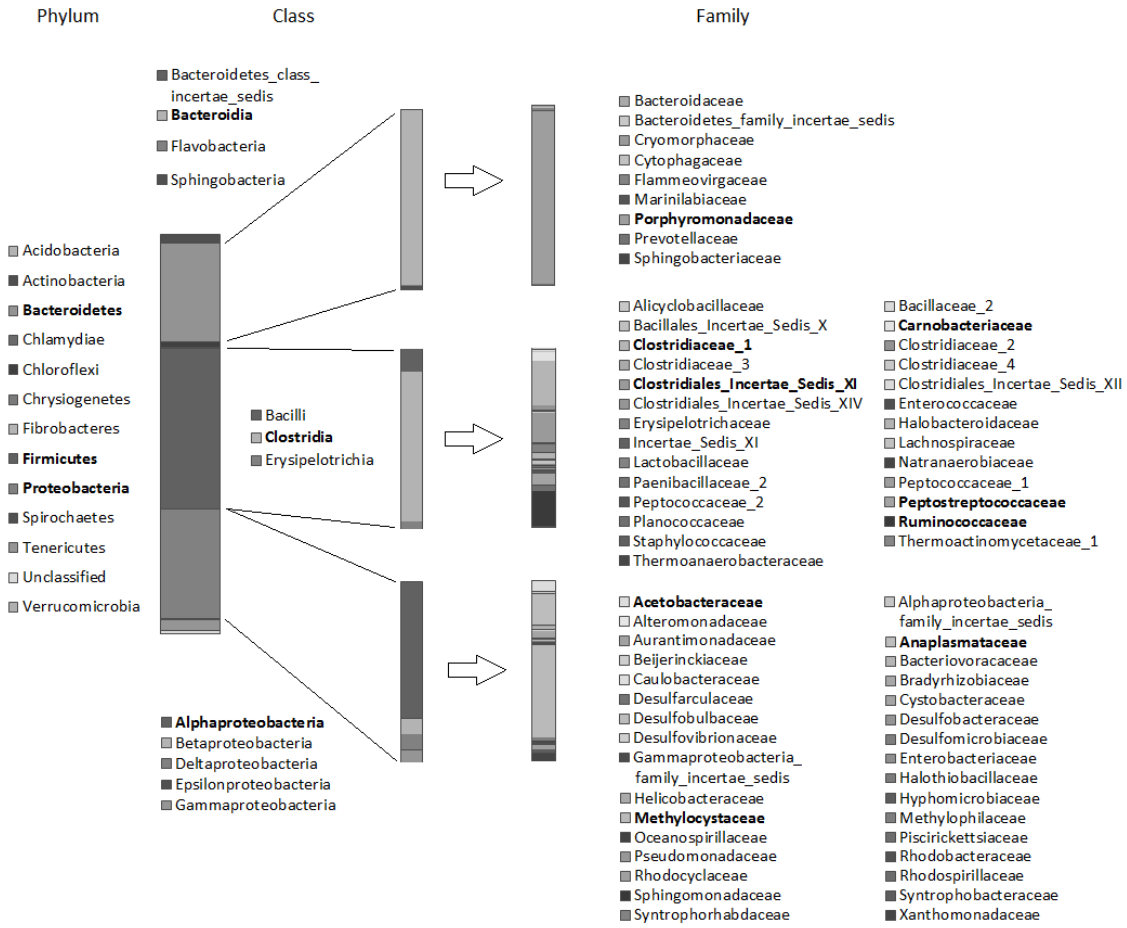

Figure 3.5. Bacterial communities of 14 foaming liquid samples (on average). Words in bold type indicate bacteria groups that take the most major portion at their level.

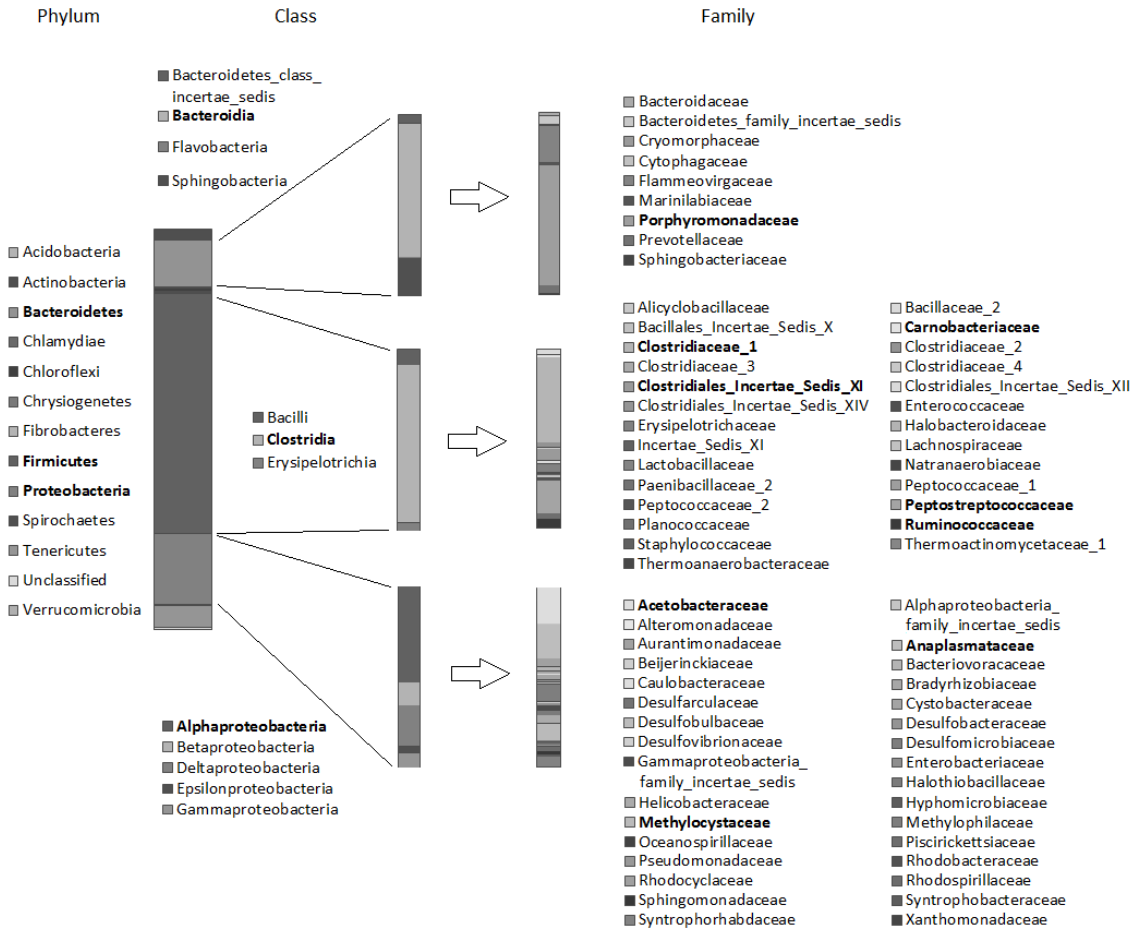

Figure 3.6. Bacterial communities of 11 non-foaming samples (on average). Words in bold type indicate bacteria groups that take the most major portion at their level.

The bacterial communities on samples collected from farm 3 were also investigated to verify if the bacteria distribution is affected by foaming process. These 15 samples were categorized into four groups by their sampling depth in pit. Result shows that the bacteria communities among these four groups of samples were similar (Fig. 3.7). *Bacterioidia*, *Bacilli*, *Clostridia* and *Alphaproteobacteria* are the same major classes, and their relative percentages are similar in these four groups of samples. This similarity indicates that the bacteria distribution in this foaming pit is homogeneous. No specific

bacteria species were found to be accumulated in the top layer that induce the foaming, and foaming in swine manure has higher possibility to be triggered by the change of manure properties.

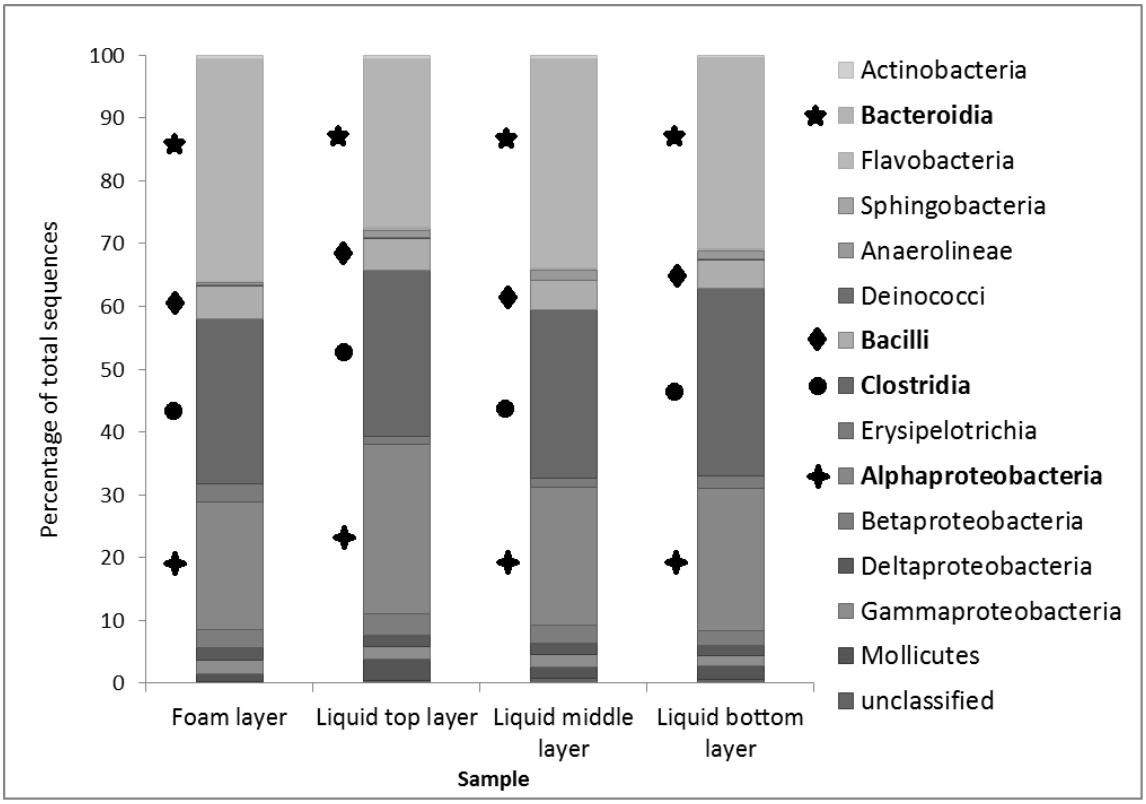

Figure 3.7. Distribution of bacteria classes in four sample layers collected from farm 3 (in average). Words in bold type indicate bacteria groups that take the most major portion.

3.4 Discussion

Foaming in wastewater treatment plants has been extensively studied for a long time. Former research on these foaming cases described the problem as the result of a combination of the presence of filamentous bacteria, surfactant and biosurfactants, in which filamentous bacteria is the primary contribution. Their hydrophobic membrane helped to increase the surface activity and promote stable foam (Kragelund, C., et al., 2007). Based on these research experiences, it is initially hypothesized that amplified

filamentous bacteria in manure is the most important cause of swine manure foaming. Before the sequencing was conducted in this project, a preliminary microbial community examination using DGGE was done on a few manure samples taken from one foaming farm and one non-foaming farm. Gel image results showed the similarity in microbial diversities between the foam layer samples and foaming liquid samples, and significant differences between foaming and non-foaming samples were also observed. The preliminary examination partially confirms the assumption that microbial community difference exists between these two types of samples. However, it is doubtful if filamentous bacteria are the leading cause of manure foaming. FISH analysis in this study shows a high abundance of filamentous bacteria in one of the non-foaming sample, with concentration similar to foaming sample collected on the same farm. Moreover, no significant amount of filamentous bacteria was observed from illumina sequencing, not even from any foam layer sample. This result indicates that the abundance of filamentous bacteria is not an essential component in the foaming process, and samples without filamentous bacteria can also obtain a strong foaming capability. With insignificant amount of filamentous bacteria in the system, there are few hydrophobic membranes existing to create stable foam. In order to induce foaming in swine manure, other specific compounds serve as the surfactant and stabilizer. Compared to filamentous bacteria, these components might be more frequent and important in manure foaming.

Together the detail distribution of both the phylum and family level based on the illumina sequencing result, the primary shift of bacteria community can be briefly described as a higher abundance of *Bacteroidia* and *Proteobacteria* in foaming farms. In

Bacteroidia, typical species under the *Porphyromonadaceae* family is *Butyricimonas synergistica*, which is a gram negative bacteria that obligatory anaerobic growth and produces butyric acid as a major end product (Sakamoto, M., et al., 2009). *Flammeovirgaceae* was found to be in abundance in non-foaming farms, and bacteria under *Flammeovirgaceae* family discovered from sequencing are *Perexilibacter*, a strictly aerobic gram-negative bacterium (Yoon J et al., 2007). In *Proteobacteria*, the most abundant family *Acetobacteraceae*, *Anaplasmataceae* and *Methylocystaceae* all belong to the *Alphaproteobacteria* class, which is a diverse class of organisms with stalked, stellate, and spiral morphologies. A variety of metabolic strategies are found in alphaproteobacteria, including photosynthesis, nitrogen fixation, ammonia oxidation, and methylotrophy (Williams K.P., et al., 2007). For *Acetobacteraceae*, all members are aerobic gram-negative rods. Their common feature is the aerobic oxidation of ethanol to acetic acid, and further oxidized to CO_2 and H_2O (Kersters et al., 2003). For *Anaplasmataceae*, bacteria discovered from sequencing are *Wolbachia*, one of the world's most common parasitic microbes and is possibly the most common reproductive parasite in the biosphere (Werren J.H., et al., 1995). For *Methylocystaceae*, all members are identified as methanotrophs, which utilize methane and methanol to obtain carbon and energy, and also are involved in the assimilation of ammonia and nitrate (Bowman, J., 2000).

In contrast with *Bacteroidia* and *Proteobacteria*, *Firmicutes* were found to have higher abundance in non-foaming farms. At the family level, *Clostridiaceae 1* and *Peptostreptococcaceae* have higher abundance in non-foaming farms, in which

Clostridium (sensu stricto) and *Clostridium XI* are the core genus found during sequencing. *Clostridium* is an anaerobic gram-positive bacteria and the main microorganism in anaerobic digestion, participating in the hydrolysis of lignocellulosic material, protein, lipid and other substrate. *Carnobacteriaceae*, *Clostridiales_Incertae_Sedis_XI* and *Ruminococcaceae* were found to have higher abundance in foaming farms, in which *Clostridiales_Incertae_Sedis_XI* and *Ruminococcaceae* belongs to the *Clostridiales* order and have similar function to *Clostridiaceae* and *Peptostreptococcaceae* as acidogenic bacteria in anaerobic digestion (Biddle A., et al., 2013). Compared to the families discussed above, *Carnobacteriaceae* represent a small portion of *Firmicutes* in these samples. Typical microorganism under the *Carnobacteriaceae* family is *Carnobacterium*, which is an anaerobic bacterium and involved in food spoilage under low temperature (Leisner, J.J., 2007).

Although significant microbial community shift was discovered, none of the abundant bacteria discussed above were reported as foam inducing bacteria. In this situation it is more possible that the shift of the bacteria community is the consequence of foaming rather than the cause of manure foaming. It is noticed that *Bacteroidia* and *Proteobacteria* are both gram negative bacteria, their enrichment in foaming manure may be considered as an indication that manure composition in foaming manure may be changed, resulting in an inhibition of gram positive bacteria. Chemical analytes on the 41 samples also indicate that zinc had a significant negative effect on the relative abundance of *Clostridiales* (Spearman's $r = -0.391$, $P = 0.011$), while potassium (K_2O) and copper (Cu) had significant positive effects on the relative abundance of *Bacteroidales* (P =

0.038 and 0.011, respectively). The composition of manure sample may have a strong influence on microbial community, and the possible change of composition could be a more important factor on manure foaming compare to filamentous bacteria.

On the other hand, does the shift of microbial community only serve as a passive consequence of foaming? Compared to gram positive bacteria, a typical gram negative bacterial envelope comprises of the plasma membrane, periplasm, peptidoglycan and the outer membrane. Due to the outer membrane, surfaces of gram negative bacteria have a higher content of lipid, lipoprotein and lipopolysaccharide. Cell wall with these components may not be able to serve as surfactants, but it is worth trying if they are relatively easier to bind with the surfactant and get settled in the liquid film to block the pathway of liquid drainage. This serves to further confirmation if gram negative bacteria involved in foaming process.

3.5 Conclusion

FISH analysis shows that filamentous bacteria exists in both foaming and non-foaming manure sample in a relative similar abundance, and sequencing result indicates that filamentous bacteria do not occupy a significant portion in manure samples. Difference in microbial community exists between foaming and non-foaming manure, but there is no direct evidence to claim the relative enriched bacteria as the major inducing factor in foaming phenomenon. The shift or change of the bacteria community is highly possible to be the consequence of foaming.

Chapter 4

Laboratory Storage Simulation to Study Swine Manure Foaming

Outline

Foaming in deep manure pits beneath swine buildings has become a serious safety concern during the past few years in the Midwestern U.S. In addition to the loss of manure storage capacity, this foaming creates a serious safety risk of flash fires and explosions. In order to understand the mechanism of manure foaming, manure samples taken from foaming and non-foaming pits were studied to reveal potential causes. Among various compositional components found in the foaming and non-foaming manure samples, long-chain fatty acids (LCFAs) were found to be a major contributing factor. Adding or removing LCFAs in swine manure samples led to a significant change in their foaming capability. A significantly higher concentration of LCFAs was also detected in the foam layers of foaming manure samples. LCFA surfactants are stimulating foaming, and the sources of these surfactants need to be determined in order to develop long-term mitigation plans for manure foaming.

4.1 Introduction

Grow-finish swine buildings in the upper Midwestern states of Minnesota, Iowa, and Illinois are primarily built with deep-pit manure storage; thus, the barns act as both animal housing and manure storage structures. Slatted floors in these barns allow manure to drop to the deep pit below, and manure is pumped out of these barns either one or two times per year and applied as fertilizer on cropland. In recent years, a significant increase in foaming in deep-pit manure storages has been observed in Midwestern swine production facilities (Schmidt and Jacobson, 2010; Burns, 2010). When viewed in a commercial barn, the foaming manure is slightly lighter in color than the dark-colored non-foaming manure, with small and stable gas bubbles in the top layer. The persistent foaming can grow to depths greater than 1.2 m (4 ft), trapping high concentrations of methane (60% to 65%) due to the anaerobic degradation processes in the stored manure (Moody et al., 2009). Foaming reduces the storage capacity of the manure pit. In some cases, foam comes up through the slats and forces removal of the pigs from the barn (Fig. 4.1). In addition, when the foam is disturbed by manure pumping or agitation, the sudden release of entrapped methane inside the barn can cause an explosion or flash fire when an ignition source (e.g., heater pilot light, spark from grinding, welding, light switch, or cigarette) is present. This has become a human safety hazard, causing serious and anxious concerns for swine producers, employees, and insurance companies. Foaming manure has been implicated as the underlying cause of several barn explosions (referred to as flash fires) in Minnesota, Iowa, and Illinois (Dehdashti, 2011). Recent fires associated with foaming manure have caused extensive building damage, with pigs being relocated or

marketed early, or immediately euthanized if severely burned (typical capacity is 500 to 2500 pigs per building). Workers have been injured by being propelled by the blast or exposed to intense heat; in one case, two workers were hospitalized with second- and third-degree burns (Moody et al., 2009). In addition to the Midwestern U.S., foaming in manure pits has also been reported in animal facilities in Canada, the United Kingdom, and France (personal communication).

To understand the severity of foaming, a survey was conducted in 2010 to estimate the foaming situation in southern Minnesota and northern Iowa. Of 153 producers surveyed, 94 responded, and 25% of the farms reported foam in their manure pits, along with two fires and one explosion (Jacobson et al., 2011). Meanwhile, analysis of three pairs of manure samples (two pairs came from foaming and non-foaming barns on the same site, and one pair came from barns that fed distillers dried grains with soluble (DDGS) in one barn and not in the other barn, with neither barn having foam at the time of sampling) showed no large difference between foaming and non-foaming manures, except a slight increase of total fat concentration in foaming manure (26% to 40% higher) and higher levels of trace metals, including magnesium, copper, iron, manganese, and zinc (Jacobson et al., 2011). From the paired samples, there was no statistical evidence to correlate management, diet, genetics, building age, or design features to foaming. However, the dramatic increase of manure foaming over the last few years appears to coincide with the recent dietary addition of DDGS. DDGS is a major co-product of ethanol production from Midwestern corn dry-milling facilities. DDGS production has increased with the recent development of large-scale bioenergy facilities to convert corn

starch and soybean oil to fuel ethanol and biodiesel. DDGS has about the same energy content as corn. It is used in the swine industry as a feed ingredient to partially replace soybean meal, corn, and inorganic phosphorus in swine diets for cost savings, while pig performance is only slightly affected (Whitney et al., 2006). The addition of DDGS to swine diets is normally at a rate ranging from 10% to 20%, but DDGS can be included at higher levels (40%) in some grow-finish diets without negative impacts on pork production and quality (McClelland et al., 2012).

Figure 4.1. Foam inside a swine finishing facility, Receive from Dave Preisler

The cause of manure pit foaming is unknown, but similar foaming cases have been observed in wastewater treatment plant for decades, especially in activated sludge systems (Blackall et al., 1991; Pagilla et al., 1997; de los Reyes et al., 2002; Rossetti et al., 2005; Thomas, 2006; Shen et al., 2007; Ganidi et al., 2009; Petrovski et al., 2011). Most research identified massive growth of filamentous bacteria and actinomycetes containing mycolic acid as the primary cause; de los Reyes and Raskin (2002) even proposed threshold concentrations of these filamentous bacteria cells for the initiation of foaming. However, studies also showed evidence that undue emphasis has been given to

filamentous mycolata in association with foaming (Davenport and Curtis, 2002), and the mycolata cell threshold concentrations may only be applicable to mycolata members with more hydrophobic cell walls (Davenport et al., 2008). Thus, as a result of these recent studies, other non-filamentous bacteria and surfactants are being investigated, rather than targeting specific filamentous bacteria, to explain foaming in wastewater treatment plants (Schilling and Zessner, 2011).

The formation of foam requires three components: (1) gas bubbles surrounded by a liquid film, (2) surfactants that reduce surface tension, preventing liquid drainage from the gas bubble walls, and (3) small hydrophobic particles for long-term foam stabilization (Heard et al., 2008; Petrovski et al., 2011). The surfactant in solution that initializes the foam formation could be either a synthetic surfactant from the influent or a bio-surfactant generated by microorganisms, such as the glycolipids produced by various sludge bacteria, e.g., actinomycetes, *Pseudomonas* species, or *Acinetobacter* species (Lemmer et al., 2005). In swine pit manure, gas bubbles are constantly generated during the natural anaerobic degradation process, but the surfactant and hydrophobic particle stabilizer are unidentified. The purpose of this research is to identify the key components that contribute to manure foaming and look for possible correlations between foaming and dietary DDGS. This research will help to understand the cause of manure foaming, and possibly provide clues on how to prevent or mitigate manure foaming.

4.2 Material and Method

Measurement of Foaming Index

Swine producers are usually not aware of the foaming situation until the foam literally comes up through the floor slats. Before the foam come up, accurately measuring the depth of the foaming layer in the manure pit is difficult because of the nonhomogeneity of the manure and the limited accessibility of the pit for measurement. This leads to errors and inaccurate assessment in foaming reports provided by barn managers about the level of foaming in manure pits. In this study, a foaming index (FI), defined as the foaming capability of manure samples, was determined by adding 25 mL of manure sample to a 100-mL volumetric graduated cylinder with an inner diameter of 2.54 cm. Nitrogen gas was pumped into the cylinder bottom through a plastic pipette (1 mm tip diameter) at a constant flow rate of 100 mL min^{-1} to generate bubbles. Nitrogen gas was used instead of air so that no oxygen was introduced for potential oxidation during tests, and the density of the gas is approximately the same as biogas. The volume that stable bubbles reached was recorded as the FI, with a maximum of 80-mL in the volumetric graduated cylinder. To increase the upper limit of 80 mL for the FI, a 1 m length transparent pipe with 2.54-cm inner diameter was used to reach a maximum detection limit of 450 mL for some samples. The FI of each manure sample was measured in triplicate when possible, and the average and standard error were calculated for comparison.

Study of factors that may affect foaming

A lab-scale manure storage test was developed using composite additions to simulate the manure pit storage process seen in the field and to determine if a composite

addition can affect the foaming performance of manure. The manure samples used in this experiment were collected from two barns using similar diets and management: one with foam and the other without foam. A third set of samples came from anaerobically digested swine manure collected at the swine facilities of the UMN South Central Research and Outreach Center in Waseca, Minnesota; these samples were used to explore if anaerobic digestion has an impact on foaming.

The lab-scale manure storage was conducted based on the following steps: 125 mL of swine manure was poured into a 250 mL flask, covered with aluminum foil, and left at room temperature for four weeks with slight shaking every two days. Assuming that filamentous bacteria species may exist only in foaming manure, two sets of experiments were conducted: one with non-foaming manure (125 mL) and the other with non-foaming manure (100 mL) seeded with an additional 25 mL of foaming manure. The manure storage tests included addition of the following composites: volatile fatty acids (VFA), trace metals (Mg, Cu, Fe, Mn, and Zn), corn oil, DDGS, and yeast extract. Of these composites, the trace metals and corn oil (lipid) were parameters that differed between foaming samples and non-foaming samples. The composites were added to the manure at the beginning of the lab-scale storage tests. Manure from the flasks was used to determine the FI at the end of the first week and fourth week.

The first set of lab-scale storage samples identified lipid as a significant factor in foaming, so another set of lab-scale storage samples was made with additions of oleic acid (a corn oil hydrolyzate) of 0.3, 0.5, and 0.7 mL and DDGS additions of 3.0, 5.0, and

7.0 g. The same method as described above was followed, using 100 mL of non-foaming manure and composite addition. All storage tests were conducted in triplicate.

Foaming and non-foaming manure sampling

A second round of sampling of foaming and non-foaming manure was conducted to test differences in FI and LCFA concentration using a different manure source. Field manure samples for the compositional analysis were collected from five farms in Iowa and Minnesota: four of the farms were reported to have foaming, and the fifth farm was free of foaming. Manure samples were taken from four pit depths in each barn. From highest level to lowest level, the samples were marked as (1) foaming layer (taken directly from the foam), (2) liquid top (the top layer of liquid manure), (3) liquid middle (the intermediate layer of liquid manure), and (4) liquid bottom (the bottom layer of liquid manure). The manure samples collected at the non-foaming farm lacked a foaming layer. Based on inquiries with the farm managers, no anti-foaming or defoaming agents were applied at these five sampling sites. Altogether, 41 samples were collected, chilled immediately after collection, and stored at -20 ℃ until analyzed.

Measurement of LCFA in Manure

The LCFA concentration in each of the 41 manure samples was measured by gas chromatography (GC) based on the following steps: the manure samples were freeze-dried, ground to powder, and mixed with chloroform and methanol to extract fatty acids (Folch et al., 1957). Approximately 0.3 g of dry manure powder was added to 10 mL of

chloroform and methanol solvent (chloroform:methanol = 2:1), spun at 150 rpm for 16 h, and then 2.5 mL water was added to the mixture. The mixture was then vortexed for 1 min and centrifuged at 7000 rpm for 7 min to separate the chloroform into the bottom layer. The chloroform layer was then filtered through a 0.45 μm filter to remove solid particles. LCFA was detected in the chloroform layer using GC (6890, Agilent Technologies, Santa Clara, Cal.) with a flame ionization detector and DB-FFAP capillary column. The oven temperature was set at 100 ℃, held for 5 min, raised to 240 ℃ at a rate of 10 ℃ min^{-1}, and held at 240 ℃ for 20 min. The injector temperature was set at 250 ℃, and the detector temperature was set at 300 ℃. Hydrogen was used as the carrier gas at a flow rate of 0.75 mL min^{-1}. Separate LCFAs in the samples were identified and quantified by comparing the peak area with standard chemicals (palmitic acid, stearic acid, oleic acid, and linoleic acid; Sigma-Aldrich, St. Louis, Mo.).

Statistical Analysis

All analyzes except LCFA measurements were done in triplicate. Means and standard errors of results were calculated to evaluate the differences between samples. In the LCFA analysis, the mean and standard deviation (SD) of each category was calculated for all 41 field manure samples. Results were analyzed with SPSS software to evaluate differences obtained from each sample, and a p-value of 0.05 was used to distinguish differences as significant or insignificant.

4.3 Results

Effects of Composite Additions on Foaming

Non-foaming manure showed small but positive FI values, while foaming manure samples had significantly higher FI values than non-foaming manure samples (Fig. 4.2). Adding corn oil to foaming manure immediately dropped the FI to nearly zero, while DDGS and yeast extract additions to foaming manure did not affect the FI (Fig. 4.2). This result suggests that corn oil can serve as an immediate defoamer when added to foaming manure. For long-term (four weeks) storage, addition of composites such as yeast extract, DDGS, VFA, and trace metals did not significantly affect the FI values of foaming or non-foaming manure (Fig. 4.3). However, dramatic changes were recorded for the manure samples with corn oil addition after four weeks of storage. After one week of storage, the manure samples with corn oil addition had FI values similar to the other samples, although the corn oil manure samples seeded with foaming manure had a slightly higher FI than the other samples. After four weeks of storage, all samples with corn oil addition showed significant and consistent foaming capability. The FI values exceeded the measuring limit of 80 mL, while all other manure samples with the addition of composites other than corn oil did not show an FI increase and remained non-foaming (Fig. 4.3). Addition of foaming manure did not increase the FI values of these samples. The anaerobically digested manure samples typically had lower FI values than the non-foaming raw manure samples. However, after corn oil addition and four weeks of storage, even the anaerobically digested manure samples had high FI values.

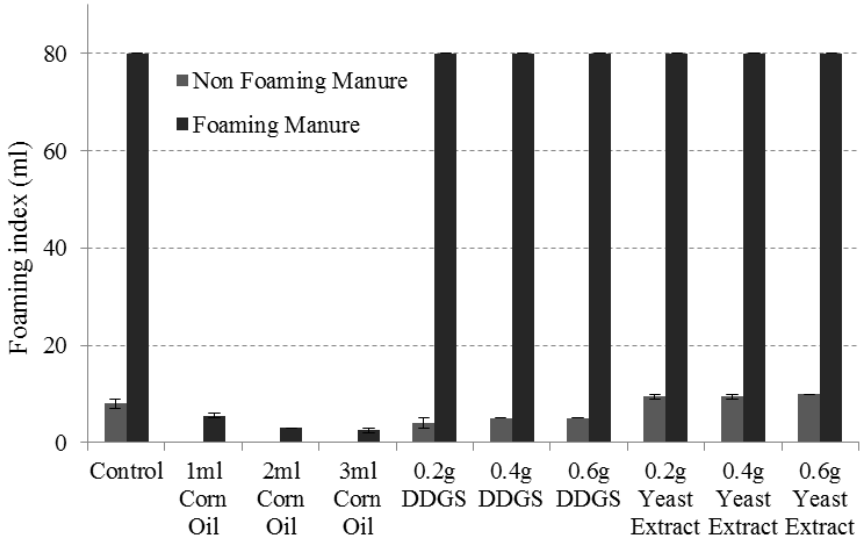

Figure 4.2. Foaming index of manure at the time of adding supplements. A: non-foaming manure. B: foaming manure

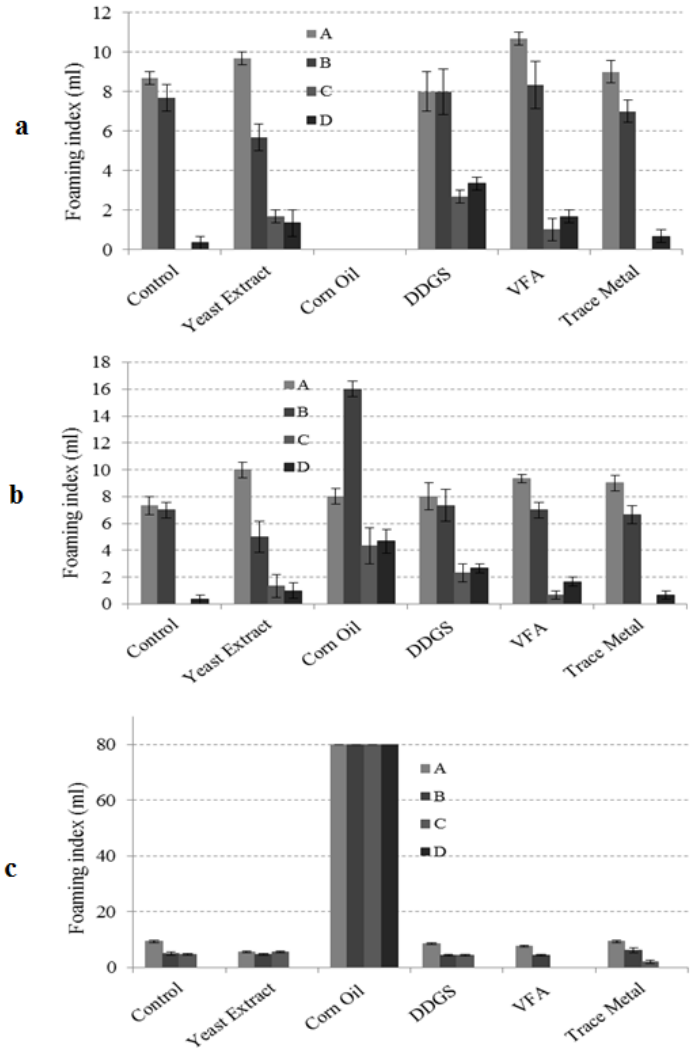

Figure 4.3. Lab scale manure storage with different nutrient supplements (A: Raw non-foaming manure, 125 ml; B: 25 ml foaming manure added into 100 ml no foaming manure. C: Digested non-foaming manure, 125 ml; D: 25 ml foaming manure added into 100 ml digested non-foaming manure) a: Starting point after adding nutrient supplements. b: One week storage (cultivation). c: Four weeks storage (cultivation).

List of addition:

Yeast extract: 0.2 g into 125 ml manure

Corn oil: 2.5 ml into 125 ml manure

DDGS: 0.2 g grounded DDGS into 125 ml manure

Fatty acid: 125 mg acetic acid into 125 ml manure

Trace metal: 0.7g L-1/L Mg^{2+}, 0.03g L-1/L Cu^{2+}, 0.1g L-1/L Fe^{2+}, 0.02g L-1/L Mn^{2+}, 0.07g L-1/L Zn^{2+} solution, 5 ml stock solution into 125 ml manure

Effects of Oil Hydrolyzates in Foaming

The manure storage experiment identified corn oil as a significant foaming enhancement factor during long-term (four weeks) storage. This result suggests that oleic acid, linoleic acid, and glycerol, as the major hydrolyzates of corn oil, are possible foaming inducers. To verify their impact on manure foaming, a quick experiment was conducted by adding them to non-foaming manure (Fig. 4.4). When oleic acid and linoleic acid were gradually added to non-foaming manure, a drastic increase of FI was detected immediately after the fatty acid concentration reached certain thresholds (Fig. 4.4). These thresholds for the tested non-foaming manure were recorded as 2 mL L^{-1} manure for oleic acid and 8 mL L^{-1} manure for linoleic acid. When the oleic acid and linoleic acid concentrations reached their threshold values, the FI value was proportional to the amount of fatty acid added to the manure. However, increasing the LCFA concentration does not create unlimited foaming capability. Although the threshold values were different for oleic acid and linoleic acid, adding excessive amounts of these two fatty acids to manure decreased the FI to the original value. This defines the LCFA concentration range in which manure foaming occurs (Fig. 4.4). Glycerol was also evaluated but did not show any positive effect on foam generation.

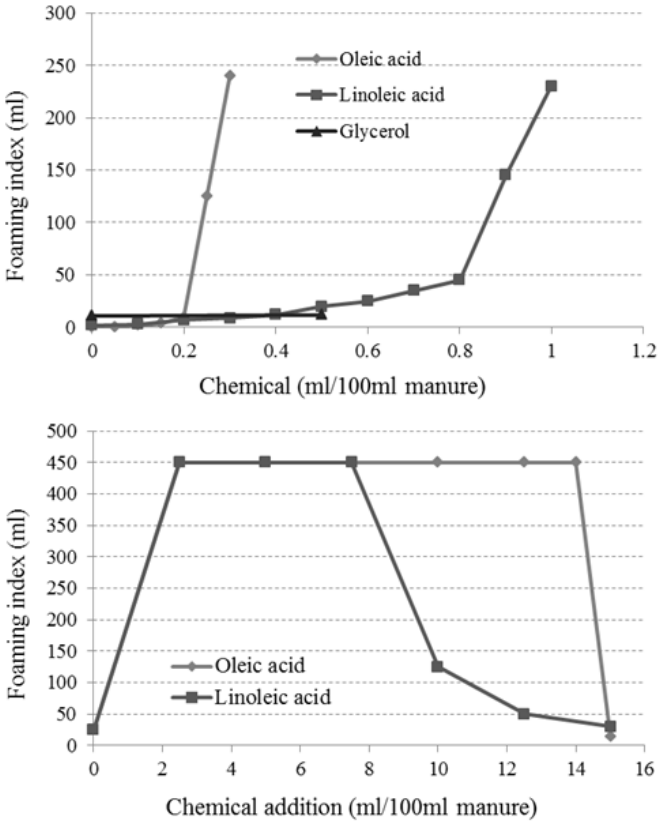

Figure 4.4. Impact of oleic acid and linoleic acid to manure foaming.

Long-Term Impact of LCFAs and DDGS

The addition of LCFAs to non-foaming manure immediately increased the FI value to the detection limits and then gradually decreased the FI to nearly zero within three weeks of storage. The highest FI was recorded after the first week (Fig. 4.5). The LCFA concentrations in the samples started to decrease after the first week, and greater additions of oleic acid slightly slowed the FI decrease over the storage time (Fig. 4.5). Adding DDGS to non-foaming manure did not initially result in foaming, but the FI slightly increased from the first week. The highest FI was recorded after the fourth week, and the LCFA concentrations in the samples also started to decrease after the fourth week. Compared with oleic acid, DDGS addition led to lower FI values, but the FI was longer

lasting, and the LCFA concentration was more stable (Fig. 4.5). It is assumed that the manure samples stored in the flasks were anaerobically digested, and the oil was degraded to LCFA and then further degraded to shorter-chain fatty acids. After further degradation of LCFAs, the FI value dropped, and the manure became non-foaming.

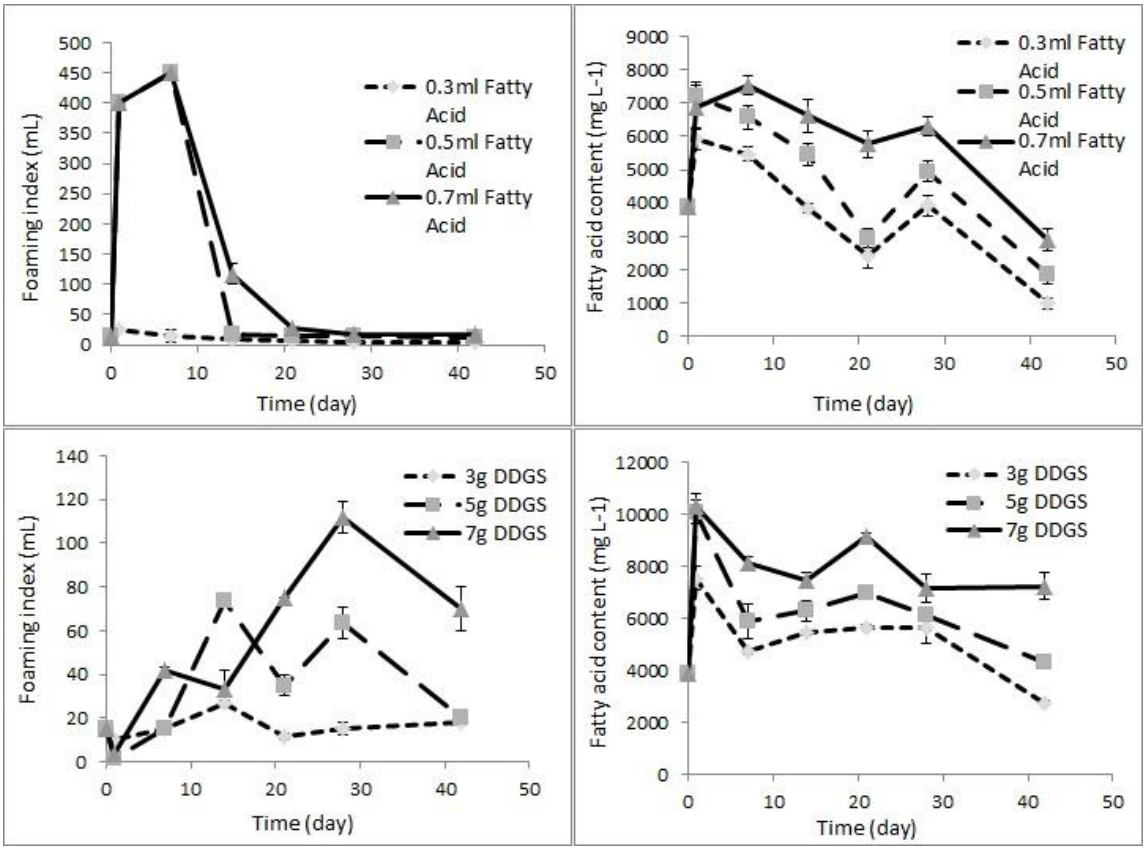

Figure 4.5. Foaming index (left) and fatty acid content (right) with additions of oleic acid (top) and DDGS (bottom).

LCFA Measurement of Field Manure Samples

Although manure samples from different pits varied in their fatty acid concentrations, the measurement of LCFAs in the field manure samples showed that the foam samples contained higher LCFA concentrations than the liquid portions of foaming and non-foaming samples ($p = 0.001$) (Table 4.1). This difference was especially

significant between the foam layer and the liquid portion of foaming manure ($p = 0.000$). Comparing the mean values in these three sample categories shows that LCFAs were highly accumulated in the top layer in foaming manure pits. The correlation between sample FI and LCFA concentration provides evidence that LCFAs are more likely to be a necessary factor in manure foaming (Fig. 4.6). Although not every sample with a high LCFA concentration had a high FI value, all samples with high FI values also had high LCFA concentrations.

Table 4.1. LCFA and FI analysis of field manure samples.

Sample	Palmitic Acid (mg L^{-1})	Stearic Acid (mg L^{-1})	Oleic Acid (mg L^{-1})	Linoleic Acid (mg L^{-1})	Sum of Acids (mg L^{-1})	Foaming Index (mL)
Foam layer of foaming manure						
Mean	650.5	1652.4	1706.6	148.4	4147.4	246.9
N	14	14	14	13	14	13
SD	309.9	1019.8	978.7	91.7	2183.0	214.2
Liquid layer of foaming manure						
Mean	212.8	555.1	483.0	62.3	1286.0	4.4
N	16	16	16	9	16	16
SD	212.1	522.0	778.1	69.1	1541.2	3.4
Non-foaming manure						
Mean	736.3	1395.5	943.5	89.5	3156.8	1.2
N	11	11	11	10	11	11
SD	257.2	392.6	311.0	123.2	882.3	1.5

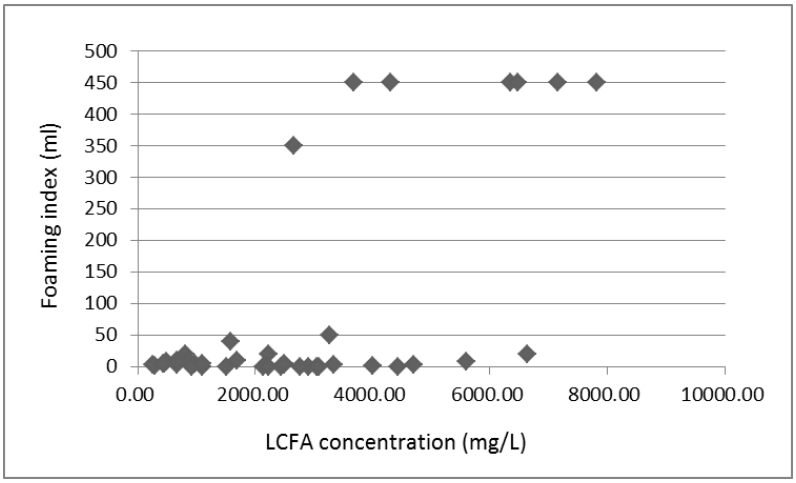

Figure 4.6. Relationship of LCFA concentration and foaming index.

4.4 Discussion

Biological Foaming or Chemical Foaming?

Foaming needs three components: a gas, a surfactant, and a stabilizer (Petrovski et al., 2011). Foaming that occurs in various industrial processes is traditionally categorized into two types, each with different components serving as surfactants and stabilizers. The first type is chemical foaming, which is primarily caused by high concentrations of chemical surfactants, e.g., detergents in municipal wastewater. This type of foaming is generally white in color and light in quality. The gas bubbles are usually not very stable and can be easily disintegrated by spraying water on the surface. The second type is biological foaming, which is generally dark brown in color with small, stable gas bubbles (Srinivasan and Viraraghavan, 2007). Massive growth of certain filamentous bacteria and actinomycetes (Shen et al., 2007) is generally found in this type of foaming. *Microthrix parvicellatha*, which have fast growth at high lipid concentrations and low temperature conditions (Srinivasan and Viraraghavan, 2007), are considered the most commonly seen bacteria among these filamentous bacteria species (Xie et al., 2007). This traditional classification of foaming relies on the type of stabilizers in the foam, such as non-microbial hydrophobic particles in chemical foaming and filamentous bacteria with hydrophobic cell membranes in biological foaming.

In swine manure pits, continuous anaerobic digestion produces biogas, which floats to the manure surface and acts as the gas source. Deep-pit manure foaming was originally suspected to be biological foaming because the foam is very stable and dark. However,

the difference in FI between samples seeded or not seeded with 25% foaming manure never exceeded 5 mL in this study, which is hard to describe as a large difference. This is not an indication of biological foaming, and therefore doubtful that the foaming is induced by certain filamentous bacteria. In addition to the potential filamentous bacteria in the manure samples, the high concentrations of solids, such as undigested fine fibers, in the manure may abundantly serve as hydrophobic particles. It was discovered that the foaming manure samples contained significantly higher concentrations of total lipids in the foaming layer, which may lead to an increase of LCFAs in the manure solution and contribute to the foaming process. Meanwhile, other types of surfactant components may also exist in foaming manure samples, as the LCFA concentration could not be directly correlated to the foaming capability of manure, as shown in Figure 4.6. In this situation, manure pit foaming should not be simply categorized as purely biological foaming, since bacteria may not play a key role in inducing the foam.

Oil: Defoamer of Foam Inducer?

In the experiment described, corn oil could be considered a defoamer since it immediately removed the foaming bubbles. However, corn oil induced foam formation after only one week of storage, even in the anaerobically digested manure samples. During storage in the flasks, the oil could be initially degraded to LCFA and glycerol, and then further degraded to shorter carbon chain derivatives. Adding glycerol to the non-foaming manure did not affect the FI value, but the addition of both oleic acid and linoleic acid led to high FI values with very stable foam bubbles. Thus, adding LCFA to a

non-foaming sample converted the sample to a foaming manure sample. From an earlier survey (Jacobson et al., 2011), numerous swine producers have reported that adding plant oils to foaming manure pits is an effective practice to initially eliminate the foaming. However, from this study, this defoaming effect is lost after a certain time period, and foaming in manure pits could be even worse because of the oil addition.

Figure 4.6 suggests that LCFA concentration is not linearly related with FI, as some manure samples had a low FI even with a high LCFA concentration. Therefore, LCFAs may not be the only components that are inducing foaming. Other components in manure may also impact the foaming process. The LCFAs detected in the manure samples included palmitic acid, stearic acid, oleic acid, and linoleic acid. Oleic acid and linoleic acid both have low solubility in water, and palmitic acid and stearic acid both have a melting point higher than 60 ℃ and should maintain a solid state at room temperature. Therefore, there is a high probability that these fatty acids were attached to some hydrophobic solid particles instead of being well dissolved in the manure samples. This is further supported by the fact that the foaming layer samples had higher contents of both solids and LCFA. Future studies on solid particles in manure may provide more detailed information and may reveal other factors that impact manure foaming in addition to LCFA.

DDGS's Role in Manure Foaming

The DDGS used in swine diets during the time when the foaming pits were reported had around 10% fat, much higher than the 4% in the original corn (Stallings, 2009). In

the storage experiments with addition of DDGS, DDGS increased the lipid concentration in manure and therefore induced foaming when the lipid was hydrolyzed to LCFA. In Figure 4.5, the FI and LCFA concentration in samples have a generally similar trend, but this trend is not followed completely. One possible reason is that the extraction efficiency of LCFAs from DDGS may not high. Although it is obvious that the addition of DDGS increased the LCFA concentration in manure, The LCFAs were not fully released into the manure solution, and only parts of the LCFAs in DDGS were detected by the GC analysis. DDGS is added as part of the animal feed, and the undigested components end up in the manure pit. Recent use of DDGS in the U.S. swine industry has increased the fat content in animal diets and consequently the LCFA concentration in animal manure. DDGS can increase the softness of bacon (Whitney et al., 2006), but how it affects manure composition is unknown. Compared to corn, DDGS has higher energy concentration and higher crude fat content. The higher energy concentration makes DDGS similar to corn in digestible and metabolizable energy content, despite its lower digestibility, and the higher crude fat content increases the oil concentration, especially unsaturated fatty acids, in animal diets. If DDGS is the cause of manure foaming, then the DDGS production process can probably be improved by reducing the crude fat content and utilizing the fat for other value-added products.

4.5 Conclusion

Studies on lipids and solid particles provide clear evidence that lipids and solid particles have a strong correlation with swine manure foaming process. From the

simulation in laboratory setting, LCFAs were found to be a major contributing factor, possibly serve as surfactant in manure pit. This find makes DDGS a high potential cause of foaming happened in manure pits.

Chapter 5

Exploration of surfactant and stabilizer in swine pit foaming

Outline

The solid components that can be separated from foaming manure samples were shown to have the capability to induce foaming. Separation of solids with different centrifuge speed indicates that smaller size of solid particles have higher foaming inducing capability, and also a higher hydrophobicity. Protein was shown to have a strong correlation with swine manure foaming in this study. Meanwhile, a high concentration of total bile acids was also discovered in manure foaming layer, providing the hypothesis that bile acids may be a major surfactant existed in foaming process.

5.1 Introduction

Grow-finish swine buildings in upper Midwestern states including Minnesota, Iowa and Illinois are primarily built with deep manure pit storage below animal housing as manure storage structures. In recent years, a significant increase in foaming in deep pit manure storages has been observed in Midwestern swine production facilities (Schmidt & Jacobson, 2010; Burns, 2010). Foam reduces storage capacity of the manure pit and creates manure management and animal respiration health concern. In some cases the foam came up through the slats and forced the removal of pigs from the barn. The cause of swine manure pit foaming is still unknown, and current strategies to reduce this hazard is addition of de-foamer to break the surface foam or application of additives to decrease the generation of biogas. None of these are permanent treatment as the foam always re-generates in a few weeks, and it is necessary to develop a direct and low-cost strategy to completely eliminate this risk.

The formation of foam requires three components: (i) gas bubbles surrounded by liquid films, (ii) surfactants which reduce the surface tensions, prevent liquid drainage from gas bubble walls and (iii) small hydrophobic particles responsible for the long-term foam stabilization (Heard et al., 2008; Petrovski et al., 2011). In some cases it is hard to clearly distinguish between surfactant and stabilization particles. To understand why manure foams and identify surfactants contained in the manure, we have tried to add different components detected from foaming manure into non-foaming manure samples and evaluate their impact on foaming by detecting sample's foaming capability. It was determined that different foaming capabilities existed between foaming manure and non-

foaming manure, and the addition of long chain fatty acid (LCFA) will greatly enhance manure's foaming capability in the short term (Yan M. et al., 2014). However, LCFAs are very hydrophobic and cannot serve as surfactant by itself. Addition of oleic acid into water will form a thin lipid layer on the surface and will not induce foam. This suggests that LCFAs need to bond with specific components in manure in order to function as a surfactant.

A preliminary test with 5 foaming manure samples shows that the removal of the solid components by high speed centrifugation will greatly reduce the samples' foaming capability. Under this situation, it is important to explore the solids components in manure samples and identify which are the candidates that enhance foaming. Since swine manure is normally used as fertilizer in croplands, most researchers are focused on its nutrition content instead of composition. As a comparison, the major components in dairy manure dry matter include bacteria, crude protein, crude lipid, fatty acids and neutral detergent fiber (Møller H.B. et al., 2014), which are also detected in human feces and expected to be detected in swine manure dry matter. Many researchers have identified massive growth of filamentous bacteria as the primary cause of foaming in wastewater treatment plants (de los Reyes and Raskin, 2002; Rossetti et al., 2005; Shen et al., 2007; Ganidi et al., 2009; Petrovski et al., 2011); and some even proposed threshold concentrations of these filamentous bacteria cells for the initiation of foaming (de los Reyes & Raskin, 2002). However, no filamentous bacteria were discovered during microbial community analysis of manure foaming samples, and filamentous bacteria was not included in our consideration for this particular situation. Lipid and fat have shown to

have a strong foam inducing capability, while the impact of proteins and neutral detergent fibers are yet to be determined. Another frequent detected component in swine manure is bile salt, which has been identified as a typical biological surfactant for a long time (Hofman and Myselsa, 1987). In this study, these three components in manure solids were evaluated to explore their possible effect to foaming in manure pit.

5.2 Material and Method

Sample source

The first set of manure samples were collected from one south Minnesota farm in July 2013. Three barns at this farm were chosen as the sampling site. Barn 1 and barn 2 had foaming occurring on top of the manure stored in the pit while barn 3 was a non-foaming barn with a crust layer on top of the manure surface. Samples from the foaming layer and liquid layer underneath the foam were collected from both barns 1 and 2, marked as 1A (foaming layer), 1B (liquid layer), 2A (foaming layer) and 2B (liquid layer). Samples from liquid layer of barn 3 were collected, marked as 3B. Before the analysis of solid particles, a foaming capability measurement was applied to make sure that suitable samples have been selected. As expected, the top layer of the foaming manure had the most significant foaming capability, and liquid manure had much less foaming capability (Table 5.1).

Table 5.1. Foaming capability of collected original sample

Sample	Foaming capability (ml)	Length of time (min)
1A	450	20
1B	75	1
2A	450	5
2B	25	0
3B	75	2

Solid particles were separated from the second set of manure samples at three different centrifuge speeds (1000 rpm [150 g], 7000 rpm [7000 g], 11000 rpm [20000 g]). This provided three categories of solid samples: solid particles separated by 1000 rpm centrifugation, solid particles separate between 1000 rpm and 7000 rpm centrifugation, and solid particles separate between 7000 rpm and 11000 rpm centrifugation. In total, 15 particle samples were collected from the 5 manure samples for analysis. To maintain the original characteristics, the drying process was skipped and solid samples used for later analysis were mixed slurry with solid content ranging from 11% to 19%.

The second set of manure samples were collected at 20 farms from Illinois and Iowa during Jan 2013 to Sep 2013. Among these 20 farms, 7 farms were defined as foaming sites that had constant foaming problem during the sampling period, 7 farms were defined as non-foaming sites that had no foaming problem. The remaining 6 farms either switched from foaming to no-foaming or switched from no-foaming to foaming, and were defined as switch sites. Samples were collected from different depths in the deep manure pits, categorized as A layer (directly from the foam layer), B layer (from the upper liquid layer) and C layer (from the lower liquid layer). Since A layer was the foam layer, there were no A layer sample collected from non-foaming farms. A total of 108 collected samples were chilled immediately and kept at -20^{o}C before analysis.

Impact of solid particles on foaming capability

The impact of solid particles on foaming was evaluated by testing the foaming capability of manure sample after the addition of solids. Foaming capability is measured by a transparent plastic pipe (ID: 2.54 cm) with a PVC tube cap (ID: 2.54 cm) at the bottom. A gas tube was connected through the cap, equipped with an air stone to provide constant small gas bubbles (Fig. 5.1). The transparent pipe can be extended by coupling another pipe to increase the measurement limitation, and the measurement limitation in the laboratory is 450 ml due to the restriction of laminar flow hood height. Five grams of solid particles was added into ~20 ml of the non-foaming 3B sample creating a total volume of 25 ml. This mixture was then added into the instrument, and N_2 gas was blown into the cylinder at a constant flow rate (100 ml min^{-1}) to generate bubbles. The volume that stable bubbles reached was recorded as the sample's foaming capability.

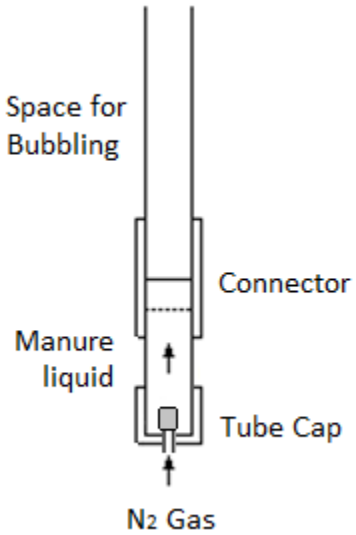

Figure 5.1. Schematic diagram of instrument for foaming capability measurement.

The solid particles' surface energy was measured by a contact angle meter (Kyowa Interface Science MCA-3, Japan). Solid particle samples were firstly coated on glass slides to form a thin flat layer, and then kept at room temperature till they were completely dried. The contact angles were measure by both diiodomethane and distilled water, an average value of five measurements were used to calculate the surface energy and the distribution between dispersive and polar components. Solid particles centrifuged at 1000rpm contain very coarse particles and large fibers, which are very difficult to make into a thin layer and thus were not included in this analysis.

Impact of protein and fiber structure

Protein denaturation and enzyme digestion was conducted to see the impact of protein content destruction on the sample's foaming capability. In the denature step, samples were put into a 80^{o}C water bath for 1 hour and occasionally shaken, and then naturally cooled to room temperature before measuring their foaming capability. In the digestion step, samples were added with Proteinase K (75 mAnson units per 25 ml sample) and put into a 35^{o}C water bath for 6 hours and occasionally mixed, and then naturally cooled to room temperature before measuring their foaming capability.

Cellulose and hemicellulose degrading enzyme were combined (50 units of cellulase, 50 units of hemicellulose and 50 units of pectinase per 25 ml sample) to evaluate the impact of fiber structure on sample's foaming process. In cellulase digestion, samples were also put into 35^{o}C water bath for 6 hours before their foaming capability

measurement. Optimum temperature for cellulase activity is 50^{o}C, but this temperature proved to reduce foaming capability, and was not selected for cellulase digestion.

Detection of protein content in manure samples

Protein concentration detection was conducted using RIPA lysis. For each sample, a 0.8 ml solution was added into a 2 ml centrifuge tube, mixed with 1 ml RIPA lysis buffer, spun for 1 minute followed with a 20 minutes ice bath. This ice bath step was repeated 3 times and then centrifuged at 10,000 rpm at 4^{o}C for 10 minutes. Supernatants were carefully pipetted into a fresh centrifuge tube, using Bio-rad protein assay kit (Bio-rad 500-0002, USA) to detect the protein concentration in lysates.

Detection of LCFA in manure sample

The LCFA concentration in manure samples was measured by Gas Chromatography (GC) method. A 5 ml manure solution was added into a 50 ml centrifuge tube, the pH was adjusted to around 3.0 by concentrated phosphoric acid, and 20 ml of diethyl ether was then added into the centrifuge tube. This mixture was spun for 2 minutes and put on tube rotator (Scientific Equipment Products 60448, USA) for 2 hours. After rotation the contents were centrifuged at 7000 rpm for 5 minutes, and the top diethyl ether layer was filtered through a 0.45 μm filter to remove residual solid particles. LCFA in diethyl ether was detected through GC (Agilent 6890, USA) equipped with flame ionization detector and DB-FFAP capillary column. The oven temperature was set at 100 ℃, held for 5 minutes, then raised to 240 ℃ at a rate of +10 ℃ min^{-1}, and held at 240 ℃ for 20 minutes.

The injector temperature was set at 250 ℃ and the detector temperature was set at 300 ℃. Hydrogen was used as the carrier gas at a flow rate of 0.75 ml min^{-1}. Separate LCFAs in the samples were identified and quantified by comparing the peak area with standard chemicals (palmitic acid, stearic acid, oleic acid and linoleic acid, Sigma).

Detection of total bile acid (TBA) content in manure samples

The analysis method to analyze bile acids consisted of three steps: alkaline hydrolysis to desorb, hydrolyze and deconjugate bile acids; acidification and extraction of unconjugated bile acids into diethyl ether; and evaporation of the ether and dissolve bile acids into methanol for enzymatic assay (Porter J. K., et al., 2003). Hydrolysis and extraction steps are adapted from De Wael L., et al, 1977. In the hydrolysis step, the manure solution was oven dried at 105^{o}C overnight and ground into powder. 50 mg of this powder was accurately weighed into a screwed test tube, and then 1 ml of KOH-ethylene glycol was added and the tube heated at 165^{o}C for 60 minutes and occasionally mixed. After cooling, 1 ml of NaCl solution and 0.2 ml concentrated HCl are added and mixed well. During the extraction step, 3 ml diethyl ether was added into the acidified solution, spun at maximum speed for 1 minute, centrifuged at 2000 g for 3 minutes and collected the upper diethyl ether layer. This extraction step was repeated 3 times and the combined extracts were completely evaporated at 40^{o}C, dissolve residue in 4 ml methanol for enzymatic assay. Enzymatic assay is conducted by colorimetric TBA kit (Diazyme DZ092A-K, USA) following the standard procedure, using NBT method to monitor the formation of formazan dye with the presence of bile acid.

5.3 Result

Samples from the first sample set were used to evaluate the importance of solid particles in foaming process. Higher centrifuge speed isolate particles with smaller sizes, and their capability to induce foaming is stronger (Table 5.2). 1A 7000 rpm, 1A 11000 rpm and 2A 11000 rpm are the most significant foaming inducing sample observed. In surface energy analysis, dispersive components can briefly be considered as hydrophobic, while the polar component can briefly be considered as hydrophilic. All samples have surface energy between 20 and 30 mJ m^{-2}, which are lower than water's surface energy (72.8 mJ m^{-2}). The difference between samples exists in the ratio of dispersive and polar components. Generally, A layer manure samples have smaller portion in the polar component than other layers, and the solid fragment with smaller particle size have a smaller portion in polar component. For example, in both B layer samples, solid separated from higher centrifuge speed have highly reduced value on polar components. Meanwhile, 1A 7000 rpm, 1A 11000 rpm and 2A 11000 rpm samples all have very small portion in polar component compare to their dispersive components. Surface energy is shown to be important in manure foaming process, and in general, less portion of polar component is required to help manure gain the foaming characteristic. The roughness of the thin layer surface was not measured in this test, and it is believed that roughness measurement have small impact on this study.

Table 5.2. Foaming induce capability and surface energy of solid particles separated from manure sample.

Particle added	Solid content (%)	Manure's Foaming capability after solid addition (ml)	Contact angle measurement with water (^{o})	Surface energy (mJ/m^2)	Surface energy polar component (mJ/m^2)
1A 1000 rpm	18.24	100	N/A	N/A	N/A
1A 7000 rpm	19.16	275	99.74	20.58	1.27
1A 11000 rpm	16.82	300	115.68	26.68	0.67
1B 1000 rpm	13.90	25	N/A	N/A	N/A
1B 7000 rpm	14.26	50	72.34	31.96	14.62
1B 11000 rpm	11.01	75	92.67	30.73	0.96
2A 1000 rpm	16.50	50	N/A	N/A	N/A
2A 7000 rpm	17.27	50	102.03	25.12	0.22
2A 11000 rpm	12.53	325	109.66	31.99	0.47
2B 1000 rpm	15.77	50	N/A	N/A	N/A
2B 7000 rpm	15.29	50	74.82	30.06	13.48
2B 11000 rpm	12.33	50	78.82	30.97	7.37
3B 1000 rpm	16.54	75	N/A	N/A	N/A
3B 7000 rpm	17.80	75	80.28	26.35	10.53
3B 11000 rpm	14.73	75	81.38	28.32	7.15

The application of protein denaturation and enzyme digestion clearly exhibit the impact of protein and fiber structure on foaming. Both the high temperature treatment and proteinase digestion seriously reduces samples foaming capability (Table 5.3). The high temperature water bath may lead to some other effect besides the denaturing of protein, but proteinase under 35^{o}C will only destroy the protein structure and decompose them into polypeptides (control samples under 35^{o}C do not have a strong impact on foaming capability). Compared to samples after proteinase degradation of protein, samples after cellulase degradation maintained stable foaming capability, shows that the impact of fiber is not as strong as protein in manure samples.

Table 5.3. Impact of protein and fiber in foaming process.

Sample	Original		High temperature Denature (80^{o}C)		Low temperature incubate (35^{o}C)	
	Foaming capability (ml)	Lasting time (min)	Foaming capability (ml)	Lasting time (min)	Foaming capability (ml)	Lasting time (min)
1A	450	20	75	0.5	450	25
1B	75	1	60	0.5	30	0.5
2A	450	5	125	1	450	15
2B	25	0	20	0	20	0
3B	75	2	60	0.5	50	0.5
Sample	Original		Proteinase digestion (35^{o}C + enzyme)		Cellulase digestion (35^{o}C + enzyme)	
	Foaming capability (ml)	Lasting time (min)	Foaming capability (ml)	Lasting time (min)	Foaming capability (ml)	Lasting time (min)
1A	450	20	50	0.5	450	20
1B	75	1	35	0.5	40	0.5
2A	450	5	30	0.5	450	10
2B	25	0	20	0	25	0
3B	75	2	60	0.5	50	0.5

Manure samples from the second sample set were used to evaluate the concentration of potential foaming inducing chemicals (protein, LCFA and TBA) in manure solution. Other than the original classification method grouping samples as A layer, B layer and C layer, these 108 samples were also categorized into three groups based on their sampling location: foaming sample, non-foaming sample and transition sample. This classification clearly shows the difference between foaming and non-foaming samples, but it also has the drawback that samples in each group were collected from different layers, which probably resulted in the large standard deviation.

Based on these two classification methods, a brief impression is that these potential foaming inducing chemicals have higher concentrations in the top layer of foaming pits.

All three chemicals have a higher concentration in foam layer (Fig. 5.2a). Compared to the slight difference of protein between layers, concentration differences of TBA and LCFA between layers are more obvious. Considering that the foam layer has a higher solid content compared to samples from other layers (average of 9.2 in A layer, 6.2 in B layer and 7.7 in C layer), the percentages of these three chemicals on a per dry mass basis are also evaluated (Fig. 5.2b). TBA and LCFA also have higher percentage in the foam layer, and B layer samples have the highest protein content on a dry mass basis. With another classification method, TBA and LCFA have the highest content in foaming farms both on concentration and percentage in dry mass (Fig. 5.2c and Fig. 5.2d), and transition farms have an obvious higher percentage of TBA and LCFA on a dry mass basis compared to non-foaming farms. Protein concentrations are about equal in these three farm types, but the protein percentage on a dry mass basis is especially higher in transition farms.

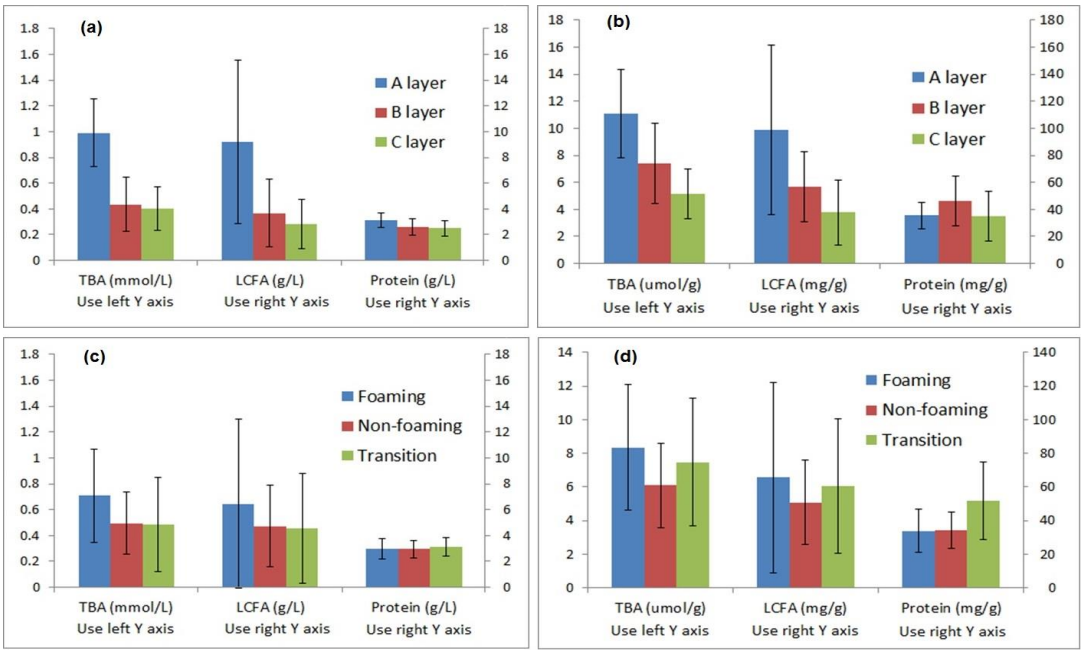

Figure 5.2. TBA, LCFA and protein concentration comparison

Correlation between TBA and the other two chemicals are also evaluated using the data obtained (Fig. 5.3). Protein concentrations of nearly all samples are located in a relative stable range between 2g/L and 4g/L. Correlation between TBA and protein is statistically significant (p=0.000) but the slope of trend line is fairly small. The linear regression only has an R^2 value of 0.234 for this dataset while no better regression can be found. LCFA concentrations are more wildly distributed from 0.3g/L to 27g/L, while 90% of samples have values between 0.6g/L and 15g/L. Similar to correlation between TBA and protein, the best regression obtained between TBA and LCFA is a linear regression with an R^2 of 0.302.

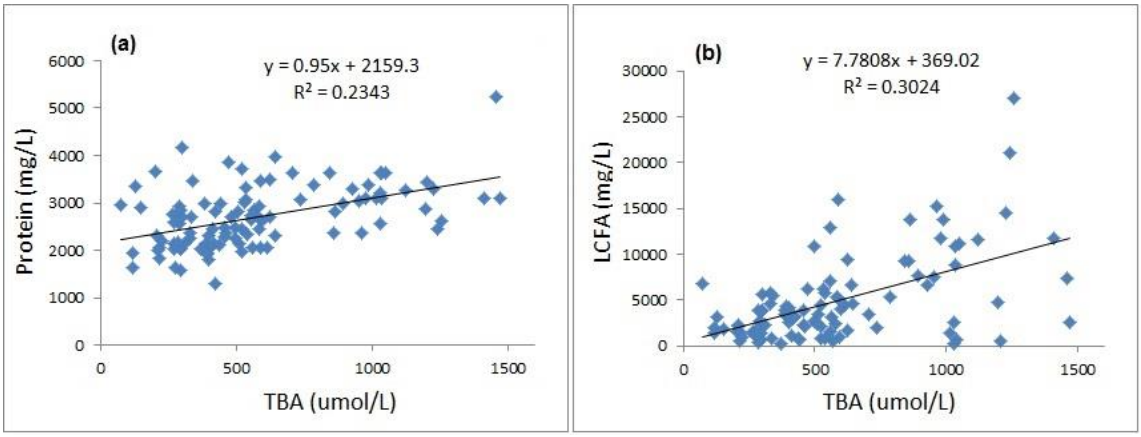

Figure 5.3. Correlation between TBA and other chemicals.

5.4 Discussion

Solid particles have been used as foaming and emulsion stabilizing species in recent years. The absorption of particles at bubble interfaces is considered as the basic driving mechanism in many dynamic foaming processes, and different types of particles are known to cause foaming in rivers, dispersed sludge, and other water environments

(Hunter, T. N., et al., 2008). Typically, solid particles were one of the major components, estimated to account for 5% in deep pit manure. In preliminary analysis on foaming manure samples, the solid particles fraction within were shown to have a strong impact on manure's foaming capability, as the removal of particles by centrifuge significantly reduced the sample's foaming capability (Fig. 5.4). In this study, the addition of solid particles from foaming manure was also shown to improve non-foaming manure's foaming capability (Table 5.2), showing that solid particles are an essential components in the foaming process.

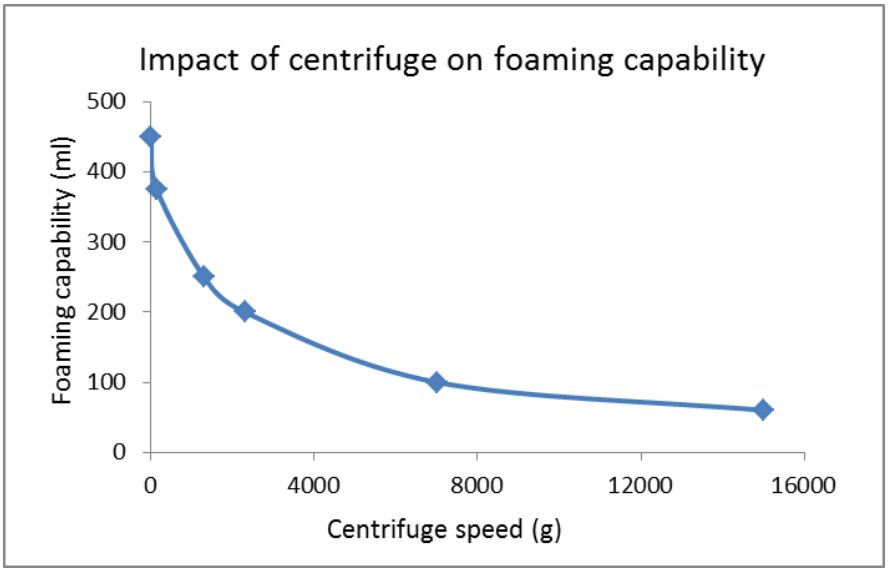

Figure 5.4. Impact of centrifuge speed on foaming capability of sample 1A

Since swine manure is primarily utilized as fertilizer to meet crop nutrient demands, most research of manure focuses on its nutrient content and nutrient balance instead of the actual components. It is reported that fiber is about 40% of swine manure dry matter, while crude protein is about 22~25% of swine manure dry matter (Chen S., et al., 2004;

Xiu S., et al., 2009). Fatty acids are also reported as a major component in swine manure solid fraction (Loughrin & Szogi, 2006). In contrast with swine manure, research on solids in human feces is more detail. Undigested fiber and solidified components of digestive juices (30%), bacteria (30%), fat (10% to 20%), inorganic matter (10% to 20%) and protein (2% to 3%) are reported as the most abundant components in human feces (Charles C., et al., 2014). Considering the feeding material (corn-soybean meal diets as major components) and digestion process, fiber, microorganisms, lipids/fat and protein are expected as the major solid components in swine manure samples. These four major components have all been discussed for their foaming capability by other researchers. Fibers, mostly wood fiber, were primarily researched in the construction of natural fiber reinforced polymer composites, while lipids, microorganisms and proteins have also been correlated with foaming in water environment (Kuboki et al., 2009; Frauk et al., 2007; Rossetti et al., 2005; Kragelund C, et al., 2007; Zayas J. F., 1997).

Besides composition, another special characteristic that draws attention is surface energy. All solids samples that are able to use sessile drop technique for surface energy measurement have lower surface energy than pure water. Meanwhile, lower polar energy was generally observed on samples with higher foaming inducing capability, indicates that solid particles with higher surface hydrophobicity were observed in foaming sample. This high surface hydrophobicity may result from hydrophobic components, or surfactants with hydrophobic parts. Hydrophobic components normally act as antifoaming agents and are widely used for such purposes (Miller C.A., 2008; Karakashev and Grozdanova, 2012). However, each antifoaming agents has its specific

optimal concentration, below which they become less effective and above which they act as foam stabilizer (Karakashev and Grozdanova, 2012). Compared to hydrophobic components, surfactants contain both hydrophobic parts and hydrophilic parts, and are widely applied as detergents, emulsifiers and foaming agents. It is hard to distinguish which components lead to the higher surface hydrophobicity because these two types of particles all exist in swine manure. Consider the observation that smaller size particles have higher contact angle, we hypothesize the hydrophobic components are primarily linked with solid particles and suspended in foaming manure. Besides linking with particles with hydrophobic regions, these hydrophobic particles could also conjugate with surfactants and dissolved in liquid.

Among the major expected solid components in manure, lipids and proteins are the most suspect components and they all have been discussed as foaming agents previously (Rossetti et al., 2005; Kragelund et al., 2007; Zayas J. F., 1997). Filamentous bacteria with hydrophobic cell wall have been considered as the main reason of foaming in wastewater pretreatment plants for a long time. However, a screening of bacteria community in foaming manure samples didn't find any significant amount of filamentous bacteria. Enzyme digestion on fibers do not significantly change manure's foaming capability (Table 5.3), so we cannot build a clear relationship between fiber and manure foaming at this time. The impact of lipids in foaming have been discussed in our previous study that long chain fatty acids have been observed to have a strong influence in manure foaming (Yan et al., 2014), and the impact of protein in foaming also have been discussed by other researchers, as soluble globular proteins can diffuse to air/water interface, unfold

and orient its hydrophilic and hydrophobic groups, then interact with one another to form stabilizing film (Yin et al., 2014). The importance of protein has also been revealed in this study, as treatments on protein either by heating denaturation or by proteinase degradation all leads to a decrease on manure's foaming capability (Table 5.3). However, protein concentration in foaming and non-foaming manure samples are in the same level (Fig. 5.2), which indicate that protein concentration is not directly correlated to manure's foaming capability.

To further verify the connection between protein and foaming, we conducted a small test adjusting the pH of manure sample to around four and tested their foaming capability again. For every sample we tested (foaming manure, non-foaming, raw collected sample and supernatant after solid removal by centrifuge at 20000g), their foaming capability after pH adjustment all increased. The stability of foam in supernatant is weaker than other samples, which is probably due to the quick liquid drainage from the film because of the low concentration of solid particles. There is the possibility that pH adjustment impacts other components besides protein, but we couldn't find any candidates other than protein at this time, especially considering that most solid components have been removed from manure supernatants during high speed centrifuge. Low pH ionized individual groups, leads to pH-dependent unfolding and destabilize proteins (Yang and Honig, 1993). This unfolding state exposed the hydrophobic part, which may help protein easily obtain the capability of foam stabilization by the theory stated above. The pH adjustment probably drives much of the protein in manure sample into foam stabilizer,

which is certainly not the real situation in foaming manure. But it is reasonable to hypothesis that specific types of protein are playing a key role in foaming process.

Another interesting characteristic of protein is that it can bind with other components to form complex compound. One example is the protein/polysaccharide complexes which originate from electrostatic interactions, and the complexes have combined hydrophobic/hydrophilic character of the original two compounds (Schmitt C., et al., 1998). Such complex compound can therefore be used as ingredients to stabilize the air/water or the oil/water interface in a variety of complex food systems (Schmitt and Turgeon, 2011), like glycoprotein is considered to retard film drainage, increase the air/water interface stability and increase foam stability (Schmidt, I., et al., 2010). Protein-lipid complexes are also very common, and especially important in membrane-embedded proteins to maintain the diffusion barrier. Interplay of lipids and membrane proteins facilitates basic processes of respiration, photosynthesis, protein and solute transport, signal transduction, and motility (Palsdottir and Hunte, 2004). The mobile lipid molecules can adhere to the membrane protein surface and flexibly adjust to conformational changes and structural rearrangements (Hunte C., 2005). This lipid-protein interaction could also happen between proteins and LCFAs in swine manure and increase foaming capability when certain content of such complex was accumulated. We can also detect a general higher LCFA content in foaming manure samples, which can support such possibility.

Besides protein, another suspicious foaming agent is TBA. Bile acids synthesis in liver cells and have long been known to facilitate digestion and absorption of lipids and

fat-soluble vitamins (Lefebvre P., et al., 2009). Prior to secretion, liver cells conjugate them with one of two amino acids to form conjugated bile acids. These conjugated bile acids are amphipathic molecules, contains both hydrophobic and hydrophilic regions, and were able to form micelles and solubilize lipids in small intestine under certain concentrations. Although most of excreted bile acids were reabsorbed in the ileum and recycled back to the liver, there are still a portion of bile acids entering the manure pit which undoubtedly can serve as surfactants to enhance manure's foaming capability. Coincidentally, a much higher TBA content has been observed in foaming layer (Fig. 5.2). Comparison between samples' TBA and protein/LCFA concentration was also conducted to reveal the possible correlation between these components (Fig. 5.3). It is clear that correlation between TBA and lipids is stronger than the correlation between TBA and protein, and the relative low R^2 value of both linear relationships may be the result of inconsistency of sample collected from various farms. Such correlation provides a hypothesis that higher amount of lipids in feeding material is the major reason for increased TBA concentration in swine manure. Since the primary lipid components in swine diet is vegetable oil, such higher lipid content will not only release more LCFA but also help to raise the TBA concentration in swine manure, and finally help to create stable foam in manure pit.

5.5 Conclusion

The solid components that separated from foaming manure samples were shown to have the capability to induce foaming. Separation of solids with different centrifuge

speed indicates that smaller size of solid particles have higher foaming inducing capability, and also a higher hydrophobicity. Protein, together with LCFA that was discussed in previous research, was proved to have a strong correlation with swine manure foaming in this study. A high concentration of total bile acids was also found in manure foaming layer, providing the hypothesis that TBA may be a major surfactant in foaming process.

Chapter 6

Conclusion

6.1 Summary of this research

To find out why foaming occurs in manure pits, we initially conducted a compositional analysis of foaming and non-foaming manure samples to identify potential differences between these two types of manure. Of all the parameters analyzed, pH remained at a similar level among the manure samples, and potassium and sodium were found to have similar concentrations between foaming and non-foaming manure. The foam layer, due to its high solids content, concentrates the following components: organic nitrogen, phosphorus, sulfur, calcium, magnesium, LCFAs, acid hydrolysis, and crude protein. Ammonium-nitrogen and trace metals were found to have higher concentrations in foaming manure and higher percentages in foaming manure solids. Some of these parameters have not been connected with foaming before, while others have been studied for their effects on foam generation, such as crude protein in wine and beer foaming. From this compositional analysis, parameters that were considered potential factors for foaming in swine manure pits included solids, lipids, trace metals, and proteins.

Studies focused on the bacteria community was then conducted, a search for filamentous bacteria in both foaming and non-foaming manure samples were done with the idea that filamentous bacteria are the leading cause of manure foaming. FISH analysis shows that filamentous bacteria may exist in both foaming and non-foaming manure samples in a relative similar amount, and sequencing result indicates that filamentous bacteria do not occupy a significant portion in both manure samples. Difference in microbial community exists between foaming and non-foaming manure, but there is no direct evidence to claim that enriched bacteria is the major inducing factor of foaming. The shift of bacteria community is highly possible to the consequence of foaming. This result indicated that filamentous bacteria might not be the main reason for foaming in deep pits, and it is more likely that foaming is induced by the change of manure composition compare to the impact of microorganisms.

Studies on lipids and solid particles provide clear evidence that lipids and solid particles have a strong correlation with swine manure foaming. From the simulation in laboratory setting, LCFAs were found to be a major contributing factor, possibly serve as the surfactant in manure pits. Proteins were also found to have a similar function in foaming, as the degradation of protein in manure samples will drastically destroy a sample's foaming capability. Another well-known surfactant, total bile acids, were found to have a higher concentration in foaming manure than non-foaming manure samples, although we lack the laboratory methods to see if the removal of bile acids will affect sample's foaming capability. Currently these surfactants have a higher possibility than filamentous bacteria to induce foaming in manure pits.

6.2 Recommendations for further research

Besides the studies described in this research, there are also studies on swine manure foaming conducted by other research groups, with other interesting results. Feeding trial conducted by Dr. Shurson's research group found that larger feed particle size tends to increase manure's foaming potential, increased lipid content in feed also has a similar effect. Experiments conducted by Dr. Anderson's research group at Iowa State University, found a higher biogas generation ratio in foaming manure samples. Besides their research, some additional facts not mentioned above are also interesting. Foaming manure samples collected from manure pits always have higher viscosity than non-foaming manure sample, and collected fresh manure samples never have a stable foaming capability. These findings reveal that swine manure was not generated with foaming capability. During their storage in deep pits, sufficient surfactants were diffused, or even generated to help swine manure develop a stable foaming capability. After finding the surfactants in foaming, it is also needed to reveal how these surfactants were accumulated in the manure pit.

The following suggestions for future research emerge from the findings of this thesis:

1. A detailed research effort to determine whether protein and LCFAs have binding ability in manure sample. LCFAs are extremely hydrophobic, and the addition of oleic acid into pure water will only foam a thin oil surface layer. However, a similar oil layer on swine manure disappears quickly after gentle shaking, and the addition of LCFAs into manure was shown to instantly increase foaming capability. There is a high probability that these fatty acids were attached to some hydrophobic solid

particles, and it will be valuable to reveal whether LCFAs are binding with proteins or just form LCFA salt to act as a surfactant.

2. Detailed experiments monitoring the concentration of each candidate surfactants during foaming. Currently, the most laboratory controllable foaming method is to add lipids into manure samples. No significant foaming can be generated in this process (not enough biogas was generated), but the foaming capability of manure samples can be detected by periodically sampling and the measure the foaming capability. Such experiments can be conducted by addition of different compounds that may induces foaming, like lipids, while monitoring the concentration change of each candidate surfactants, including TBA, LCFAs, protein and other potential components. This may not be the real situation that happened in deep pits, but it will directly show how these surfactants functions during a foaming-diminishing process. Compared to periodically sampling from deep pits, this method is more controllable, needs less labor, and can avoid the expectation that foaming will happen in the deep pit. Moreover, this single-manure-source method can also avoid the differences of manure composition naturally occurring on different farms.
3. Considering feeding material that generates less protein and lipids in manure. It is assumed that protein and lipids are the main surfactant in foaming manure samples, and a practical method that reduces the protein and lipid content should be an effective way of reducing the risk of foam generation. Meanwhile, inorganic nitrogen and phosphorus, instead of protein and lipids, are more desired nutrients in swine manure that act as fertilizer for cropland. There are some techniques that can be

applied, like extraction of lipids from DDGS or grinding feed into small particle size for increased digestibility. If these methods are shown to be efficient after a period of time, it would be easier to manage a large amount of manure that already exists in pits.

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