FOAMING PHENOMENON IN BENCH-SCALE ANAEROBIC DIGESTERS

by

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ABTRACT

The Madison Metropolitan Sewerage District (The District) in Madison, WI has been experiencing seasonal foaming in their anaerobic biosolids digesters, which occurs from mid-November to late June for the past few years. The exact cause(s) of foaming is unknown. Previous research findings are unclear as to whether applications of advanced anaerobic digestion processes reduce the foaming potential of digesters. The object of this study was to investigate how configurations of thermophilic and acid phase-thermophilic anaerobic digestion would affect foaming at the bench-scale level compared to single stage mesophilic digestion. Bench-scale anaerobic digesters were fed with 4-4.5 % by dry weight of solids content blend of waste activated sludge (WAS) and primary sludge from The District. Foaming potential was monitored using Alka-Seltzer and aeration foaming tests. The bench-scale acid phase-thermophilic digester had a higher foaming potential than the bench-scale mesophilic digester. These results indicate that higher temperatures increase the foaming potential of the bench-scale anaerobic digesters. The bench-scale thermophilic and acid phase-thermophilic digesters had a greater percent (2-8%) volatile solids destruction and a greater percent (2-9%) total solids destruction when compared to the bench-scale mesophilic digester.

CHAPTER 1: INTRODUCTION

The Madison Metropolitan Sewerage District (The District) has been experiencing seasonal (mid-November to late May) foaming for the past few years. The research presented in this paper aimed to examine the microbial ecology of foaming and various other parameters used in The District's anaerobic digestion process. The goal was to identify the site-specific factors affecting foaming using bench-scale anaerobic digesters. This information will be used to design strategies to mitigate or control foaming at The District.

Foaming is identified as a chocolate-brown scum that accumulates on the surfaces of aeration basins and secondary clarifies, the foam enters the anaerobic digester through the waste activated sludge (WAS) (Jenkins *et al*. 2003). Many researchers have suggested that foaming is site-specific to each wastewater treatment plant and the exact causes of foaming are unknown. The literature has identified several parameters that could potentially contribute to foaming in anaerobic digesters which are: filamentous organism, organic overloading of digesters, accumulation of volatile fatty acids, and inadequate mixing of the digesters (Barjenbruch *et al.* 2000, Bates *et al.* 2006, and Pagilla *et al.* 1997).

The aim of this study was to investigate how the destruction of filamentous organisms that enter the anaerobic digester through the WAS would affect foaming at the bench-scale level. The filamentous organisms contribute to foaming in anaerobic digester because these filamentous organisms are hydrophobic due to the presence of mycolic acid contained in their cell walls. This allows the filamentous organism to attach to the gas bubbles produced in the anaerobic digester, and rise to the liquid-air interface of the digester creating a foam blanket (Jenkins et al. 2003)

Previous research findings are unclear as to whether applications of advanced anaerobic digestion processes reduce the foaming potential of anaerobic digesters. This study was conducted in four phases to investigate the ability of thermophilic digestion and acid phase-thermophilic digestion to reduce foam. Phase 1 examined favorable conditions for foam-causing organisms to try and promote foaming in the bench-scale anaerobic digesters at mesophilic (37^{o}C) conditions. This would allow year round examination of foaming, and not during foaming season experienced at The District only (mid-November to late June). Phase 2 and 3 investigated how thermophilic (55^{o}C) and acid phase-thermophilic anaerobic digestion would affect foaming at the bench-scale level compared to single stage mesophilic (37^{o}C) digestion. Phase 4 investigated the effects of steam treatment of phosphorus-release WAS on the foaming potential of the bench-scale anaerobic digesters.

The bench-scale anaerobic digesters were fed with 4-4.5 % by dry weight of solids mixture of WAS and primary sludge from The District. The bench-scale anaerobic digesters were fed daily and had a solids retention time (SRT) of 18-days. Alka-Seltzer and aeration foaming potential tests were used to monitor the foaming potential of the bench-scale anaerobic digesters. Throughout the study, chemical analyze were conducted (alkalinity, pH, total Kjeldahl nitrogen, total phosphorus, % total solids, % total volatile solids, and total volatile fatty acids) on the bench-scale anaerobic digesters to determine if a correlation between foaming potential and chemical level exist.

CHAPTER 2: LITERATURE REVIEW

ANAEROBIC DIGESTION

Anaerobic digestion stabilizes organic matter in an oxygen-free environment utilizing anaerobic microorganisms, which reduce the quantity of solids to be disposed of, and producing methane and carbon dioxide (biogas). Raw biogas typically consists of 30-40 percent carbon dioxide and 60-70 percent methane and small trace amounts of hydrogen sulfide (McFarland 2001). The biogas can then be used as a fuel source within the wastewater treatment plant. Other benefits of anaerobic digestion include reduction in biosolids mass, minimization of odors, improving dewatering properties of the fermented solids, and reduction in pathogen content (Metcalf and Eddy 2003).

Two types of sludge are stabilized in anaerobic digesters, primary sludge and waste activated sludge (WAS). Primary sludge is essentially raw solids, which come from the bottom of the primary clarifier, which usually contains a higher proportion of fats and proteins and a lower proportion of carbohydrates (Sykes 2003). This results in a higher gas yield than digestion of WAS; however, the gas contains a lower percentage of methane. Waste activated sludge (WAS) is more difficult to digest than primary sludge. WAS is produced in the secondary wastewater treatment process and is typically light and fluffy. It is composed of flocculated microorganism organic matter, mainly bacteria and protozoa (Metcalf and Eddy 2003).

Anaerobic digestion involves a complex biochemical process in which a mixed culture of anaerobic organisms breaks down the organic matter. In Figure 1, a simplified scheme for anaerobic digestion of organic matter is shown.

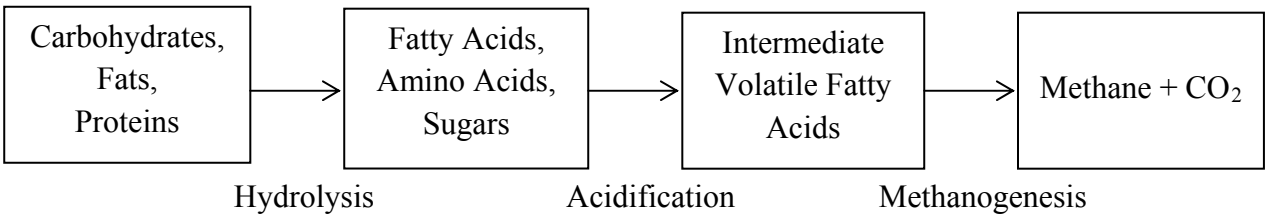

Figure 1: Simplified scheme for anaerobic digestion of organic matter (after Murk *et al*. 1980)

As shown in Figure 1, anaerobic sludge digestion can be modeled as a three stage process to convert the organic matter into carbon dioxide and methane. With each step being performed by different microorganisms.

Sludge contains polymeric substances, which are large insoluble molecules consisting of many smaller soluble molecules held together by chemical bonds (Gerardi 2003). These insoluble compounds include carbohydrates, fats, and proteins. During the first step of sludge stabilization, hydrolytic bacteria or facultative anaerobes break down these insoluble compounds by hydrolysis (Seviour and Nielsen 2010). This process allows for smaller soluble molecules, including fatty acids, amino acids, and sugars to be further digested by other bacteria.

The second step is the acid phase (acidification), acid forming organisms convert soluble organic matter, which include fatty acids, sugars, and amino acids, to organic acids. The most common organic acids or volatile fatty acids are: acetic, propionic, butyric, and valeric acids. These organic acids will then be fermented further to yield acetate, hydrogen, and carbon dioxide, the final products of acidification (Metcalf and Eddy 2003). Propionate oxidation to acetate is energetically unfavorable unless the partial pressure of hydrogen is low. In contrast to acetate, propionate can't be directly utilized by methanogens in the final stage of digestion and will be broken down to hydrogen and carbon dioxide (Dong *et al.* 1994).

During the final digestion stage, also known as the methanogenic phase, methanogenic bacteria reduce the simple carbons of the intermediate volatile fatty acids, primarily acetate, to methane (Metcalf and Eddy 2003). After digestion, the initially added primary sludge and waste activated sludge is referred to as digested sludge or biosolids, which is reduced in mass, less odorous, and contains reduced levels of pathogens.

Anaerobic digesters are operated at conditions that favor the proliferation and activity of these acid- and methane-forming bacteria. Methane production can occur at various temperatures; the most critical for anaerobic digestion are bacteria that can survive in mesophilic (30-38^{o}C) and thermophilic (50-60^{o}C) ranges (Gerardi 2003). Evidence has shown that acid- and methane-forming bacteria are sensitive to temperature changes. Temperature fluctuations greater than 1^{o}C/day in the anaerobic digester, are especially harmfully to the methane-forming bacteria (Metcalf and Eddy 2003).

The pH range for anaerobic digestion is also vital for the methanogens. The desired pH range for a healthy population of methanogens in anaerobic digesters is 6.5-7.6. The typical pH ranges for anaerobic digestion are 6.8-7.2, within the range for the methanogens (Metcalf and Eddy 2003). Since methanogens are sensitive to acid conditions and can be inhibited by a drop in the pH (Rittmann and McCary 2001), fluctuations of pH in anaerobic digesters are not recommended.

Changes in temperatures and pH can inhibit the growth of the acid- and methane-forming bacteria in the anaerobic digesters. If methane production decreases an accumulation of volatile fatty acids can be observed (Gerardi 2003). This increase is suggested by the literature to be one cause of increased foaming potential in anaerobic digesters (Barber 2005).

BACKGROUND OF FOAM

Foaming is a serious issue and many wastewater treatment plants around the world have struggled with bulking and/or foaming with their anaerobic digesters. Foaming can lead to serious operational problems, hazardous working conditions, and disrupt solid separation. The exact cause of foaming is unknown; however, many researchers suggest that the proliferation of filamentous organisms in the aeration basins is one cause (Soddell and Seviour 1990).

The widespread occurrence of foaming can be illustrated through surveys; it was determined by the Central States Water Environmental Association that there are 216 Publicly Owned Treatment Works (POTWs) that use anaerobic digestion for biosolids in the states of Wisconsin, Minnesota, and Illinois. With a 44% response rate, a total of 53% of the respondents reported that their anaerobic digesters have experienced significant foaming issues in the past ten years. Pitt and Jerkins (1990) conducted a U.S. nationwide survey with a response rate of 48% (134 responses) out of the initial 282 distributed surveys. One hundred forty four of those who responded where from activated sludge plants, with 66% indicating they have experienced foaming issues in the past. In France a survey was conducted on the 6013 wastewater treatment plants and determined that 1192 of the activated sludge plants were affected by foaming (Pujol *et al.* 1991). A survey conducted in Australia by Seviour *et al.* (1994) reported that 44 plants out of 65 plants had experienced foaming. Foaming has been reported in activated sludge plants in Denmark, Japan, Sweden, Austria, Saudi Arabia, Hong Kong just to name a few (Seviour and Nielsen 2010).

These surveys demonstrated that foaming occurs worldwide and is an important problem facing the wastewater industry. Foaming is characterized by the formation of a thick, stable, chocolate-brown scum, which floats on the surfaces of aeration basins and secondary clarifiers in

wastewater treatment plants (Jenkins *et al.* 2003). Foaming can also be associated with anaerobic digesters and has severe impacts on digester functionality. Foam can accumulate to such an extent that the foam overflows the basin freeboard and covers walkways, handrails, surrounding areas, creating hazardous conditions, and unacceptable odors. The following are other operational problems associated with foaming at various wastewater treatment plants:

- blockage of gas mixing devices;
- inversion of digester solids profiles;
- gas binding of sludge recirculation pumps leading to inability to heat the digesters;
- foam penetration between floating covers and digester walls;
- pressurization of digesters due to blocked gas piping; and
- risk of environmental contamination from bio-aerosols.

(Bates *et al.* 2006, Jenkins *et al.* 2003, and Pagilla *et al.* 1997)

Figure 2 shows an anaerobic digester at the Madison Metropolitan Sewer District in Madison, WI that had experienced excessive foaming.

Figure 2: Foaming incident, Madison, WI

The foaming incident caused biosolids to overflow the walls of the digester and to enter the biogas collection system, which interfered with the operation of the gas mixing system. Also, causing unpleasant odors and unsafe working conditions at the wastewater treatment plant.

The exact cause of foaming is unknown. Since foaming is extremely site specific the literature reports conflicting results. Barber (2005) states that the prerequisites for foaming must include a sludge mixture that contains surface active agents, a gaseous phase and hydrophobic material which are all present in an anaerobic digester. Researchers have identified numerous parameters that could potentially contribute to digester to foam, which are as follows:

- filamentous organisms;
- organic overloading;
- digester feeding regime;
- accumulation of volatile fatty acids in digesters;
- excessive grease and scum in digester feed;
- temperature fluctuation;
- insufficient mixing of digesters; and/or
- gas mixing.

(Barber 2005, Barjenbruch *et al.* 2000, Bates *et al.* 2006, and Pagilla *et al.* 1997)

The complexity of foaming results from the various combinations of parameters observed to contribute to a foaming incident. Any single or combination of the proposed parameters may contribute to foaming at a specific wastewater treatment plant. Understanding those factors on a site-specific basis presents a significant engineering challenge.

ANAEROBIC DIGESTER FOAMING

Foaming in anaerobic digesters is a site-specific problem depending upon climate, sludge quality, and wastewater treatment design. This section will examine the current knowledge of the causes of foaming in anaerobic digesters. Aiming to identify the most common parameters that researchers have predicted could increase the foam potential in anaerobic digesters.

Filamentous Organisms

Foaming in anaerobic digester can result from filamentous organisms entering the anaerobic digester in the waste activated sludge (WAS). Van Niekerk (1987) reported that 26 (54%) of the original 54 wastewater treatment plants in California surveyed, had foaming problems that could be attributed to filamentous organisms in the WAS feed. Activated sludge samples from wastewater treatment plants with foaming issues normally contain multiple different filamentous organisms (Jenkins *et al*. 2003). These filamentous organisms are generally bacteria, fungi, or algae whose cells do not become detached from each other after cell division and therefore grow in the form of filaments (Seviour and Nielsen 2010).

The unstable, brown foam that Westlund *et al.* (1998) classified as being caused by filamentous organisms was thought to be mainly attributed to *Gordonia amarae* (formerly called *Nocardia*). However, recent studies have suggested that a diverse population of mycolic acid containing Actinomycetes cause foaming in anaerobic digesters; which include *Skermania piniformis*, *Rhodococcus rhodochrous*, *Tsukamurella spp.* (Nam *et al.* 2003), and *Millisia* (Soddell *et al.* 2006). Mycolic acid is a high molecular weight 3-hydroxy fatty acids with a long alkyl branch in the 2-postion, located on the cell wall of these organisms (Stratton *et al*. 1998). Since it is difficult to specify the causative organism a general term, “mycolata foaming” has

been given to describe foam caused by mycolic acid containing Actinobacteria, whose specific identification may be unknown. Where Actinobacteria, are easily identified by their Gram-positive, right-angled branching (Seviour and Nielsen 2010).

Filamentous organisms classified as Actinomycetes have hydrophobic tendencies which contribute to foaming in anaerobic digesters because of the presence of mycolic acid. If these filamentous organisms grow in abundant quantities, they are able to attach to solid particles rendering them hydrophobic. The filamentous organisms then attach to gas bubbles present in anaerobic digesters and rise to the surface of the liquid phase. Creating a foam blanket in the anaerobic digester at the liquid and gas interface (Jenkins *et al.* 2003). Particles must be less than 300um in diameter, in order to be lifted by the buoyancy of bubbles (Soddell and Seviour 1990). It should be noted that bacterial cells in an aqueous media have a fundamentally hydrophilic cell surface or they would spontaneously aggregate to minimize cell-water surface (Heard *et al.* 2009).

For many years Actinomycetes were considered to be the only filamentous organism to cause foam; however, another group of filamentous organisms that are gaining a lot of attention is *Microthrix parvicella*. Previously thought to be only associated with bulking (inadequate separation of biosolids and liquid effluent phase), but more recently have been identified in activated sludge wastewater treatment plants associated with severe foaming incidents. And is now one of the most common foam-causing organism worldwide (Blackall *et al.* 1996).

Microthrix parvicella are a long, thin, non-branched and unsheathed filamentous bacterium with a diameter of 0.6-0.8 μm (Rossetti *et al.* 2005). They can be easily recognized by their Gram positive reaction and coiled appearance in Gram stains, which is shown in Figure 3.

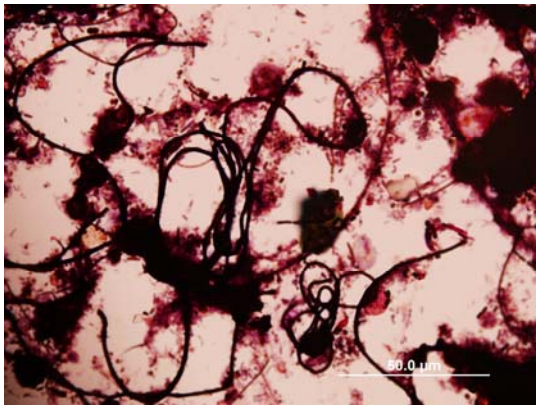

Figure 3: Gram Stain containing Gram-positive filaments

Two species of *Microthrix* have been identified; *Candidatus Microthrix parvicella* and *Candidatus Microthrix calida.* The more common of the two is *Candidatus Microthrix parvicella*, which is known to predominate in wastewater treatment plants utilizing biological nitrogen and phosphorus removal systems. The *Microthrix parvicella* can utilize the long chain fatty acids as an energy source to favor growth under anoxic and/or anaerobic conditions (Mamais *et al.* 1998). The other, *Candidatus Microthrix calida*, is thinner and grows at slighter higher temperatures (30-40 ^{o}C) than *Candidatus Microthrix parvicella* (Rosetti *et al.* 2005).

Mamais *et al.* (1998) used bench-scale biological nitrogen removal activated sludge systems to conclude that *Microthrix parvicella* is hydrophobic and utilized long chain fatty acids as its carbon source. Studies have shown these organisms to be more hydrophobic than most other bacteria, allowing these organisms to out compete other bacteria for insoluble lipid uptake (Nielsen *et al.* 2002).

Environmental Factors Affecting Growth

Many researchers have determined that temperature plays a critical role in the growth of Actinobacteria and *Microthrix* in wastewater treatment plants. These filamentous organisms proliferate in the activated sludge systems in the wastewater treatment. One of the first

researchers to correlate temperature and foaming problems in wastewater treatment plants was Le Chevalier in 1975 who observed an increase in foaming at temperatures above 17 ^{o}C. Soddell and Seviour (1990) determined that *Gordonia* sp. grew at a wide range of temperatures by isolating the filaments from foaming activated sludge and cultivating them in the lab at various temperatures. The foam-causing organism *Gordonia* sp. grew best between 23 and 37 ^{o}C. Pitt and Jenkins (1990) reinforced these findings using bench-scale activated sludge experiments to determine optimal growth temperatures for *Gordonia* sp. They determined that 18, 20, and 25 ^{o}C were optimal growth temperatures and saw little growth at 13^{o}C. Soddell and Seviour (1995) determined that *Rhodococcus* sp. could flourish at temperatures as low as 5 ^{o}C and emphasized the importance of proper identification of the foaming causing organisms since these organisms can grow in such wide ranges of temperatures.

Seviour *et al.* (1990) noticed that *Microthrix parvicella* was more common than Mycolata foam-causing organisms in plants that were in colder regions in Australia, concluding that *Microthrix parvicella* thrives in colder climates. Seviour *et al.* (1990) conclusions were strengthened when Eikelboom (1994) demonstrated that temperature greatly influenced the growth of *Microthrix parvicella* and observed higher concentrations at plants whose temperatures were less than 20^{o} C. It was also determined that *Microthrix parvicella* has an optimal growth rate between 25^{o}C and 8^{o}C, while at temperatures greater than 35^{o}C very poor growth was observed (Rosetti *et al.* 2005). This gives *Microthrix parvicella* an ecological advantage over other bacteria since lipids are less soluble at colder temperatures. Since these foam-causing organisms thrive at various temperatures; knowing the temperature of the activated sludge operating system will give insight into the possible type of foam-causing organisms at that wastewater treatment plant.

Another environmental factor affecting the growth of foaming causing organisms is pH. Rosetti *et al*. (2005) demonstrated that *Microthrix parvicella* could grow over the range of pH 7.1 to 8.0, and growth was mitigated when the pH was less than 7.1. For the majority of Actinomycetes, these organisms can grow within the range of pH of 6 and 9, but growth is diminished at pH 5. This is within the pH range of a typical anaerobic digester, 6.8-7.6 (Metcalf and Eddy 2003). However, growth at pH of 10 has been observed and studies have shown that *Rhodococcus erythropolis* and *Gordonia terrae* can grow at pH as low as 4 (Goodfellow 1990).

Organic Loading

Many researchers have agreed that organic overloading of anaerobic digesters can be one cause of foaming (Pagilla *et al.* 1997, Barjenbruch *et al.* 2000, Moen 2003, Barber 2005). These researchers believe that foaming may result from incomplete digestion of organic matter in the anaerobic digester. Determining the optimal feed rates for the anaerobic digester is crucial to prevent excess foam. Overloading the digester can cause an accumulation of hydrophobic substances and an increase concentration of filamentous bacteria in the digester resulting in foam. Brown (2002) suggest that anaerobic digesters need to be operating at an organic loading rate no higher than 4.5 kg VS $m^{-3}d^{-1}$ to help minimize foam; even though the suggested operating range is 1.6-4.8 kg VS $m^{-3}d^{-1}$ (Metcalf and Eddy 2003).

Ganidi *et al.* (2011) strengthened Brown's conclusions by studying the relationship between organic loading rates and anaerobic digester foaming. Bench-scale batch digesters were operated using loading rates of 1.25, 2.5, and 5.0 kg VS $m^{-3}d^{-1}$ to determine the threshold for foaming. The loading rate of 2.5 kg VS $m^{-3}d^{-1}$ was determined to be the threshold, not producing

constant foam. However a loading rate of 5.0 kg VS $m^{-3}d^{-1}$ resulted in a persistent foam occurrence.

Surface Active Agents

'Surface active agents' are surfactants that are either chemically synthesized or microbial produced (bio-surfactants). A typical municipal waste will contain 1-20 mg/L of feed surfactants but this will increase if the anaerobic digester is overloaded (Barber 2005). Surfactants include oil, grease, volatile fatty acids, detergents, proteins and particulate matter (Vardar-Sukan 1998). Surface active agents have both hydrophilic and hydrophobic properties. The hydrophobic ends of surface active agents tend to move towards the air phase, and the hydrophilic ends, tends to move towards the liquid phase. Accumulation of the surface active agents' hydrophobic end at the air-liquid interface increase the surface activity and lowers the surface tension of the solution, increasing the risk of foam (Ganidi *et al.* 2011).

Volatile fatty acids (VFAs) are an important group of surface active compounds in biosolids digestion. Volatile fatty acids (VFAs), can vary in length but are normally low molecular weight compounds, soluble in water and sludge. The seven most common fatty acids that are found in anaerobic digesters are formic acid (HCOOH), acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3(CH_2)_2COOH$), valeric acid ($CH_3(CH_2)_3COOH$), iso-valeric acid ($(CH_3)_2CHCH_2COOH$) and caproic acid ($CH_3(CH_2)_4COOH$). The suggested ranges of these volatile fatty acids in anaerobic digesters vary between 50 and 300 mg/L as total VFAs concentration. Acetic acid is the dominant acid that accounts for approximately 85% of the total volatile fatty acids concentration in a typical anaerobic digester (Metcalf and Eddy 2003).

Volatile fatty acid concentration is a good parameter to monitor in determining the stability of an anaerobic digester. Volatile fatty acid accumulation in the anaerobic digester reflects an imbalance between the acid producing and acid consuming bacteria. If the digester is overloaded and contains a high VFA concentration, greater than the methane-producing bacteria (slow-growers) can consume, biogas production will quickly rise. This increase in biogas increases the foaming potential of the anaerobic digester (Baber 2005). Many researchers have identified that this increased foaming potential is caused by hydrophobic material in the digester attaching to the gas bubbles and rising to the top of the digester, creating a foam blanket (Barjenbruch *et al.* 2000, Bates 2006, Pagilla *et al.* 1997, and Westlund *et al.* 1998).

Greater concentrations of fats, oils, and grease (FOG) can increase and improve the biogas production of an anaerobic digestion system (Kabouris *et al.* 2009). However excessive FOG can be problematic to an anaerobic digester, FOG contains long chain fatty acids that are hydrophobic and can attach to gas bubbles and form a dense foam layer in the anaerobic digester (Moen 2006). As seen in the previous section, the greater presence of hydrophobic material can increase the foaming potential of the anaerobic digester.

Other parameters that can increase an anaerobic digester's ability to foam are: temperature fluctuation and mixing, which will be discussed in the section, *Monitoring of Foam*. The various causes of foaming are extremely site-specific to each anaerobic digester. Monitoring foam provides information on how the anaerobic digesters responds to these causes and will help determine patters in foaming potential.

MONITORING OF FOAM

Based upon the major causes of anaerobic digester foaming, this section will discuss the various methods that are available in the literature to quantify the foaming potential of a digester. Comparisons of foaming measurements under different operational conditions can provide insightful factors that affect foaming.

Monitoring of Filamentous Organisms

There are two main approaches used in the scientific community to identify filamentous organisms, which include: microscopic techniques and molecular methods. Microscopic examination is useful for determining physical characteristics and the abundance of filamentous organisms. Further investigation of the molecular method, fluorescence *in situ* hybridization (FISH) is becoming more important to be able to confirm the true identity of each filamentous bacterium.

Microscopic

When foaming in anaerobic digesters was first observed, microscopic techniques were the primary means of identifying the foam-causing organisms. Early work done by Eikelboom (1975) led to the development of a simple key for the identification of 26 different filamentous organisms, grouped into 7 categories, based solely on morphological characteristics. Eikelboom (1975) obtained approximately 1100 sludge samples from 200 different wastewater treatment plants in the Netherlands. Two techniques of staining were used, one being the Gram Stain and the other the Neisser stain. Phase contrast and electron microscopy were used to visualize the filaments for characterization. It was concluded that approximately 800 of the 1100 samples

contained at least moderate amounts of one or more filaments. Since the work of Eikelboom (1975) was completed, 40 additional morphotypes have been detected at low concentrations (Seviour and Nielsen 2010). Identification of filamentous organisms requires a well-trained person or misidentification can occur.

Filament Counting is another microscopic method used to characterize the filamentous community. This method was modified by Jenkins *et al.* (2003) to predict foaming in anaerobic digesters based upon the abundance of filaments in activated sludge and digested sludge. The quantity of the filaments is assessed by counting the number of intersections the filamentous organisms make with three equally spaced lines drawn across the microscope slide. The objective is that there is a correlation between the greater amount of filaments in the digester and the foaming potential of the digester.

Pitt and Jenkins (1990) also developed a method to quantify filament abundance based upon a seven point rating scale. This technique consists of microscopically examining a Gram stained slide for Gram positive filaments. The sample is assigned an arbitrary number based upon the number of filaments observed; the numerical rating scale is summarized in Table 1. Filaments were considered dominant when their abundance was above a numerical rating of 4, which is classified as 5-20 filaments per floc. Indicating that a foaming problem was common with a rating value of 4, while a rating of 2 did not indicate a foaming problem.

Table 1: Filament Density Numerical Rating Scale

Numerical rating scale	Abundance	Filament level
1	None	none
2	Few	Filaments only in an occasional floc.
3	Some	Filaments common but not present in all flocs
4	Common	Filaments in all flocs at low density (1-5 per floc)
5	Very Common	Filaments in all flocs at medium density (5-20 per floc)
6	Abundant	Filaments observed in all flocs at high density (>20)
7	Excessive	Filaments in all flocs; more filaments than flocs;

An additional microscopic method that was modified by Jenkins *et al.* (2003) was the Total Extended Filament Length (TEFL) method. This method determines the total length of filamentous organisms extending beyond the flocs and into the bulking solution. This method used 2 mL of well-mixed activated sludge with known total suspended solids (TSS) concentration and mixed in 2 L of distilled water. The suspension is stirred at 95 rpm for 1 minute and 1 mL is transferred to a microscopic slide. Using a microscope equipped with a ocular micrometer scale, counting the number of filaments present and categorizing them by size; 0-10 µm, 10-25 µm, 25-50 µm, 50-100 µm, 100-200 µm, 200-400 µm, 400-800 µm, and greater than 800 µm. The TEFL is used to document the physical nature of the filamentous organisms. No quantitative information was provided about the filament length and its effects on foaming in anaerobic digesters.

These microscopic techniques are rapid and can establish the abundance of filamentous organisms with a wastewater treatment plant. Regular monitoring of the filamentous organism in the activated sludge could help establish warning signs for an imminent severe foaming incident

of the anaerobic digester. These tests can only give visual characteristics of the filamentous organisms and their densities but cannot determine the true identity of the organisms.

Molecular Methods (FISH)

Identification of filamentous organisms into morphotypes at the microscopic level has been inadequate to understand foaming. Molecular techniques based upon the 16S rRNA gene, have made it possible to study the microbiology community in foam without any need for culturing or phenotype identification (Seviour and Nielsen 2010).

Fluorescence *in situ* hybridization (FISH) is a newly adopted technique that allows visualization of the organism based on their phylogeny. FISH also has the ability to quantify the number of bacteria presented in the sample. This technique uses rRNA-targeted oligonucleotide probes, the targeted probe contains a fluorescent molecule and the probes hybridize with complementary 16s rRNA structure in the microbial cell (Nielsen *et al.* 2009, Seviour and Nielsen 2010). Table 2 summarizes the FISH oligonucleotide probes that target the 16S rRNA of many of the foaming causing organisms that are found in foam.

Table 2: FISH oligonucleotide probes (Seviour and Nielsen 2010)

Probe name	Target group	Target site (rRNA positions, E. coli numbering)	Probe Sequence (5' to 3')	% formamide in FISH
S-*Myb-0736-a-A-22	Mycolata	0736-0757	CAGCGTCAGTTACTACCCAGAG	30
S-*Myb-0736-b-A-22	Mycolata	0736-0757	CAGCGTCAGTTACTxCCCAGAG	30
S-G-Gor-0596-a-A-22	*Gordonia*	0596-0617	TGCAGAATTTCACAGACGACGC	20
S-G-Gam-0192-a-A-18	*G. amarae*	0192-0209	CACCCACCCCCATGCAGG	30
S-S-G.am-0205-a-A-19	*G. amarae*	0205-0223	CATCCCTGACCGCAAAAGC	30
MNP1	*Mycolata*	0152-0172	AACCCATGCAGGCCGTAGTCC	50
DLP	*Dietzia, Rhodococcus*	0182-202	CCACCATGCGGVAGGAGCTCA	40
GLP2	*G. amarae*	0178-0197	AAGGGCAGGTCATATCCGGT	45
Rco1	*Rhodococcus*	0181-0193	ACCATGCAACCGGAGGTCAT	40
Rco2	*Rhodococcus*	0611-0631	AGCCCGCAGTTGAGCTCCGGG	40
Myc657	*Mycolata*	0657-0672	AGTCTCCCCTGYAGTA	30 at 37^{o}C
Spin 1449	*Skermania piniformis*	1449-1466	CCGCTCCCTCCCACAAAG	35
MPA 60	*M. parvicella*	0060-0077	GGATGGCCGCGTTCGACT	20
MPA 223	*M. parvicella*	0223-0240	GCCGCGAGACCCTCCTAG	20
MPA 645	*M. parvicella*	0645-0661	CCGGACTCTAGTCAGAGC	20
MPA 650	*M. parvicella*	0650-666	CCCTACCGGACTCTAGCT	20

FISH normally consist of six consecutive steps: probe synthesis, cell fixation, hybridization, wash, pre-analysis treatment, and sample analysis. The purpose of cell fixation is to inactivate,

preserve the cells, and permeabilize them for probe penetration (Seviour and Nielsen 2010). This step is a difficult step; the cell walls of the Mycolata are very difficult to permeabilize making it difficult for the probe to penetrate the cells and getting a strong FISH signal (Kragelund *et al* 2007).

For *Actinobacteria*, mycolic-acid containing bacteria and *Microthrix parvicella*, the cells are usually treated with aldehydes or alcohols. Kragelund *et al.* (2007) recommends pretreatment of the cells using absolute ethanol, to promote the strongest FISH signal, use with a combination of achromopeptidase and lysozyme solutions when treating the cells is recommended. de los Reyes *et al.* (1997) recommends pretreatment of the cells with a 4% paraformaldehyde solution.

Hybridization occurs at an elevated temperature and once the fluorescently labeled probe penetrates the cells, the probes hybridize to the targeted sequence on the ribosomal rRNA structures via hydrogen bonding (Amann 1995). If the cells undergo successful hybridization the cells can be visualized under epifluorescence microscopy using an appropriate wavelength. It is recommend that total cell structures on FISH slides should be checked by DAPI or a universal bacteria probe such as EUBmix (Nielsen *et al.* 2009).

There are many protocols available in the literature. The previous paragraph describes a general method for FISH. Using FISH to identify foaming causing microorganisms is still in the developmental stages; more research is needed. There are many unidentified filamentous organism that are still to be identified by FISH.

Foaming Potential

Analyzing foaming potential in parallel with microscopic and molecular characterization can provide an extremely important correlation among filamentous presence and abundance,

with the foaming potential of an anaerobic digester. When evaluating foam, two of the most important factors to evaluate are how easily foam forms and the foam's stability (Fryer *et al.* 2011).

There are many foaming tests available; one that has been widely used in the literature is based upon Blackall *et al.* (1991) who developed a test that measures the foamability and stability of the foam. This test uses a glass cylinder (40 mm diameter and 500 mm height) fitted with a sintered glass disc through which 200 mL/min of air is pumped. Fifty milliliters of sample is used and the generated foam is evaluated based upon; foam volume, bubble size, speed of formation, and time of foam to collapse.

Another way to quantify foam is by measuring the foam heights. Ho and Jenkins (1991) developed a test that used a 1 L graduated cylinder containing 500 mL of well-mixed sample, which was aerated at 4 ft^3/h through a sintered silica sand diffuser. Foam heights were measured every 10 s for 20 minutes and the average foam height was recorded at 5 minutes after the air is turned off. No conclusion was reached in this study that correlated foam heights with a foaming incidents.

Many researchers have used the Alka-Seltzer foaming potential test that is designed to determine the foaming potential of influent and effluent streams (de los Reyes *et al*. 2002, de los Reyes III and Raskin 2002, Marneri *et al*. 2009, and Oerther *et al.* 2001). Jenkins *et al*. (2003) modified this test using two Alka-Seltzer tablets, which are placed into a 500 mL graduated cylinder containing 250 mL of sample, simulating foam that exist in the wastewater treatment plant. The foaming potential was determined by recording the initial foam volume and the maximum foam volume observed. Then the stability of the foam was determined by noting the half-life of the foam, which is the time it takes for half the maximum volume of foam to dissipate

(de los Reyes III and Raskin 2002 and Jenkins *et al.* 2003). Despite the number of methods available in the literature to measure foaming potential, there is no standard method available and no consensus on the values that indicate a foamy digester.

Surface Tension and Alkalinity

In 1981, the American Society of Civil Engineers survey suggested a positive correlation between alkalinity and severe foaming problems in anaerobic digesters (Niekerk 1987). Barber (2005) suggested that alkalinity is inversely proportional to surface tension of a liquid. If the sludge's alkalinity increases, out of the typical range of 2000 to 4000 mg $CaCO_3$/L (Metcalf and Eddy 2003), this would result in a decrease in surface tension and thus increasing the risk for foam. Hence, studying the surface tension of sludge can be an indirect measurement of the foaming potential of an anaerobic digester. However, the literature suggests that finding a reliable method for measuring surface tension has been difficult.

Heard *et al.* (2008) used a modified method to examine the relationship between surface tension and foam. The surface tension was measured with an Analite Surface Tension Meter that used the Wilhelmy Plate Method. This method allowed the filtrate to equilibrate in a petri dish. Heard *et al.* (2008) concluded that stable foam was observed when the surface tension was less than 55 mN/m.

One study conducted by Ross and Ellis (1992) used a Fischer Surface Tensiomat, Model 21 to determine the surface tension of various digested sludge samples. This studied the effects of the volatile acid concentration in the anaerobic digester and how that would affect the surface tension. Ross and Ellis (1992) concluded that the apparent surface tension did not decrease with an increase in acid concentration. It should be noted that they could not generate consistent

results with acetic acid spiked sludge samples. They observed the surface tension increased between trials with the same acetic acid samples. They ruled out temperature fluctuation because the temperature was taken before and after the test was conducted; since surface tension is affected by temperature.

In the literature, researchers have identified a number of parameters that could potentially contribute to digester's ability to foam and monitoring techniques. Based upon this review there is no single method to correctly analyze foam in anaerobic digesters. All the data indicates that foaming is an extremely site-specific issue. Therefore the control measures differ widely based upon the exact cause of foaming and the type of sludge that is being stabilized

CONTROL OF FOAMING

The phenomenon of foaming in anaerobic digesters has affected numerous wastewater treatment plants around the world. This section will focus on measures that have been examined in the literature to mitigate foaming. The literature is inconsistent if advanced anaerobic digestion (thermophilic and acid-phase thermophilic) has the capability to reduce the foaming tendencies when compared to the more commonly used mesophilic configuration for biosolids stabilization.

Thermophilic Anaerobic Digestion

Thermophilic digestion is operated at temperatures between 50 and 57 ^{o}C. Because biochemical reaction rates increase with temperature, thermophilic digestion is much faster than mesophilic digestion. Having multiple advantages over mesophilic temperatures which include;

increased pathogen destruction, improved dewatering, and increased solids destruction capability (Metcalf and Eddy 2003).

The temperature of the anaerobic digester greatly influences the foaming potential. Temperature fluctuation can create instability in thermophilic digesters by affecting the activity of the volatile acid-forming and methane-forming bacteria. Fluctuations as small as 2 ^{o}C, can create enough instability to create severe foaming incidents (Gerardi 2003).

One study conducted at the Denver Metro Wastewater Treatment Plant demonstrated that mesophilic digesters seemed to have a lower tendency to foam than thermophilic digesters (Niekerk *et al.* 1987). Bench-scale digesters were feed a 45/55 (wt/wt) mixture of primary solids and waste activated sludge (WAS). The treatment plant analyzed solids for ammonia, alkalinity, and volatile fatty acids from the experimental digesters and found that these values were higher in the thermophilic digester than in the mesophilic digester. The author states that high ammonia concentrations ranging from 1800 to 2700 mg/L N were toxic to the methanogens so that volatile acids accumulated to levels of 1000 to 2000 mg/L of acetic acid (Niekerk *et al.* 1987). Since volatile fatty acids are surfactants, their accumulation increased the digester's ability to foam.

A study performed by Rubia *et al.* (2006) who used 850 L pilot-plant continuously stirred-tank reactors (CSTR) to examine the effects of thermophilic conditions. This study concluded that the thermophilic CSTR had higher VFA concentration than the mesophilic CSTR. The mesophilic CSTR had total VFA values approximately 260 g/m^3 while the thermophilic CSTR had values of approximately 1200 g/m3. This paper did not address the issue of foaming in the CSTR; however, the literature is consistent that higher VFA leads to foamier anaerobic digesters.

Marneri et al. (2009) could not draw a conclusion between the foaming potential of a single-stage mesophilic bench-scale anaerobic digester and a single-stage thermophilic digester. However this study did conclude that the thermophilic anaerobic digesters had the highest filamentous destruction rate; determining that foaming potential was not reduced with increased filamentous destruction. That greater destruction of filamentous organisms increased the concentration of hydrophobic compounds in the digester with the release of mycolic acid from the destroyed organisms.

Dual-Stage Anaerobic Digestion

Dual-stage anaerobic digestion is gaining attention because thermophilic/mesophilic staged anaerobic digestion incorporates the advantages of thermophilic digestion and mitigates the disadvantages through the addition of a mesophilic phase. Allowing dual-stage anaerobic digestion to be economical to operate since the majority of the sludge stabilization is performed in the mesophilic stage (Cheunbarn and Pagilla 2000).

Marneri *et al.* (2009) investigated the foaming potential of dual-stage anaerobic digesters. In this study, bench-scale anaerobic digesters were designed with operating parameters as summarized in Table 3. All the digesters were initially filled with mesophilic digested sludge from the Psyttalia Wastewater Treatment Plant in Athens, Greece. The reactors were feed daily with a 50/50 (wt/wt) mixture of WAS and primary sludge. FISH was used to monitor the rates of filamentous bacteria destruction; *Gordonia amarae* and *Microthrix parvicella*. The Alka-Seltzer foaming test was implemented to measure the stability of the foam.

Table 3: Operating Parameters of Bench Scale Anaerobic Digester (Marneri *et al*. 2009)

	Single Mesophilic Digester	Single Thermophilic Digester	Dual Thermophilic/ Mesophilic System		Dual Mesophilic Digester	
Detention Time (d)	20	20	8	12	10	10
Temperature (^{o}C)	35.5 ± 0.6	55.2 ± 0.3	54.6 ± 0.3	35.0 ± 0.5	36.6 ± 0.7	36.1 ± 0.7
pH	7.3 ± 0.1	7.3 ± 0.1	6.7 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.5 ± 0.1

Marneri *et al.* (2009) concluded that the best overall treatment process was the dual-stage thermophilic/mesophilic system. According to FISH, there was 77.8 to 97.1% destruction for *Microthrix parvicella*, while single-stage mesophilic only observed a 51 and 76% reduction. The dual-stage thermophilic/mesophilic system was the bench-scale anaerobic digester that showed the greatest reduction in foaming potential. The dual-stage thermophilic/mesophilic system had a 65% reduction in foaming potential while the single-stage thermophilic was reduced by 17% based upon the Alka-Seltzer foaming potential test.

Acid phase/Thermophilic

Many researchers have hypothesized that acid-phase digestion preceding thermophilic digesters could possibly mitigate potential foaming problems. Ghosh *et al.* (1995) observed no evidence of foam using bench-scale acid anaerobic digesters with a pH between 5.5 and 6.5 and a total volatile fatty acid concentration of at least 5000 mg/L followed by methane anaerobic digestion. The study did observe a foaming incident when the feed percent total solids were below 3 and the entire daily sludge load was feed to the acid-phase anaerobic digester once per day. This suggests that continuously fed, mixed operation helps alleviate foam. No foaming potential tests were employed in this study. These observations were based upon visual inspection of the bench-scale anaerobic digesters.

The quantification of the filamentous organism, *Nocardia*, was examined using bench-scale single stage anaerobic digestion and bench-scale two-phase anaerobic digestion consisting of an acid digester followed by a methanogenic digester at mesophilic temperature. The immunofluorescence counting technique was used to determine the number, length, and mass of *Nocardia*. The two-phase anaerobic digester showed a faster degradation rate of *Nocardia* to approximately half of the *Nocardia* than in the single stage anaerobic digester with a residence time of 14 days. A lower foaming potential based upon aeration technique was observed for the two-phase anaerobic digester (Hernandez and Jenkins 1994).

A warning from the Madison Metropolitan Sewer District (MMSD) in Madison, WI explains that acid-phase followed by thermophilic digesters may actually increases the foaming potential of the anaerobic digesters in certain situations (Reusser 2009). In September 2006, MMSD implemented this system and within a week of start-up the acid-phase digester pH was 5.2 and the VFA concentration was approximately 3000 mg/L. Since the acid phase digester seemed to be stable, the thermophilic anaerobic digester was gradually added into the system. By mid-November the plant observed that the pH was increasing and the VFA concentration was dropping significantly and the methane gas production was increasing in the acid-phase digester. At this point the plant began to observe significant foaming, which is shown in Figure 4.

Figure 4: Acid Digester Foaming out the Top of the Digester (Reusser 2009)

The plant discovered there was at least 15 feet of foam above the sludge level in the digester during the most severe foaming incident (Reusser 2009). The literature suggests that the increased foam potential in the acid-phase digester could be caused by the hydrophobic materials attaching to the gas bubbles and rising to the top of the digester (Baber 2005). There was no information presented about filament counts in the acid-phase digester or the thermophilic digester. Based on these findings and conflicting information in the literature, more research is needed on the topic of advanced anaerobic digestion treatment and the effects on foaming potential and foam stability.

Mixing and Digester Shape

The operation and design of anaerobic digesters, especially mixing and shape, play a critical role in the foaming potential of an anaerobic digester. A sufficient mixing system within the anaerobic digester is extremely important for the efficiency of the system. Mixing allows the contents of the anaerobic digester to be in suspension and in full contact with the microbial community within the anaerobic digester and eliminates dead zones and minimizes short-circuiting of the sludge.

Niekerk *et al.* 1987 found that foam levels produced in bench-scale anaerobic digester reactors depend on the mixing methods used. Fine bubble gas mixing produced more foam than coarse bubble mixing and mechanical mixing produced the least amount of foam. This results from mechanical mixers minimize the amount of gas bubbles that enter the anaerobic digesters when compared to gas-mixed digesters. Pagilla *et al.* (1997) also identified gas-mixing systems as an operational cause of excessive foaming in anaerobic digesters. He studied two full-scale

anaerobic digesters, one mechanically mixed and one gas mixed at the Sacramento Regional Wastewater Treatment Plant in California making sure the digesters had nearly the same operating conditions. The results show that foam occurred in both the gas mixed and mechanically mixed digesters; however, there was greater foam in the gas-mixed digester (Pagilla *et al.* 1997). The average foam layer thickness in the gas mixed digester was 2.4 m where as the average foam layer thickness in the mechanically mixed was 1.3 m (Pagilla *et al.* 1997).

These findings correlate with the findings of Jenkins *et al.* (2003) that hydrophobic particles will attach to the gas bubbles and rise to surface of the digester creating a foam blanket. The formation of a foam blanket causes a solids gradient that reduces as the digester deepens. It was determined in one study that the percent total solids were 5% at the surface of the digester and about 2% at the bottom of the digester (Pagilla *et al.* 1997), resulting in inefficient sludge stabilization. Since all activated sludge systems are aerated, it is not possible to eliminate the gas phase from the activated sludge aeration basin; however, the gas entering the anaerobic digester can be minimized and hopefully minimize foaming resulting from the presence of gas bubbles by utilizing mechanically mixed digesters.

There is speculation that the geometry of the anaerobic digester could help to minimize foaming. However, there is no evidence in the literature to support a relationship between digester shape and foam occurrence to date (Dalmau *et al.* 2010). In the United States, the majority of the wastewater treatment plants use cylindrical digesters because they are more cost effective to build. Several advantages and disadvantages have been discussed in the literature between cylindrical and egg-shaped anaerobic digesters.

Cylindrical digesters have a greater headspace available when compared to the egg shaped digesters, which allows for more gas to be stored in the anaerobic digesters. This could increase the amount of foam that accumulates in the digester. Egg shaped digesters have a smaller area above the bulk liquid to accumulate foam which allows some researchers to conclude that egg shaped reduces the digesters ability to have excessive foam (Metcalf and Eddy 2003). Mixing and digester shape, which is more effective in an egg shape, potentially play a critical role in the foaming potential of the anaerobic digester (Dalmau *et al.* 2010). More research is needed.

Reduction in Sludge Age

Operating activated sludge aeration tanks with an optimal sludge age can be one control technique to limit the proliferation of the filamentous organisms in the WAS. Potentially decreasing the foam potential in the anaerobic digesters by decreasing the number of filamentous organism entering the digesters from the WAS. Pitt and Jenkins (1990) conducted a nationwide survey and concluded that there was a 73% success rate among the 44 plants that are controlling filamentous foaming by reducing the sludge age. A factor contributing to the success of this strategy is that nocardioform actinomycetes, typical filamentous organisms, are slow growing (Rossetti *et al*. 2005).

Pitt and Jenkins conducted a number of bench-scale activated sludge studies to determine if reducing the sludge age decreases filamentous organism density. They concluded that if the Mean Cell Residence Time (MCRT) was less than 5 days, the filamentous count was significantly less than the filamentous count with a MCRT greater than 5 days. They also reported that if the MCRT was less than 5 days unstable foam was observed using the aeration

foaming potential test. Cha *et al*. (1992) used the filament counting technique to demonstrate that *Nocardia* populations increased with increasing MCRT. Reducing the number of filamentous organisms in the WAS would results in less filamentous organisms entering the anaerobic digester.

Chemicals and Antifoaming Polymers

Today there are many available options to control foam using chemicals and antifoaming agents. Chlorine, the most common chemical used, has been used to kill filamentous organisms and prevent proliferation and reduce the foaming potential of anaerobic digesters. The chlorine is added to the return activated sludge (RAS) and the literature suggests that this strategy is not very effective for controlling filamentous organisms in activated sludge. One study showed a success rate of 58% at activated sludge plants attempting to mitigate foaming with chlorination (Pitt and Jenkins 1990).

A study conducted in Japan concluded that *Microthrix parvicella* showed a greater tolerance to chlorine than other filamentous organisms, requiring 10-100 times higher doses for destruction. This suggests that a chlorine dose of 200 g Cl/ kg MLSS is needed to break up approximately 50% of the coils and 500 g/kg is needed to destroy the majority of *Microthrix parvicella* (Barber 2005). Consequently, this large chlorine dose is costly and will degrade effluent quality.

Studies have shown that polyaluminium chlorides (PAX) are an effective control strategy for activated sludge foaming. PAX are hydroxide or oxyhydroxide species with more than one aluminum nucleus. The number associated with a specific PAX depends upon the aluminum

concentration in the compound (Hahn *et al.* 2002) and the PAX concentration needed varies depending upon the solids retention time (Roels *et al.* 2005). One study recommended a dose for PAX-14 of 2-3 g Al/kg MLSS/day in the return activated sludge (RAS) for a minimum of three weeks (Rossetti *et al.* 2005). While Jacobsen *et al.* (1997) recommends a higher dose, 15 g Al/kg MLSS is needed to inhibit the growth of *Microthrix.*

To examine how PAX affects foaming, samples of activated sludge were collected from aeration tanks at various full-scale wastewater treatment plants that have been experiencing significant foam (Nielsen *et al.* 2005). Using morphology and FISH, it was identified that these activated sludge samples contained the known foam-causing organism: *Microthrix*. Adding PAX-14 to the activated sludge with a concentration range of 0-80 mg/L Al per gram of suspended solids, Nielsen *et al*. (2005) evaluated the effect of PAX-14 on the potential to alter the hydrophobicity of the *Microthrix* using microsphere adhesion to cells (MAC). Studies suggest that PAX is a positively charged compound and will attach to the negatively charged surface of the *Microthrix,* affecting the hydrophobic properties of the cell wall (Rossetti *et al*. 2005). No change to the hydrophobicity of the cell wall was observed in Nielsen *et al.*'s study.

It is also hypothesized that PAX acts as a flocculation agent (Nielsen *et al.* 2005). Narayanan *et al.* (2010) used bench-scale, continuous-flow activated sludge systems and observed that PAX-14 did not decrease the *Nocardia* organism counts. When PAX-14 and a cationic polymer were dosed together, a decrease in the dispersed *Nocardia* counts was measured. This suggests that the PAX-14 and the cationic polymer have the ability to flocculate the *Nocardia* organisms reducing their ability to foam.

An additional study investigated PAX-21, The PAX-21 was added to the influent of the WAS thickener prior to the solids being fed to the anaerobic digester at a wastewater treatment

plant in Himmerfjardsverket, Sweden that was experiencing foam in their anaerobic digesters. It was emphasized that the PAX-21 must be completely mixed with WAS to work properly. Through visual inspections and observation, this study concluded that the PAX-21 was successful at minimizing the foam in their anaerobic digesters (Westlund *et al.* 1998).

An additional option for wastewater treatment plants to try to alleviate foam in anaerobic digesters is through the use of antifoaming agents. There are over 700 commercially available antifoaming agents, these chemicals work by changing the interfacial properties of the fluid, the antifoaming agent will decrease the surface elasticity of the liquid and prevent foam formation (Barber 2005). These antifoaming agents can be made of oils, fatty acids, esters, polyglycols, siloxanes, and sulfonates (Vandar-Sukan 1998). Ross *et al*. (1992) used bench-scale anaerobic digesters to determine that the optimal concentration of defoamants was 500 uL/L. This study used visual observations and recorded the height of the foam. There is little data available in the literature to the success rate of applying antifoaming agents. Anti-foaming agents are a costly quick fix to a very complicated issue.

Separated Stabilization of WAS and Primary Sludge

Westlund *et al*. (1998) suggested that separating the waste activated sludge from the primary sludge would minimize foaming from filamentous organisms. A wastewater treatment plant in Sweden implemented this idea and designed their anaerobic digesters such that; primary sludge was pumped into the first anaerobic digesters and the WAS pumped into the second digester. The fundamental idea behind this design was to minimize foaming since gas production should be lower in the second anaerobic digester that contains the filaments contained in the WAS. This minimizes the filamentous organisms being entrapped in the gas bubbles and rising

to the surface of the digester, creating foam. This plant declares that their foaming problems were eliminated when the design of separate digestion was put in operation (Sundin 2008).

RESEARCH NEEDED

There is still a lot of information that needs to be learned about the diverse microbial community that may disrupt smooth operation of anaerobic digesters. Especially understanding the specific organisms involved for each individual situation that is experiencing severe foaming. It seems fair to state that more information is known about how to prevent filamentous organisms from growing, but the causes of their dominance is unknown and more research is needed. Further examination into the many parameters that are thought to increase foaming potential of the anaerobic digesters is essential for solving the foaming epidemic.

Based upon the literature, foaming appears to be extremely site-specific, and any one or unique combination of the multiple factors listed in Section 2.0 may be the underlying cause. Therefore, standardized and systematic testing on a site-specific base is needed to understand these variations.

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CHAPTER 3: FOAMING PHENOMEMON IN BENCH-SCALE ANAEROBIC DIGESTERS

ABTRACT

The Madison Metropolitan Sewerage District (The District) in Madison, WI has been experiencing seasonal foaming in their anaerobic biosolids digesters, which occurs from mid-November to late June for the past few years. The exact cause(s) of foaming is unknown. Previous research findings are unclear as to whether applications of advanced anaerobic digestion processes reduce the foaming potential of digesters. The object of this study was to investigate how configurations of thermophilic and acid phase-thermophilic anaerobic digestion would affect foaming at the bench-scale level compared to single stage mesophilic digestion. Bench-scale anaerobic digesters were fed with 4-4.5 % by dry weight of solids content blend of waste activated sludge (WAS) and primary sludge from The District. Foaming potential was monitored using Alka-Seltzer and aeration foaming tests. The bench-scale acid phase-thermophilic digester had a higher foaming potential than the bench-scale mesophilic digester. These results indicate that higher temperatures increase the foaming potential of the bench-scale anaerobic digesters. The bench-scale thermophilic and acid phase-thermophilic digesters had a greater percent (2-8%) volatile solids destruction and a greater percent (2-9%) total solids destruction when compared to the bench-scale mesophilic digester.

INTRODUCTION

Foaming in wastewater treatment plants can be characterized by the formation of a thick, stable, chocolate-brown scum which floats on the surfaces of aeration basins and secondary clarifiers and can be carried over into the biosolids digestion processes (Jenkins *et al.* 2003). Foam can accumulate to such an extent that the foam overflows the basin freeboard and covers walkways creating hazardous conditions. Foaming also causes several operational problems, such as:

- blockage of gas mixing devices;
- inversion of digester solids profiles;
- gas binding of sludge recirculation pumps leading to inability to heat the digesters;
- foam penetration between floating covers and digester walls;
- pressurization of digesters resulting from blocked gas piping; and
- environmental contamination caused by bio-aerosols.

  (Bates *et al.* 2006, Jenkins *et al.* 2003, and Pagilla *et al.* 1997)

The widespread occurrence of foaming is best illustrated through information gathered in a recent survey of Publicly Owned Treatment Works (POTWs). It was determined by the Central States Water Environmental Association that there are 216 POTWs that use anaerobic digestion for biosolids in the states of Wisconsin, Minnesota, and Illinois (CSWEA, 2010). With a 44 % (94 responded out of 216) survey response rate, a total of 53 % (50 out of 94 responses) of those POTWs reported having significant foaming issues in the past ten years.

Prerequisites for foaming include the presence of surface active agents, a gaseous phase and hydrophobic material, which are all present in anaerobic digesters (Barber *et al.* 2005). Although the exact cause of foaming is unknown and the literature present conflicting results. Researchers have identified several parameters that could potentially contribute to digester foaming, such as:

- inadequate blending of waste activated sludge (WAS) and Primary Solids (PS);
- insufficient mixing of digesters;
- gas mixing;
- temperature fluctuation;
- filamentous organisms;
- excessive grease and scum in digester feed;
- accumulation of volatile fatty acids in digesters; and/or
- organic overloading.

(Pagilla *et al.* 1997 and Bates *et al.* 2006)

The Madison Metropolitan Sewerage District (The District) has been experiencing seasonal (mid-November to late May) foaming for the past few years. The research presented in this paper aimed to examine the microbial ecology of foaming and various other parameters used in The District's anaerobic digestion process. The goal was to identify the site-specific factors affecting foaming using bench-scale anaerobic digesters. This information will be used to design strategies to mitigate or control foaming at The District.

METHODS

Bench-Scale Anaerobic Digester Design

Bench-scale anaerobic digesters operated under a variety of conditions were constructed to investigate foaming in The Districts biosolids. The experiments were conducted in four phases over a 2-year period. This design is a modification of the bench-scale anaerobic digesters that were built at the Wastewater Research Section located at the Joint Water Pollution Control Plant (Tang, 2009) of the Los Angeles County Sanitation District, CA.

The bench-scale digesters had a capacity of 6 L (Corning, 4502-6L, Corning, NY) with a neck diameter of 120 mm, and two side arm openings of 45 mm diameter. The bench-scale anaerobic digester is shown in Figure 5. The digesters were seeded with digested sludge from The District. The temperature was maintained between 35-55^{o}C using temperature controlled water baths with daily monitoring. Styrofoam balls and Styrofoam sheets were placed around and above the digesters for insulation and to maintain incubation temperatures.

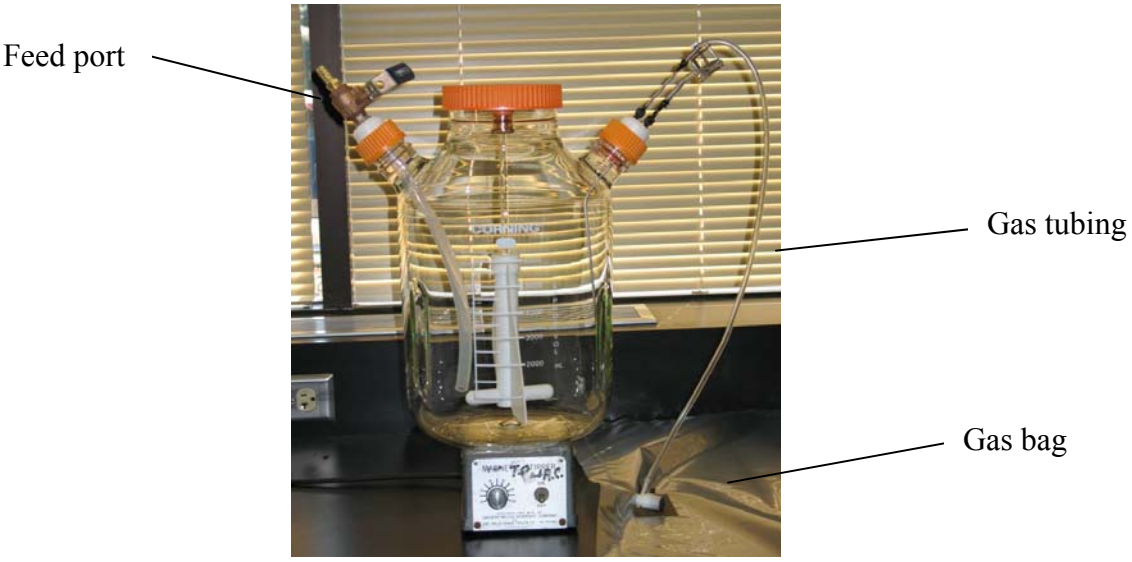


Figure 5: Bench-Scale Anaerobic Digester Design

Bench-Scale Anaerobic Digester Operation

Biosolids withdrawal and feeding were conducted daily during each phase of the experiments. Six liters of biosolids were stirred in the bench-scale anaerobic digester for 15 minutes on a magnetic stir plate prior to sampling and feeding.

The bench-scale anaerobic digesters had an 18-day solids retention time (SRT); therefore the feeding and withdrawing rate was 333 mL/day. The digester effluent was withdrawn prior to feeding to minimize short-circuiting. The digesters were fed a 4.0 to 4.5% by dry weight of solids mixtures of WAS and primary sludge which were collected weekly and stored separately at 4^{o}C. Based upon % solids measurements, conducted weekly, the mass proportion of WAS and primary sludge were prepared immediately before feeding each day. The feed varied based on operational conditions being tested. See following sections for details. After feeding, the digester were purged with helium for 2 minutes to aid in maintaining anaerobic conditions and then placed back into the temperature controlled water bath.

Bench-Scale Anaerobic Digester Configuration

Phase 1 was operated from October 2009 to February 2010 to determine "favorable ecosystem conditions" for the growth of filamentous foam-causing organisms that would promote foaming. Phase 1 included three bench-scale anaerobic digesters:

- Control digester;
- Digester spiked with cultures of *Rhodococcus* and *Skermania*, which were available from the American Type Culture Collection (ATCC); and
- Digester spiked with center well foam scum, from the activated sludge settling tank.

The three digesters were operated under mesophilic conditions (35-37ºC) with an 18-day solids

retention time. The digesters were fed with a 4.0 to 4.5% by dry weight of solids mixture of WAS and primary sludge.

Phase 2 was operated from February 2010 to June 2010 to evaluate whether thermophilic and/or acid phase-thermophilic operations would potentially mitigate foaming in the bench-scale anaerobic digesters and by inference, potentially at full-scale. Phase 2 included four bench-scale anaerobic digesters:

- Control digester (1) (mesophilic conditions with 18-day SRT);
- Thermophilic digester (2) (with 18-day SRT); and
- Mesophilic acid phase digester (3) (2-day SRT) followed by thermophilic digester (4) (with 18-day SRT).

The control digester was similar to that in Phase 1. The thermophilic digester was incubated in a 53-55ºC waterbath to maintain constant temperature. The control and thermophilic digesters were fed with a 4.0 to 4.5% by dry weight of solids from the mixture of WAS and primary sludge. The mesophilic acid phase digester was fed with a 4.0 to 4.5% by dry weight of solids from the mixture of WAS and primary sludge. pH was maintained below 5.5 in the mesophilic acid phase digester. The subsequent thermophilic digester was fed with effluent from the mesophilic acid phase digester.

Phase 3 was operated from November 2010 to March 2011 to continue evaluating potential mitigation strategies for foaming in bench-scale anaerobic digesters. Phase 3 included five bench scale anaerobic digesters:

- Control digester (1) (mesophilic conditions with 18-day SRT);
- Mesophilic acid phase digester (2) (37^{o}C with a 2-day SRT) followed by thermophilic digester (3) (with 18-day SRT); and
- Higher mesophilic temperature acid phase digester (4) (46ºC with a 2-day SRT) followed by thermophilic digester (5) (with 18-day SRT).

The control digester (1) was operated similar to that in Phase 1 and 2. The mesophilic acid phase digester and the thermophilic digester (2 and 3) were performed similar to those in Phase 2. The higher temperature mesophilic acid phase digester (4) was run similar to that in Phase 2, but temperature was increased to 46^0 C. The subsequent thermophilic digester (5) was fed with effluent of acid phase digester (46^0 C).

Phase 4 was operated from March 2011 to May 2011 to examine the effects of heat treatment of phosphorus release (P-release) WAS, proposed for implementation at The District for phosphorus recovery. The P-release WAS was prepared by adding 20 mL of 17N glacial acetic acid to 20 gallons of thinned WAS into a pilot-scale dissolved air flotation (DAF) tank. The mixture of glacial acetic acid and thinned WAS held for 5 hours and then a 3:1 ratio of whitewater to sludge was added to thicken the WAS. The p-release thickened WAS was collected and stored in 4^oC until mixed with primary sludge immediately before feeding. Phase 4 included five bench scale anaerobic digesters:

- Control Digester (1) (mesophilic conditions with 18-day SRT);
- Mesophilic acid phase digester (2) (with 1.5 day SRT) followed by thermophilic digester (3) (with 18-day SRT); and
- Mesophilic acid phase digester (4) (with 1.5 day SRT) followed by thermophilic digester (5) (with 18-day SRT).

The control digester (1) was operated similar to that of Phase 1. The mesophilic acid phase digester and the thermophilic digester (2 and 3) were performed similar to those in Phase 2. The acid phase digester (4) and thermophilic digester (5) was run similar to that in Phase 2, but was fed with P-release WAS subject to heat treatment at 71.1ºC for four hours before mixing with primary solids for feeding the mesophilic acid phase digester (4).

Analytical Methods

A suite of measurements were made to assess bench-scale process performance and foaming potential. Table 4 summarizes the physical and chemical analyses performed on the feed and digester effluent samples from the Phase 1 through Phase 4 of the bench-scale anaerobic experiments.

Table 4: Analytical Measurements

Parameters	Method	Reference	Frequency
Alkalinity	Standard Method 2320, Alkalinity by Titration	APHA *et al.* 2005	Weekly
pH	Method 4500-H, pH by Electrometric		
Total Solids	Standard Method 2540-G, Total, Fixed and Volatile Solids		
Total Volatile Solids	Standard Method 2540-G, Total, Fixed and Volatile Solids		
Volatile Fatty Acids	The District's in house method	-	Bi-weekly
Total Kjeldahl Nitrogen	EPA Method 351.2, Total Kjeldahl Nitrogen-Block Digestion	US EPA 1993	
Total Phosphorus	EPA method 365.4, Total Phosphorus	US EPA 1974	

Gram stains were used to document the visual characteristics of the filamentous organisms in the bench-scale anaerobic digester effluent and feed samples on a weekly basis using digital photography. The microscope was an Olympus Bx50 fitted with an Olympus DP 70 digital camera (Olympus, Center Valley, PA).

To examine the density of filamentous organisms in Gram stains, a 1:7 rating scale developed by Pitt and Jenkins (1990) was implemented. Filamentous organisms were dominant when their abundance was above a numerical rating of 4, classified as 5:20 filaments per floc (Jenkins *et al.* 2003).

The simplified filament counting technique was also used to determine the relative amount of filaments that are extending from flocs in anaerobic digester samples (Jenkins *et al.*

2003). A known amount (50 μL) of sample was placed on a microscope slide and the entire width of the slide was observed. The total filament count for the entire slide is determined.

Foaming Potential Tests

Alka-Seltzer Foaming Potential Test

This test involved placing 250 mL of a well-mixed biosolids sample into a 1 L graduated cylinder and adding 2 tablets of commercial Alka-Seltzer antacid. Foam height was recorded every 20 seconds until foaming ceased or up to 33 minutes. The maximum foam height was also recorded. Foaming potential was calculated using Equation 1. The higher foam volumes correlate to a higher foaming potential.

$$\text{Foaming Potential (FP)} = \frac{\text{Maximum Foam Volume Observed}}{\text{Volume of Digester Effluent}} \qquad \text{Eq (1)}$$

To determine the stability of foam, half-life of the foam was determined. The half-life was defined as the time for half of the maximum foam height to diminish (de los Reyes *et al.* 2002 and Jenkins *et al.* 2003). A longer half-life indicates a more stable foam.

Aeration Foaming Potential Test

Aeration foaming potential test was measured based on the work by Zabranska *et al.* (2002). One hundred milliliters of well-mixed anaerobic digester effluent was placed into a 500 mL graduated cylinder. A one-inch aeration stone connected to a pump with a flow rate of approximately 1 L/min was placed into the graduated cylinder. The volume of sludge was recorded before aeration (V_0). The sludge was aerated for 5 minutes and the level of foam was recorded (V_5). Maximum foam height was recorded (V_{max}). The foaming potential was calculated for both V_5 and V_{max} in the numerator of Equation 1.

Statistical Analysis

Statistical analyses (correlation and ANOVA) were performed to examine causative or associative relationships between various parameters, such as pH, alkalinity, volatile fatty acid (VFA) concentrations, etc. The statistical analyses were aimed to understand operation parameters associated with foaming potential and occurrence in The District's anaerobic digesters.

Correlation Analysis

Correlation analysis was performed using Microsoft Excel 2003. Correlation coefficients (R^2 values) were recorded. Values close to ± 1.00 indicate the parameters vary in a similar or opposite manner to each other in the reactors. Smaller correlation coefficients indicate that parameter vary in different ways.

Analysis of Variance (ANOVA)

One-way analysis of variance (ANOVA) was used to determine whether there were differences between the five foaming classifications and the measured parameters (% TS, %VS, VFA, pH, Alkalinity, TP, and TKN). The foaming classifications are: Not Foamy, Weak Foam, Fast Collapsing Foam, Weak Foam but Stable, and Strong Foam but Stable. These categories were determined based upon results of the aeration and Alka-Seltzer foaming potential tests, and summarized in Table 5. For this study a *p*-value less than 0.05 was considered to be statistically significant.

Table 5: ANOVA Foaming Classification

Classification	Experimental Reasoning
Not Foamy (NF)	Small Alka-Seltzer and aeration foam volume
Weak Foam (WF)	Alka-Seltzer or aeration gives moderate to large volume, Alka-Seltzer collapse slowly but before 11 min
Fast Collapsing Foam (FC)	Large Alka-Seltzer volume that falls off quickly before 11 min, half-life less than 1 minute
Weak Foam but Stable (WS)	Alka-Seltzer or aeration gives moderate to large volume, Alka-Seltzer falls back slowly >11 min, half-life greater than 1 minute
Strong and Stable (SS)	Alka-Seltzer and aeration give large volume, Alka-Seltzer falls back slowly >11 min

For this study a *p*-value less than 0.05 at a 90% confidence level was considered to be statistically significant.

RESULTS AND DISCUSSION

Performance of Bench-Scale Anaerobic Digesters

Bench-scale anaerobic digesters were examined to determine the effects of thermophilic and acid phase-thermophilic conditions on the foaming potential of the biosolids. The average (± standard deviations) for chemical analyses for each phase of the digesters is summarized in Table 6.

As shown in Table 6, the pH of biosolids in the thermophilic and acid phase-thermophilic digesters was as high as 7.8. The pH of biosolids in the control mesophilic digesters had a value closer to 7.2. This is a result of higher alkalinity observed in the thermophilic and acid phase-thermophilic digesters. For example, alkalinity of 3938 ± 142 mg/L for the mesophilic digester was observed in Phase 2 where the alkalinity of biosolids in the thermophilic digester was 4918 ± 231 mg/L.

Table 6: Performance characteristics of Phase 1 to 4

	pH	Alkalinity (mg/L)	Total Phosphorus (ppm)	Total Kjeldahl Nitrogen (ppm)	% Total Solids Reduction	% Total Volatile Solids Reduction	Total VFA, (mg/L)
Phase 1: October 2009 to February 2010							
Dig Control (meso)	7.2 ± 0.1	3426 ± 411	383 ± 52	1060 ± 180	39 ± 3%	48 ± 1%	144 ± 70
Digester Culture Seeded (meso)	7.2 ± 0.1	3441 ± 430	356 ± 36	1015 ± 91	41 ± 4%	49 ± 3%	134 ± 51
Digester Center Well (meso)	7.3 ± 0.1	3434 ± 424	356 ± 35	1025 ± 127	40 ± 3%	49 ± 1%	164 ± 42
Phase 2: March 2010 to June 2010							
Dig Control (meso)	7.3 ± 0.1	3938 ± 142	287 ± 22	1071 ± 93	39 ± 4%	48 ± 4%	162 ± 96
Dig Thermo	7.6 ± 0.1	4918 ± 231	290 ± 17	1323 ± 105	43 ± 7%	45 ± 7%	350 ± 157
Dig Acid phase (37^{o}C)	5.5 ± 0.1	3000 ± 214	600 ± 122	691 ± 240	16 ± 5%	20 ± 5%	5882 ± 796
Dig Acid phase-Thermo	7.6 ± 0.1	5042 ± 351	304 ± 33	1371 ± 177	52 ± 6%	55 ± 6%	396 ± 235
Phase 3: November 2010to March 2011							
Dig Control (meso)	7.3 ± 0.2	3481 ± 579	263 ± 46	932 ± 165	48 ± 12%	56 ± 11%	80 ± 39
Dig Acid phase (37^{o}C)	5.5 ± 0.1	3084 ± 308	714 ± 78	775 ± 90	17 ± 3%	21 ± 4%	4914 ± 895
Dig Acid phase (46^{o}C)	5.5 ± 0.1	3340 ± 252	700 ± 62	899 ± 95	21 ± 7%	25 ± 8%	5040 ± 970
Dig Acid phase-Thermo (46^{o}C)	7.5 ± 0.3	4619 ± 734	296 ± 29	1281 ± 175	53 ± 12%	62 ± 10%	412 ± 304
Dig Acid phase-Thermo (37^{o}C)	7.5 ± 0.3	4851 ± 726	290 ± 45	1274 ± 206	57 ± 9%	65 ± 8%	346 ± 235
Phase 4: March 2011 to May 2011							
Dig Control (meso)	7.4 ± 0.1	3896 ± 164	283 ± 155	1258 ± 314	45 ± 7%	54 ± 7%	65 ± 37
Dig Acid phase (37^{o}C)	5.4 ± 0.1	2656 ± 155	431 ± 145	840 ± 406	15 ± 9%	19 ± 9%	3805 ± 1579
Dig Acid phase (p-released)	5.5 ± 0.1	2425 ± 267	280 ± 108	661 ± 278	17 ± 7%	20 ± 7%	2560 ± 1448
Dig Acid phase-Thermo (37^{o}C)	7.7 ± 0.1	4915 ± 191	279 ± 136	1080 ± 314	48 ± 10%	58 ± 9%	186 ± 185
Dig Acid phase-Thermo (p-released)	7.7 ± 0.1	4650 ± 248	207 ± 121	956 ± 30	48 ± 10%	58 ± 8%	102 ± 79

The bench-scale measurements summarized in Table 6 demonstrate an increase in percent total solids reduction and percent total volatile solids reduction for the thermophilic and acid phase-thermophilic anaerobic digesters when compared to the mesophilic digesters. For example in Phase 2 and 3, the acid phase-thermophilic digesters on average resulted in a 52 ± 6% total solids reduction and 57 ± 9% total volatile solids reduction where the mesophilic digester resulting 39 ± 4% and 48 ± 12%, respectively. These results are in good agreement with those presented by Zabranska *et al.* (2002). That is, one major advantage of advanced anaerobic

digestion (phased acid-thermophilic digestion), higher % total solids and % volatile solids destruction when compared to mesophilic anaerobic digestion.

As presented in Table 6, the average concentration of volatile fatty acids (VFA) in the mesophilic digester in Phase 2 was 162 ± 96 mg/L, where the thermophilic digester was 350 ± 157 mg/L, and there were 396 ± 235 mg/L VFAs in the acid phase-thermophilic digester. These results show less complete stabilization in the thermophilic and acid phase-thermophilic digesters. These two digester configurations have greater concentration of VFA in the effluent than the mesophilic digester. In Phase 3, similar differences in reactor VFA concentration can be noted. Song *et al.* (2004) also reported that higher VFA concentrations in thermophilic digesters result from methanogenic bacteria that are more sensitive to environmental changes. These environmental changes reduce the methanogenic bacteria ability to convert VFA to methane.

Another disadvantage of thermophilic and acid phase-thermophilic digestion is less process stability, as observed with the high variability with the total volatile fatty acid content as shown in Table 6. For example in Phase 3 the mesophilic digester had a standard deviation of 39 mg/L where the acid phase (37 ^{o}C)-thermophilic digester had a standard deviation of 235 mg/L.

Investigation of Foaming for Bench-Scale Anaerobic Digesters

Bench-scale anaerobic digesters were investigated to determine a relationship between the digester's configuration and foaming potential. Based upon the foaming classification that was defined in Table 5, the most frequently observed classification for each bench-scale anaerobic digesters are shown in Table 7.

Table 7: Foaming Classification of Phase 1 to 4

Bench-Scale Anaerobic Digester	Weak Foam	Fast Collapsing Foam	Weak Foam but Stable	Strong and Stable
Phase 1: October 2009 to February 2010				
Dig Control (meso)	X	X		
Digester Culture Seeded (meso)	X	X		
Digester Center Well (meso)	X	X		
Phase 2: March 2010 to June 2010				
Dig Control (meso)	X	X		
Dig Thermo	X	X		
Dig Acid phase (37^{o}C)	X		X	
Dig Acid phase-Thermo	X	X		
Phase 3: November 2010to March 2011				
Dig Control (meso)	X			
Dig Acid phase (37^{o}C)			X	
Dig Acid phase (46^{o}C)			X	X
Dig Acid phase-Thermo (46^{o}C)	X	X		
Dig Acid phase-Thermo (37^{o}C)	X		X	
Phase 4: March 2011 to May 2011				
Dig Control (meso)	X			
Dig Acid phase (37^{o}C)			X	
Dig Acid phase (p-release, 37^{o}C)		X		X
Dig Acid phase-Thermo (37^{o}C)	X	X		
Dig Acid phase-Thermo (p-released)		X		

The foaming classification, on average, fluctuated between weak foam and fast collapsing foam for the 18-day SRT digesters with the exception of the mesophilic acid phase-thermophilic digester in Phase 3 which exhibited weak but stable foam. However, the acid phase 2-day SRT digesters had a foaming classification of either weak but stable foam and/or strong and stable foam. These classifications can more easily be understood through the general trends of each digester's foam stability. Data for each reactor type from different experimental Phases were pooled for this comparison. While experimental results (see Phase I discussion) demonstrate that feed character which is affected by time of year significantly controls whether the reactors foam or not, the variation among the data can account for these differences. Figure 6 shows the average (± standard deviations) of the foam's half-life from the Alka-Seltzer foaming potential tests pooled across all Phases. Bearing in mind the seasonal differences in feed, the bars

of the same color can be directly compared to each other, while comparisons among all results may not be completely valid.

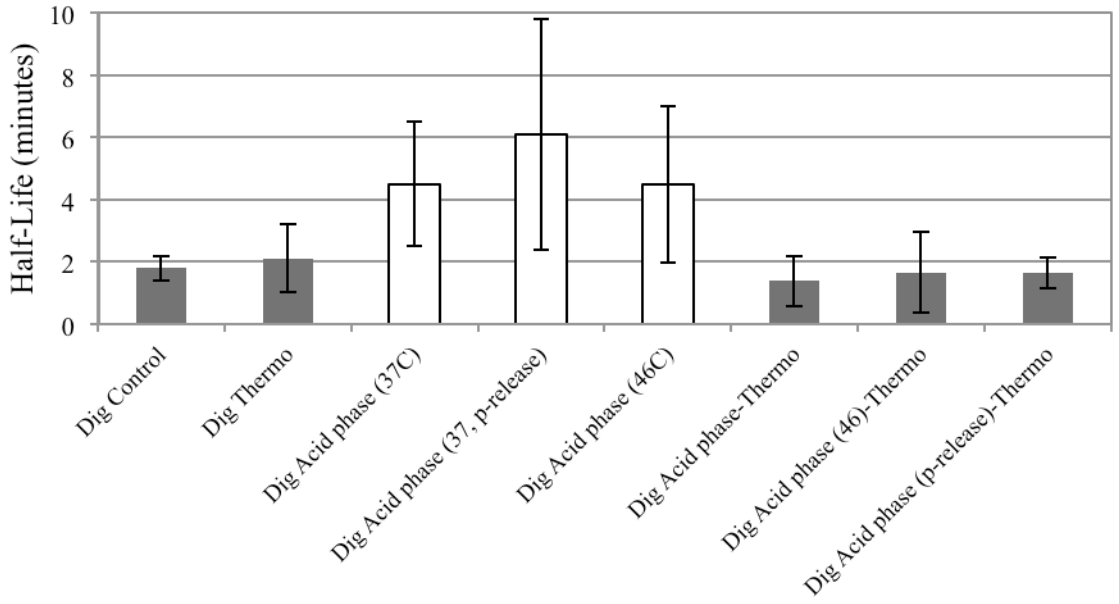


Figure 6: Half-Life by Alka-Seltzer Foaming Potential Test

As shown in Figure 6 the half-life varied approximately between 1 and 3 minutes for the 18-day SRT anaerobic digesters (gray bars). The thermophilic digester appears to have the most stable foam of the 18-day SRT bench-scale anaerobic digesters. A greater foaming stability was observed on average in the acid phase digesters when compared to the 18-day SRT bench-scale anaerobic digesters. However, the variance among the data resulting from testing being conducted at different times of year may diminish the significance of this observation. The acid phase digesters varied approximately between 3 and 10 minutes while the acid phase (37, p-release) displayed the greatest foam stability. Foam stability is hypothesized to correlate to the foams ability to maintain a foam layer (de los Reyes and Raskin 2002).

Phase 1

The purpose of this phase was first to determine if a stable foaming anaerobic digester could be achieved at the bench-scale level. As shown in Table 6, the pH, alkalinity, total phosphorus, total VFA, and percent volatile and total solids reductions are within the typical range (Metcalf and Eddy 2003) for anaerobic digesters. Therefore, the digesters were stable and functioning properly. The design of the anaerobic digesters did achieve significant foaming. This conclusion is supported by an increased presence of filamentous organisms in photos of Gram stains slides, shown in Figure 7. The increased concentration of filamentous organisms in the WAS is one major parameter that is believed to contribute to foaming in anaerobic digesters (Zabranska *et al.* 2002).

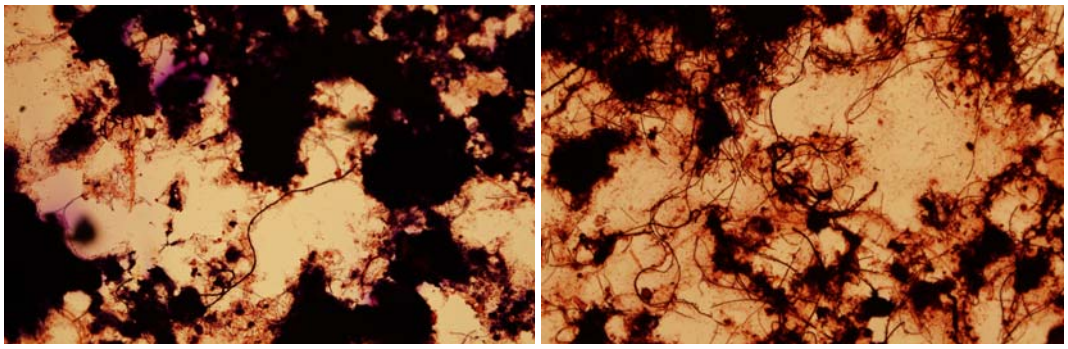


Figure 7: Phase 1 Gram Stains photos of Control Digesters. Photo on the left taken November 12, 2009 and the photo on the right taken January 13, 2010.

Figure 8 presents the foaming potential based on the Alka-Seltzer test for all three digesters in Phase 1 (note the y-axis is on a log scale). Foaming potential varies between 100 and 200 percent and is similar for all three reactors. This demonstrates that seeded filamentous organisms or center well foam cannot force foaming. The statistical analyses showed no difference between control and seeded digesters with all p-values >0.05. Temporal variations between the foaming potential were observed among all three digesters. Since foaming cannot be

forced in the bench-scale anaerobic digesters used, the time of year and quality of feed solids appeared to be more important in studying the foaming potential in the bench-scale anaerobic digesters. The results of Phase 1 exhibited that foaming potential in bench-scale anaerobic digesters could only be obtained during the foaming season, which typically occurs mid-November to June at The District.

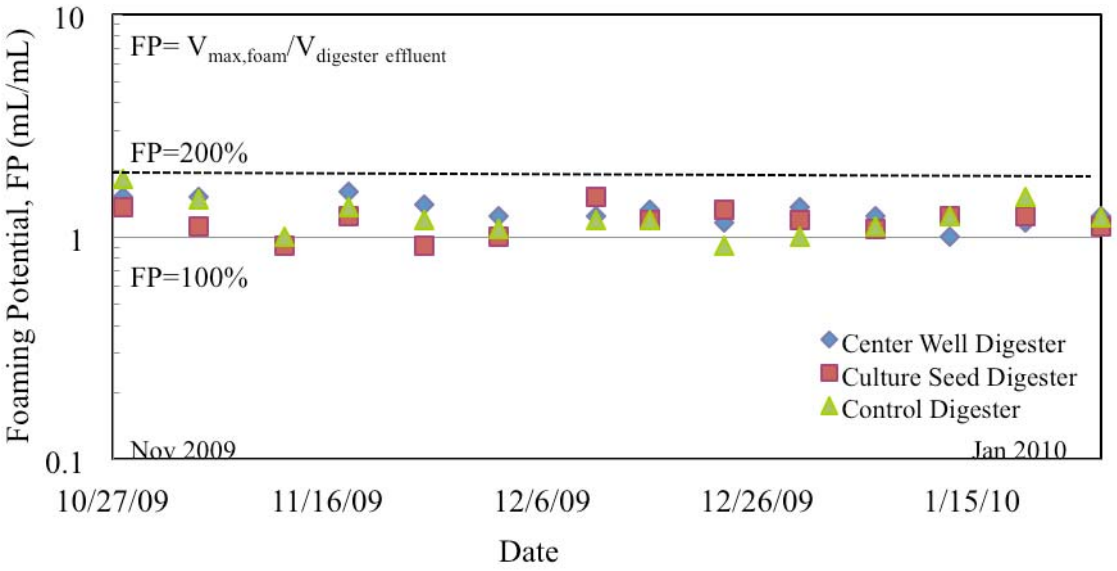


Figure 8: Phase 1 Foaming Potential by Alka-Seltzer Test

Statistical analysis of Phase 1 was performed to identify the significant parameters, such as pH, alkalinity, and VFA concentration, which may cause foaming in the District's anaerobic digesters. Based on correlation analyses, Alka-Seltzer foaming potential is statistically (p-value < 0.05) correlated with alkalinity (R^2=0.724), pH (R^2=0.724), total Kjeldahl nitrogen (R^2=0.875), percent total solids (R^2=0.816), percent total volatile solids (R^2=0.822), and total phosphorus R^2=0.863). A one way analysis of variance (ANOVA) showed that foaming classification (see Table 5) is statistically significant at a 90 percent confidence level (p-value < 0.05) with pH, total Kjeldahl nitrogen, percent total solids, percent volatile solids, Alka-Seltzer test results and

filament count. Some of these associations were positive (alkalinity and pH) and others were negative (TKN, %TS, %VS, and TP). However associations among parameters do not imply causality.

Phase 2

The objective of this phase was to determine if advanced anaerobic digestion (thermophilic and acid phase-thermophilic digestion) decreases the foaming potential when compared to the mesophilic anaerobic digester. As shown in Figure 9, foaming potential varies approximately between 100 and 200 percent for all three digesters. There is no statistical difference (p-value > 0.05) among the mesophilic (control) digester and the thermophilic digester based upon the Alka-Seltzer foaming potential test results. These results corresponded to the conclusions of Marneri et al. (2009) which did not observe a significant decrease in foaming potential of bench-scale thermophilic anaerobic digester when compared to mesophilic conditions. In Phase 2, there was a statistically significant difference observed (p-value < 0.05) between the control digester and the acid phase-thermophilic digester, indicating acid phase-thermophilic digestion has greater foaming potential. Once again, correlations do not infer causality.

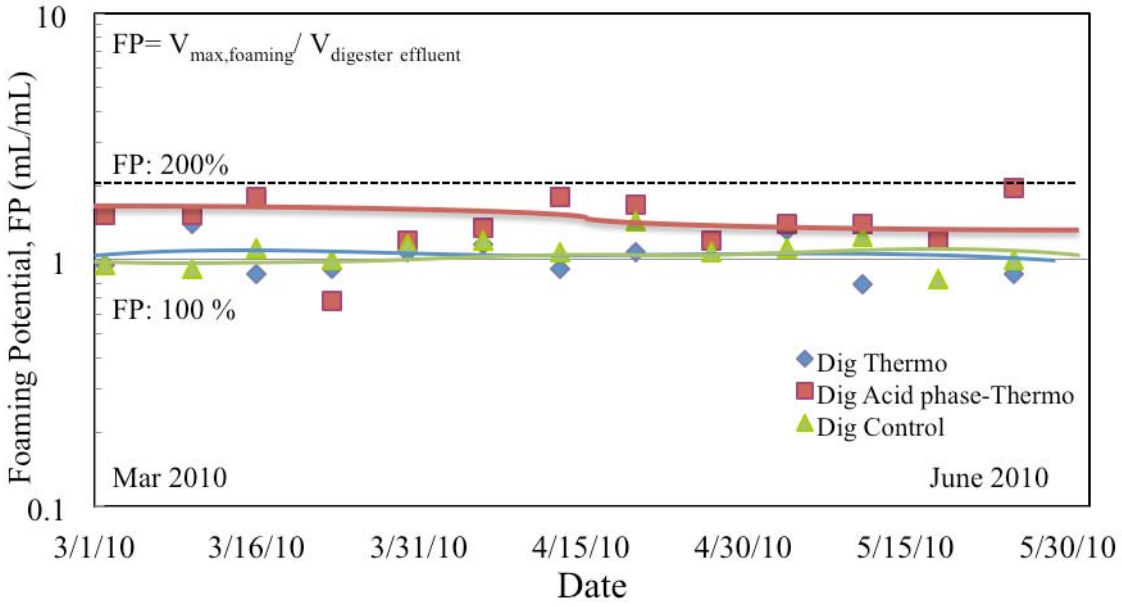


Figure 9: Phase 2 Foaming Potential by Alka-Seltzer Test

Figure 10 shows that foaming potential by aeration varies from 140 to 200 percent during the foaming season as experienced at The District. There is no statistical difference (p-value > 0.05) among the 3 bench-scale anaerobic digesters during foaming season. However, there was a statistical difference (p-value < 0.05) among the three digesters out of foaming season with the thermophilic and acid phase-thermophilic digesters demonstrating greater foaming potential.

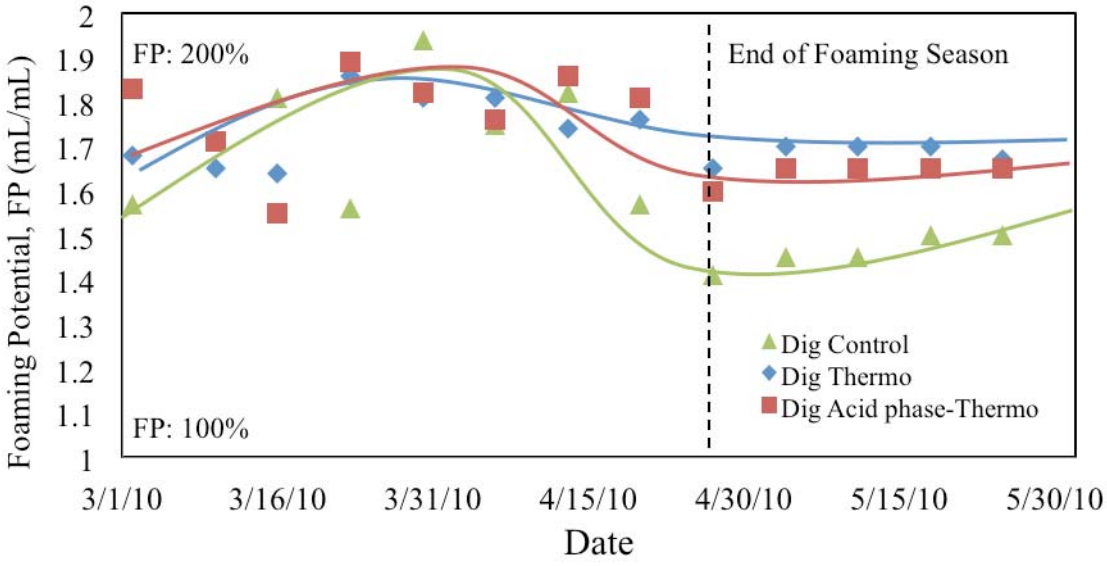
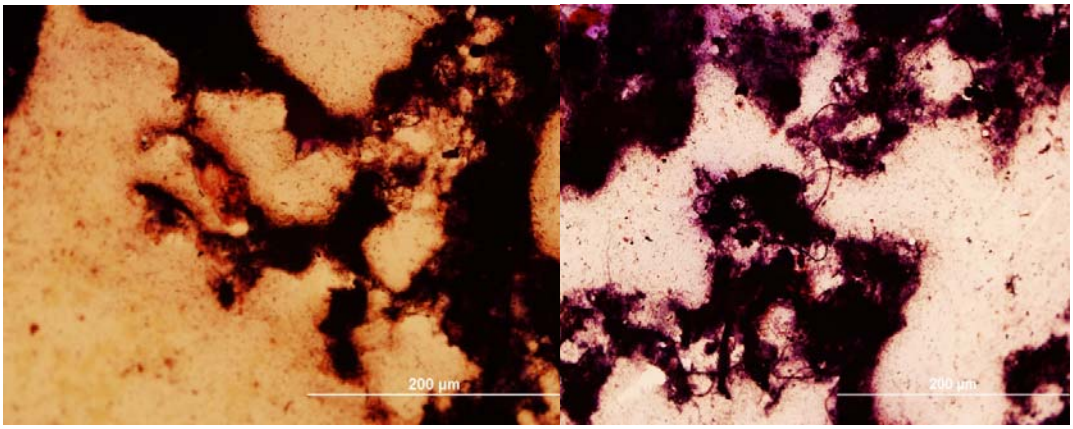


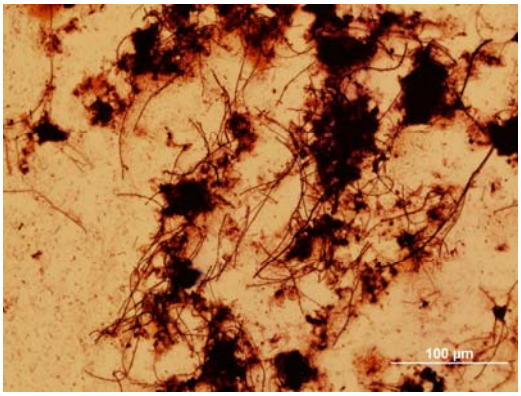
Figure 10: Phase 2 Foaming Potential by Aeration Test

From the results of Phase 2, at the bench-scale level, thermophilic and acid phase-thermophilic digestion did not reduce the foaming potential when compared to bench-scale mesophilic digestion. van Niekerk *et al.* (2006) and Rubia *et al*. (2006) also indicated that greater foaming potential in thermophilic digesters was associated with the greater volatile fatty acid accumulation. As shown in Table 6 for Phase 2, the thermophilic digester and acid phase-thermophilic digester have greater total volatile fatty acid content (350 ± 157 and 396 ± 235 mg/L) than the mesophilic digester (162 ± 96 mg/L).

From the Gram stain slides presented in Figure 11, the acid phase-thermophilic digester and thermophilic digester had a decreasing presence of filamentous organisms when compared to the mesophilic (control). Zabranksa *et al.* (2002) also determined that thermophilic digestion had a greater efficiency of destroying the filamentous organism than mesophilic digestion based upon Gram stain slide analysis. The greater destruction of filamentous organisms in thermophilic and acid phase-thermophilic anaerobic digesters increases the concentration of colloidal hydrophobic compounds, like mycolic acid in the digesters (Marneri *et al.* 2009). Mycolic acid is located on the cell walls of these filamentous organisms allowing the filamentous organisms to attach to solid particles rendering them hydrophobic. The filamentous organisms attach onto the gas bubbles present in anaerobic digesters and rise to the surface of the liquid creating a foam blanket (Jenkins *et al.* 2003). Fragmented filamentous organisms can more evenly distribute foam-associated compounds throughout the digester, increasing foam (Marneri *et al.* 2009).



Acid phase-Thermophilic Digester Thermophilic Digester



Control Digester

Figure 11: Phase 2 Gram Stain Photos of Filamentous Organisms from April 22, 2010

Correlation analysis on Phase 2 data showed that alkalinity (R^2=0.737), pH (R^2=0.381), percent total solids (R^2=0.731), percent volatile solids (R^2=0.740), and filament counts (R^2=0.333) that correlated with the Alka-Seltzer foaming potential results. A one way analysis of variance (ANOVA) showed that foaming classification (see Table 5) is statistically significant at a 90 percent confidence level (p-value < 0.05) with alkalinity, pH, percent total solids, percent volatile solids, and aeration test results. These associations; however, do not necessarily imply cause and effect.

Phase 3

The purpose of Phase 3 was to continue to evaluate the effects of acid phase-thermophilic digestion on the foaming potential and confirm results observed in Phase 2. As shown in Figure 12, the foaming potential from Alka-Seltzer tests varied approximately from 70 to 200%. There was a significant statistical difference (p-value < 0.05) in foaming potential between the acid digesters (37 vs. 46ºC). The acid digester at 46°C had a greater foaming potential than the acid digester at 37°C.

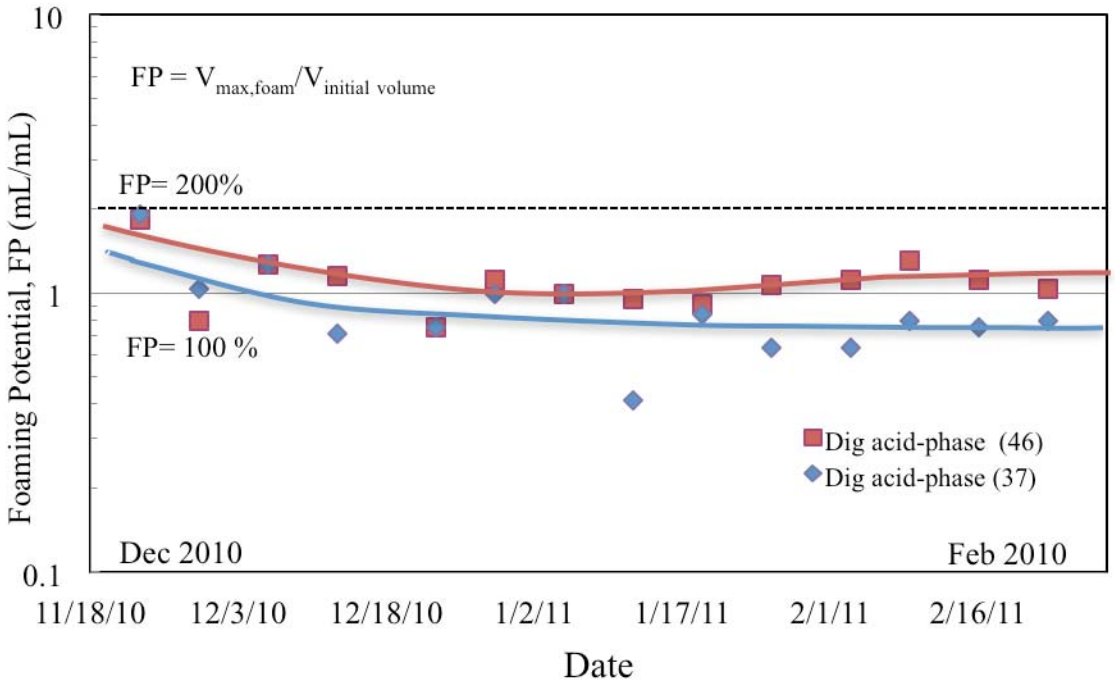


Figure 12: Phase 3 Foaming Potential of Acid-Phase Digesters by Alka-Seltzer Test

Comparing Figures 9 and 13, the trend in foaming potential for the Phase 3 Alka-Seltzer test results for the control digester and two acid phase-thermophilic digesters is similar to that in Phase 2. Figure 13 shows that increased temperature and increased filament destruction increase the foaming potential at the bench-scale level. These results are consisted with Phase 2.

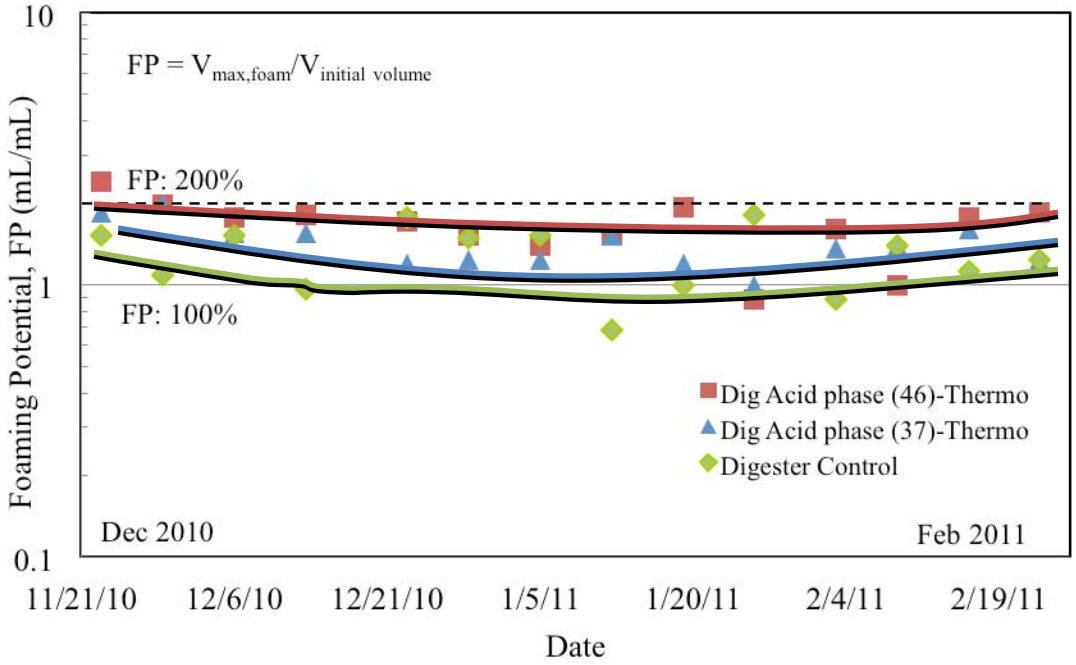


Figure 13: Phase 3 Foaming Potential by Alka-Seltzer Test

The results from the aeration tests for Phase 3 also demonstrated similar trends as was observed from Phase 2 (Figures 14 and 10). When foaming was not present at full-scale, there was a statistically significant difference (p-value < 0.05) when comparing the two acid phase-thermophilic digesters with the mesophilic (control) digester. There was no difference among the three digesters during foaming season (foaming present at full-scale).

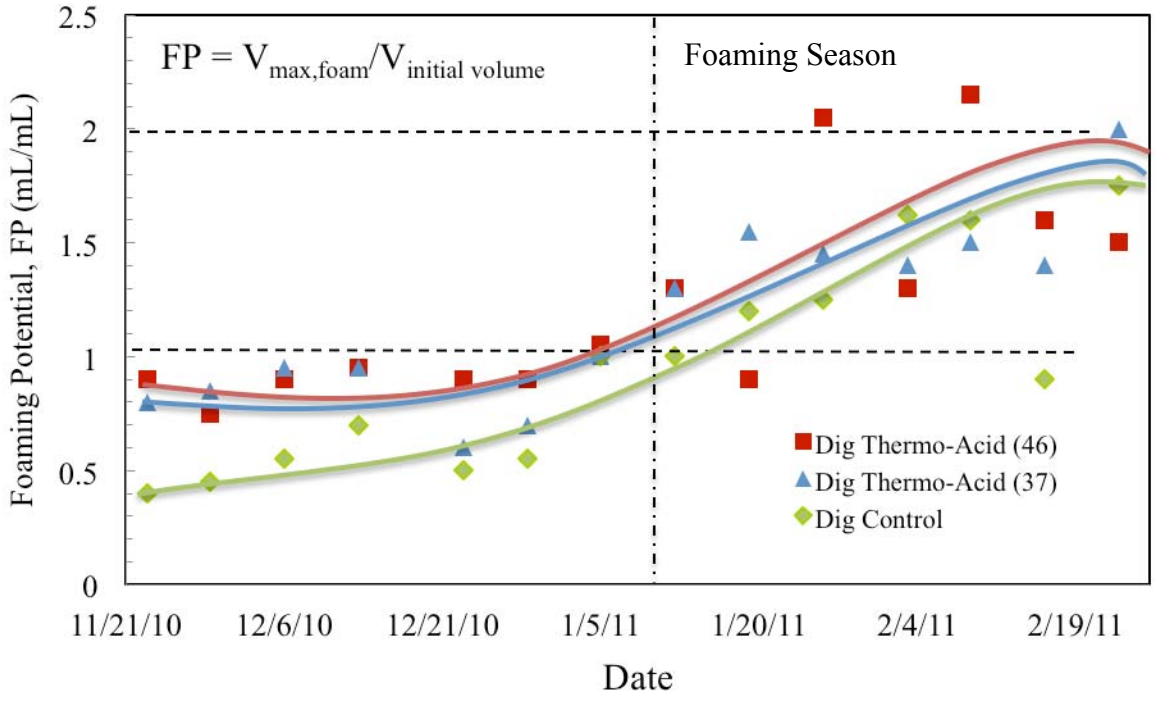


Figure 14: Phase 3 Foaming Potential by Aeration Test

Based upon statistical analyses alkalinity (R^2=0.442), pH (R^2=0.201), percent total solids (R^2=0.582), percent volatile solids (R^2=0.608), total Kjeldahl nitrogen (R^2=0.346) and total phosphorus (R^2=0.532) significantly correlated with Alka-Seltzer foaming potential. These results deviate from Phase 2 where alkalinity was correlated but total Kjeldahl nitrogen was not. While ANOVA showed that alkalinity, pH, percent volatile solids, and percent total solids, results are statistically significant for foaming classification. These results deviated slightly from Phase 2 where aeration test results were also significant.

Phase 4

The purpose of Phase 4 was to evaluate the effect of phosphorus release (P-release) and thermal treatment (71.1 ^{o}C for 4 hours) of the WAS on foaming potential. Utilizing P-release and thermal treatment of the WAS has the potential to alter the sludge structure by breaking down the

filamentous organisms' cell walls and possibly decreasing the foaming potential (Barjenbruch *et al.* 2000). This process is under consideration for use at The District to recover phosphorus. As shown in Figure 15, foaming potential varies approximately between 100 and 200%. Figure 15 shows that greater destruction of filamentous organisms and increased temperature increase foaming potential. The bench-scale anaerobic digester that was fed P-released WAS demonstrated a greater foaming potential than the mesophilic digester (control). There is no statistical difference among the acid phase-thermophilic digester and the acid phase-thermophilic digester fed the P-release WAS; however, both of these digesters showed a statistical difference (p-value < 0.05) when compared to the mesophilic (control) digester.

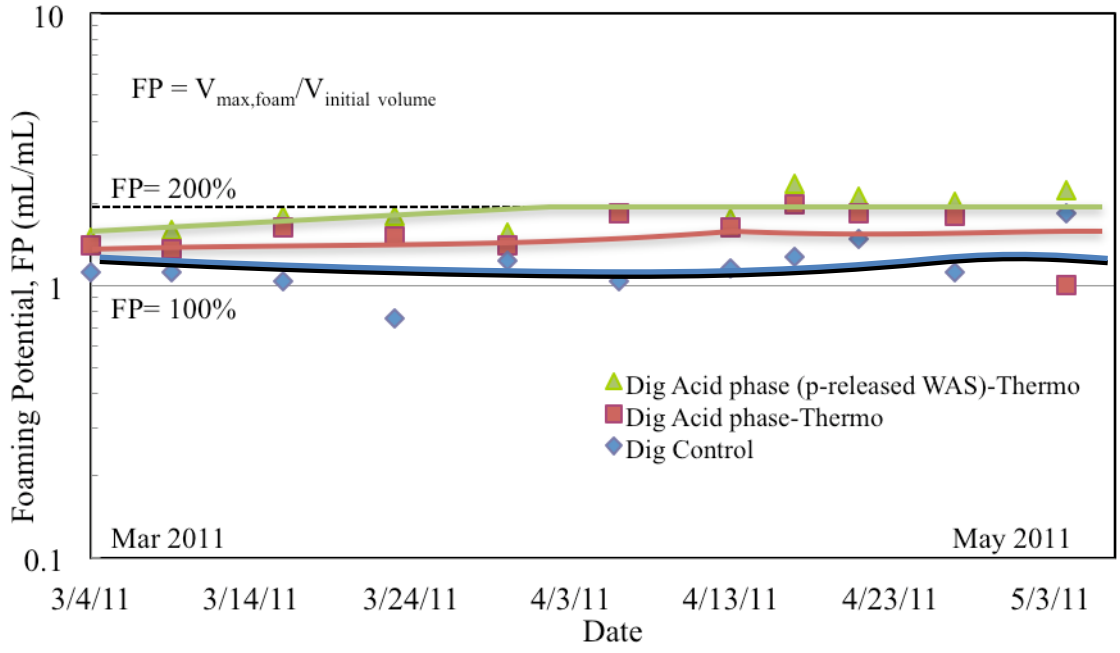


Figure 15: Foaming Potential by Alka-Seltzer Test

Correlation analysis shows that there is a significant correlation between the Alka-Seltzer foaming potential and alkalinity (R^2=0.732), pH (R^2=0.392), total Kjeldahl nitrogen (R^2=0.338), total phosphorus (R^2=0.637), percent total solids (R^2=0.689), percent volatile solids (R^2=0.700), filament numerical rating scale (R^2=0.160), and filament counting (R^2=0.310). ANOVA revealed

a statistical significance between the foaming classifications and alkalinity, pH, percent total solids, and percent volatile solids.

Pooled Statistical Analyses in Bench-Scale Anaerobic Digesters

Keeping in mind that the four Phases of experimentation were conducted at different points in the foaming season, analysis of pooled data can provide an overview of reactor characteristics associated with foaming. Thus, statistical analyses were performed pooling all the data from all four experimental phases. Alkalinity, pH, total VFA, and percent volatile solids were found to be statistically related to foaming potential by correlation analysis, i.e., p-value < 0.05. ANOVA analyses confirmed that foaming classification is related to differences in alkalinity, pH, total Kjeldahl nitrogen, total phosphorus, percent volatile solids and percent total solids (p-value <0.05). The correlation between foaming potential and alkalinity ($R^2 = 0.165$) is consistent with what has been reported by van Niekerk (1987). Alkalinity is inversely proportional to the surface tension of a liquid (Barber 2005). Increased alkalinity results in a decrease in surface tension and a greater risk of foaming. While these parameters are associated, it is unclear if there is a cause and effect relationship.

Total VFA concentrations are statistically correlated ($R^2 = 0.149$) with foaming potential in the studied bench-scale anaerobic digesters. VFAs are surfactants meaning they have both hydrophilic and hydrophobic properties. The hydrophobic end of the surfactant tends to move towards the air phase, and the hydrophilic ends, tends to move towards the liquid phase. Accumulation of surfactants in anaerobic digesters increases the surface activity and lowers the surface tension of the biosolids increasing the risk of foam (Ganidi *et al.* 2011). Thus, conditions that favor maximizing methanogenesis are potentially beneficial.

Foaming Potential in Bench-Scale v. Full-Scale Anaerobic Digesters by Alka-Seltzer Test

A number of parameters affecting an anaerobic digester's ability to foam are difficult to mimic using bench-scale anaerobic digesters, such as solids feed rates, mixing, and temperature fluctuation. Figure 16 illustrates the ability of the Alka-Seltzer foaming potential test to be able to determine if bench-scale anaerobic digesters can determine the foaming potential of the wastewater treatment plant's anaerobic digesters. The bench-scale mesophilic anaerobic digester using Alka-Seltzer tests is able to mimic a similar foaming potential trend seen in The District's full-scale mesophilic anaerobic digesters. The foaming potential in bench-scale and full-scale mesophilic anaerobic digesters are approximately between 100 and 200%. However, The District's full-scale mesophilic anaerobic digester displays a greater foaming potential than the bench-scale mesophilic anaerobic digester, although the trends overtime parallel eachother.

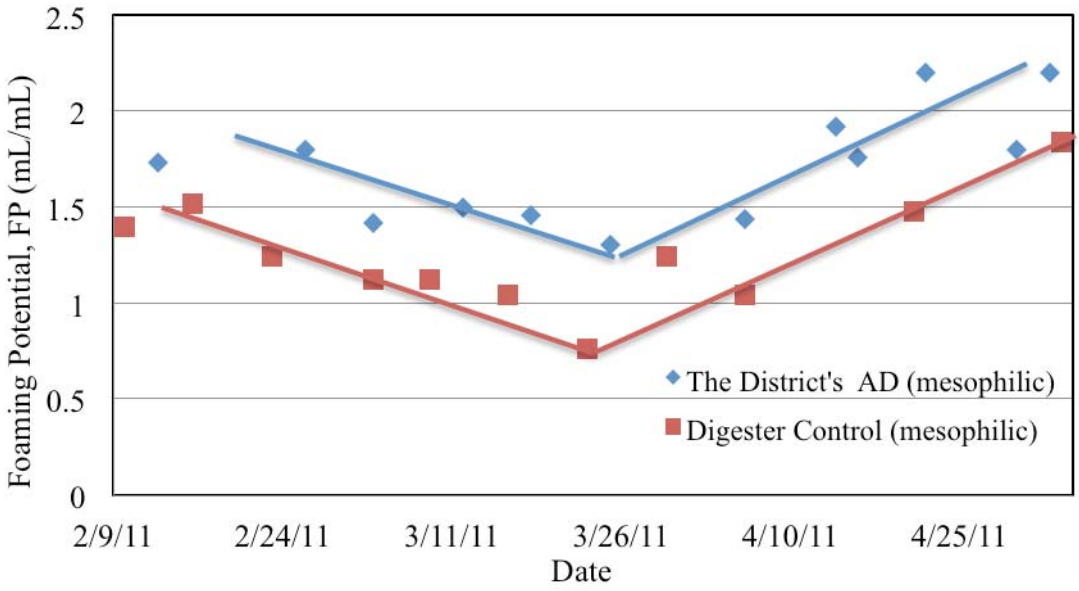


Figure 16: Foaming Potential by Alka-Seltzer Test

CONCLUSIONS

In this study, the performance of bench-scale mesophilic, thermophilic, and acid phase-thermophilic biosolids anaerobic digesters were evaluated with respect to foaming potential. The causes of foaming at any given plant may be numerous and complex. The bench-scale system presented here appears to be useful to evaluate process variation on a site-specific basis.

It was concluded that for The District, foaming could not be forced by seeding in foam-causing organisms. It was the time of the year and quality of the feed solids that dictated the foaming potential of the anaerobic digesters at the bench-scale level. Additionally it was determined that bench-scale thermophilic and acid phase-thermophilic anaerobic digestion increased the foaming potential in bench-scale anaerobic digesters. These results can be explained by the fact that greater destruction of filamentous organisms does not change the hydrophobic nature of the cell walls of the filamentous organism it just more evenly distributes them throughout the digester causing an increase in foaming potential. Further studies are needed to identify all of the site-specific cause(s) of foaming so that appropriate mitigation measures can be designed.

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APPENDIX A: Phase 1

Table 8: Phase 1 Summary Table of Foaming Results Over Time

Sample	Foam Volume Max. Alka-seltzer (mL)	Foam Half life Alka-seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
10/28/09				
Feed	50 mL	1:20	-	-
Dig Control	455 mL	3:40	30 mL	-
Dig Culture Seed	380 mL	2:40	10 mL	-
Dig Center Well	340 mL	3:20	30 mL	-
11/4/09				
Feed	95 mL	2:20	-	-
Dig Control	370 mL	1:00	22 mL	-
Dig Culture Seed	380 mL	1:40	14 mL	-
Dig Center Well	280 mL	1:00	24 mL	-
11/12/09				
Feed	75 mL	0:40	-	-
Dig Control	250 mL	4:00	20 mL	-
Dig Culture Seed	240 mL	3:00	25 mL	-
Dig Center Well	230 mL	4:20	20 mL	-
11/18/09				
Feed	105 mL	6:00	-	-
Dig Control	340 mL	2:00	22 mL	-
Dig Culture Seed	400 mL	1:20	24 mL	-
Dig Center Well	310 mL	1:20	25 mL	-
11/25/09				
Feed	90 mL	4:00	-	-
Dig Control	300 mL	1:40	24 mL	-
Dig Culture Seed	350 mL	1:40	20 mL	-
Dig Center Well	230 mL	1:40	19 mL	-
12/2/09				
Feed	65 mL	5:00	-	-
Dig Control	270 mL	4:40	13 mL	-
Dig Culture Seed	310 mL	1:00	20 mL	-
Dig Center Well	250 mL	2:40	12 mL	-
12/11/09				
Feed	85 mL		-	-
Dig Control	300 mL	2:40	33 mL	-
Dig Culture Seed	310 mL	2:20	40 mL	-
Dig Center Well	380 mL	1:00	30 mL	-

Sample	Foam Volume Max. Alka-seltzer (mL)	Foam Half life Alka-seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
12/16/09				
Feed	90 mL	-	-	-
Dig Control	300 mL	2:20	39 mL	-
Dig Culture Seed	330 mL	1:20	35 mL	-
Dig Center Well	300 mL	1:00	40 mL	-
12/23/09				
Feed	45 mL	2:40	-	-
Dig Control	230 mL	4:20	39 mL	49 mL
Dig Culture Seed	290 mL	3:40	41 mL	46 mL
Dig Center Well	330 mL	3:00	30 mL	40 mL
12/30/09*				
Feed	45 mL	-	-	-
Dig Control	250 mL	6:40	34 mL	39 mL
Dig Culture Seed	340 mL	3:40	54 mL	64 mL
Dig Center Well	300 mL	2:40	52 mL	77 mL
1/6/10				
Feed	65 mL	8:00	-	-
Dig Control	280 mL	3:00	54 mL	79 mL
Dig Culture Seed	310 mL	4:20	61 mL	91 mL
Dig Center Well	270 mL	3:00	100 mL	110 mL
1/13/2010*			-	
Feed	55 mL	8:20	-	-
Dig Control	310 mL	2:20	54 mL	109 mL
Dig Culture Seed	250 mL	3:20	70 mL	120 mL
Dig Center Well	310 mL	3:20	81 mL	121 mL
1/20/10				
Feed	60 mL		-	-
Dig Control	380 mL	2:00	76 mL	141 mL
Dig Culture Seed	290 mL	2:00	87 mL	122 mL
Dig Center Well	310 mL	2:00	79 mL	99 mL
1/27/10				
Feed	55		-	-
Dig Control	305	2:40	100 mL	155 mL
Dig Culture Seed	310	4:00	95 mL	160 mL
Dig Center Well	280	2:20	79 mL	124 mL

Table 9: Phase 1 Summary Table of Sample Chemistry Over Time

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
10/28/09						
Feed		6.5	-	-	3.26	-
Dig Control	2530	7.1	-	-	1.66	-
Dig Culture Seed	2290	7.1	-	-	1.8	-
Dig Center Well	2390	7.1	-	-	1.81	-
11/4/09						
Feed	892	6.5	2710	1430	3.14	361.68
Dig Control	3180	7.1	890	374	1.79	171.81
Dig Culture Seed	3180	7.2	886	327	1.75	157.13
Dig Center Well	3080	7.0	846	343	1.74	107.89
11/12/09						
Feed	710	6.6	-	-	3.37	-
Dig Control	3060	7.4	-	-	1.79	-
Dig Culture Seed	3210	7.2	-	-	1.78	-
Dig Center Well	3090	7.2	-	-	1.74	-
11/18/09						
Feed	649	6.5	2550	1270	3.69	334.83
Dig Control	3180	7.3	829	342	1.82	104.25
Dig Culture Seed	3220	7.3	988	399	1.78	192.1
Dig Center Well	3310	7.2	947	347	1.79	147.46
11/25/09						
Feed	689	6.4	-	-	3.84	-
Dig Control	3160	7.1	-	-	1.87	-
Dig Culture Seed	3300	7.1	-	-	1.86	-
Dig Center Well	3270	7	-	-	1.87	-
12/2/09						
Feed	674	6.4	2600	1320	3.44	339.04
Dig Control	3360	7.2	1380	498	1.68	248.05
Dig Culture Seed	3420	7.2	1160	433	1.73	226.55
Dig Center Well	3310	7.1	1240	409	1.7	243.55
12/11/09						
Feed	694	6.6	-	-	3.54	-
Dig Control	3440	7.1	-	-	1.8	-
Dig Culture Seed	3440	7.2	-	-	1.76	-
Dig Center Well	3470	7.2	-	-	1.75	-

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
12/16/09						
Feed	610	6.6	2460	1200	3.35	182.49
Dig Control	3410	7.3	1010	362	1.85	126.98
Dig Culture Seed	3380	7.3	970	330	1.75	141.64
Dig Center Well	3390	7.3	939	331	1.75	110.53
12/23/09						
Feed	624	6.6	-	-	3.68	-
Dig Control	3260	7.3	-	-	1.81	-
Dig Culture Seed	3460	7.3	-	-	1.74	-
Dig Center Well	3500	7.3	-	-	1.81	-
12/30/09*						
Feed	644	6.5	3800	1710	3.16	133.6
Dig Control	3820	7.3	1070	356	1.77	23.21
Dig Culture Seed	3910	7.5	1100	351	1.76	93.32
Dig Center Well	3730	7.3	1070	355	1.76	123.14
1/6/10						
Feed	514	6.5	-	-	3.26	-
Dig Control	3840	7.3	-	-	1.81	-
Dig Culture Seed	3750	7.3	-	-	1.74	-
Dig Center Well	3810	7.3	-	-	1.75	-
1/13/2010*						
Feed	613	6.6	2680	1190	3.41	111.18
Dig Control	3840	7.5	1100	369	1.78	163.84
Dig Culture Seed	3820	7.4	1030	355	1.68	176.14
Dig Center Well	3780	7.3	1060	339	1.68	108.77
1/20/10						
Feed	850	6.3	-	-	3.9	-
Dig Control	4010	7.3	-	-	1.8	-
Dig Culture Seed	3820	7.4	-	-	1.73	-
Dig Center Well	3950	7.3	-	-	1.79	-
1/27/10						
Feed	929	6.4	2630	1180	3.53	169.08
Dig Control	3870	7.3	1140	381	1.82	168.6
Dig Culture Seed	3970	7.5	973	350	1.73	164.2
Dig Center Well	4000	7.4	1070	315	1.79	98.89

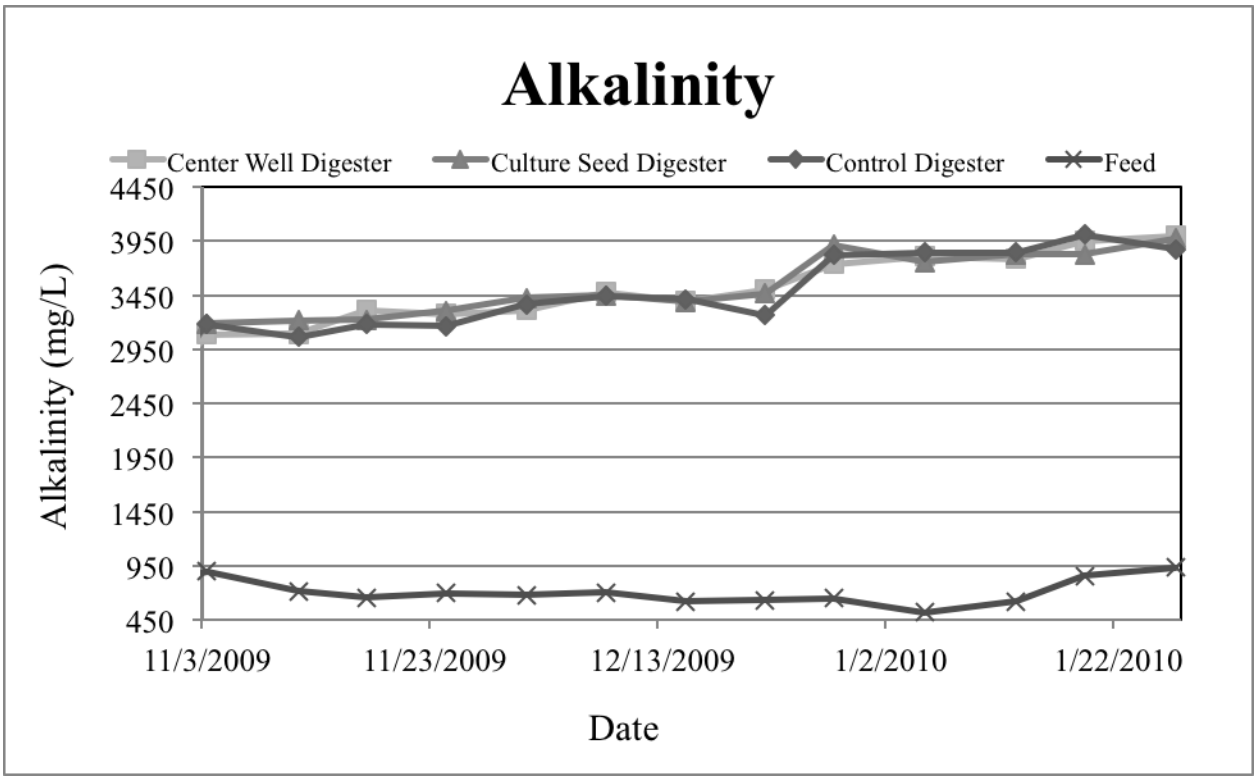


Figure 17: Phase 1 Alkalinity

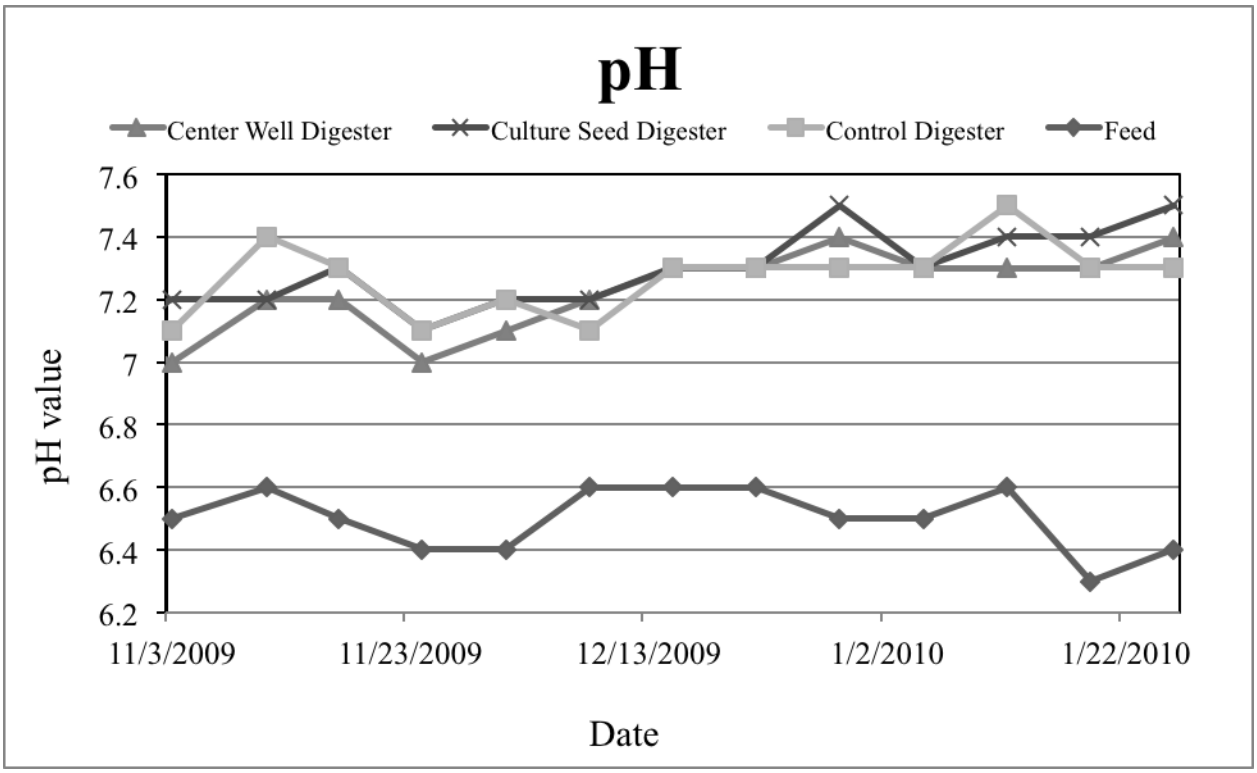


Figure 18: Phase 1 pH

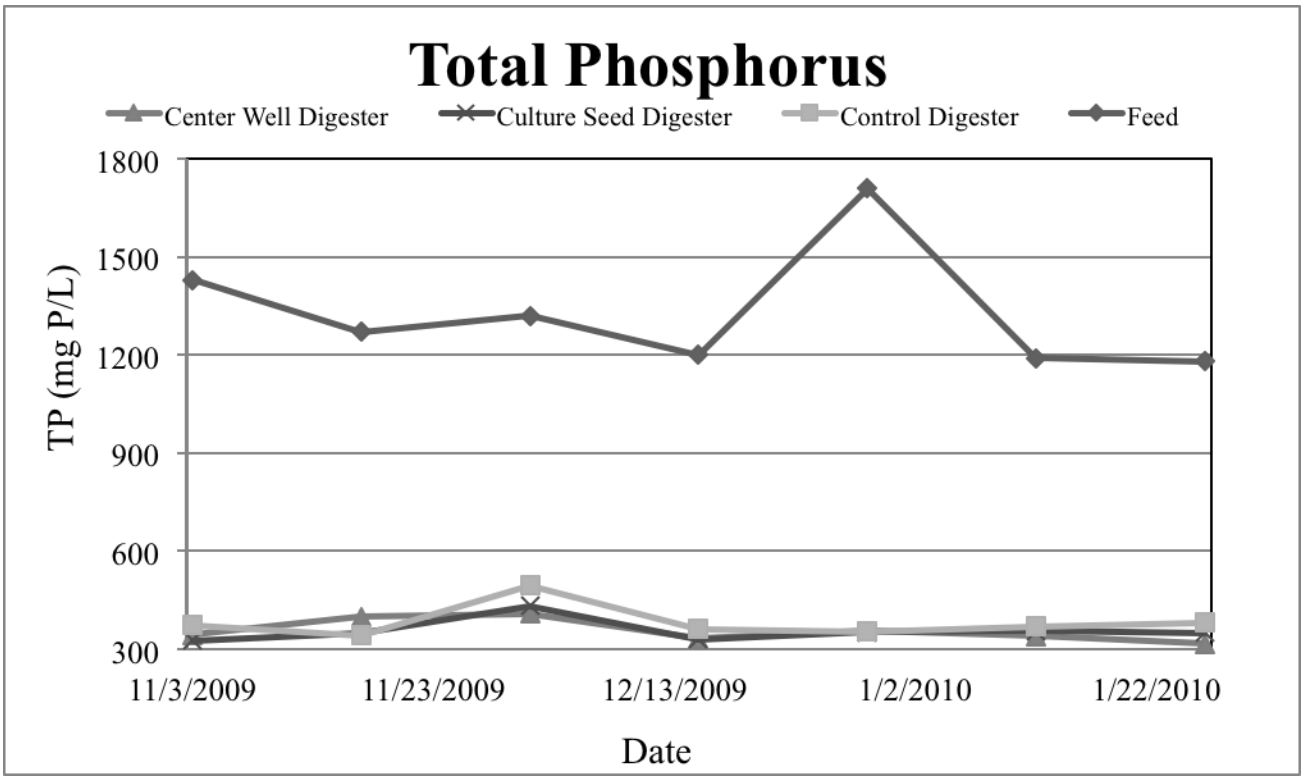


Figure 19: Phase 1 Total Phosphorus

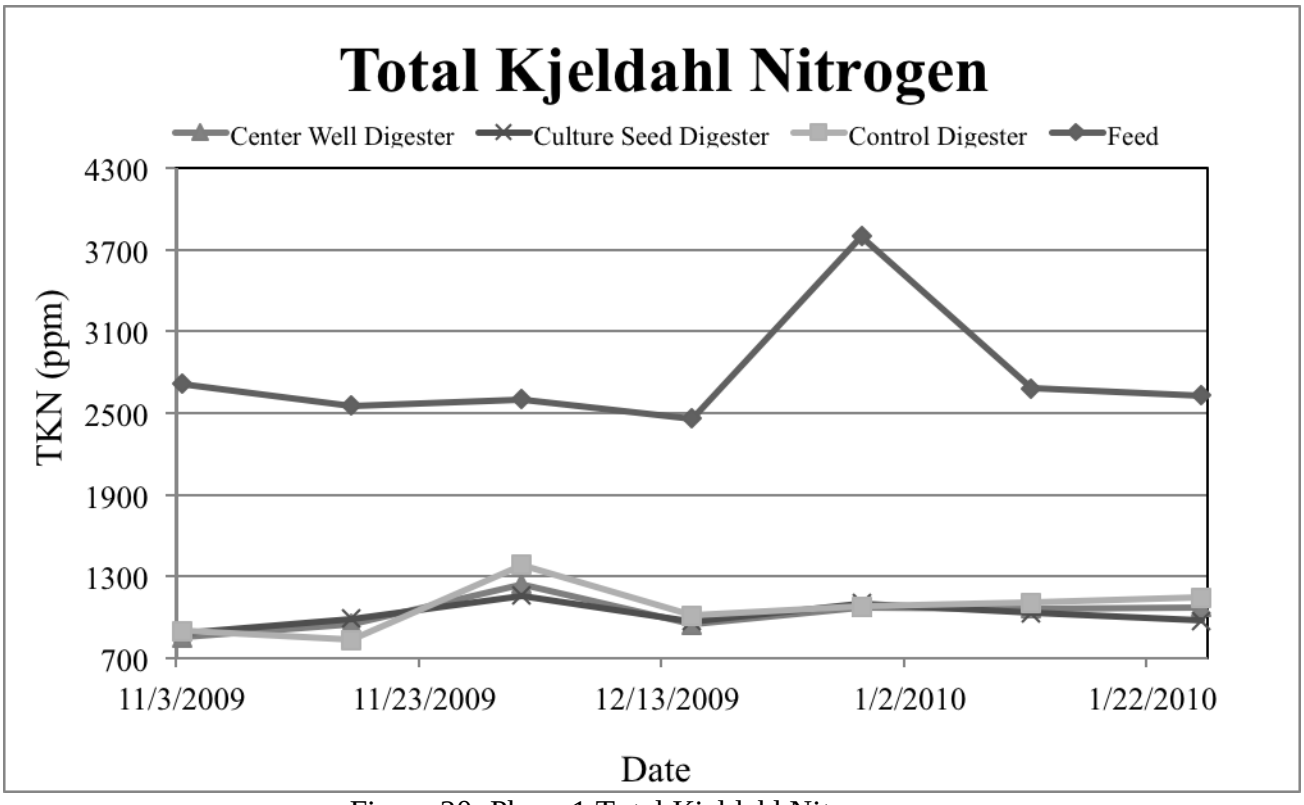


Figure 20: Phase 1 Total Kjeldahl Nitrogen

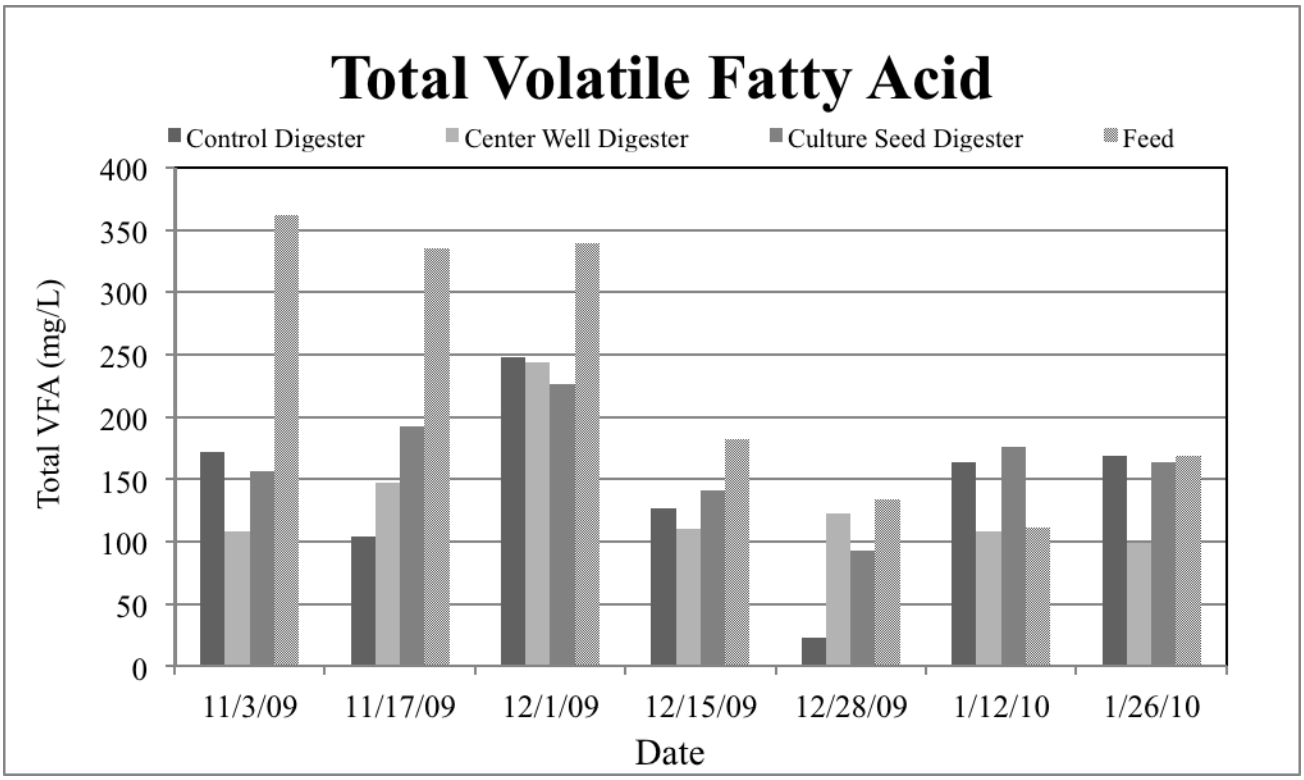


Figure 21: Phase 1 Total Volatile Fatty Acid

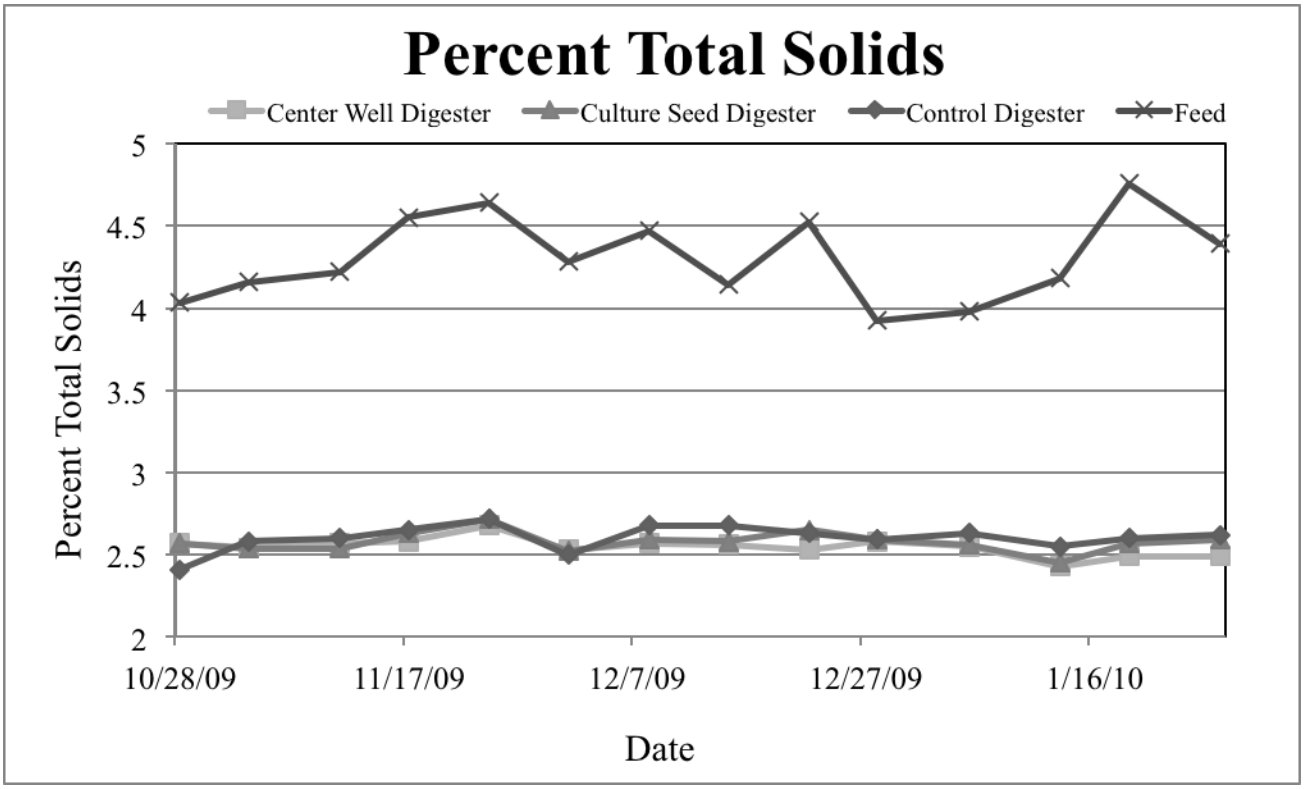


Figure 22: Phase 1 Percent Total Solids

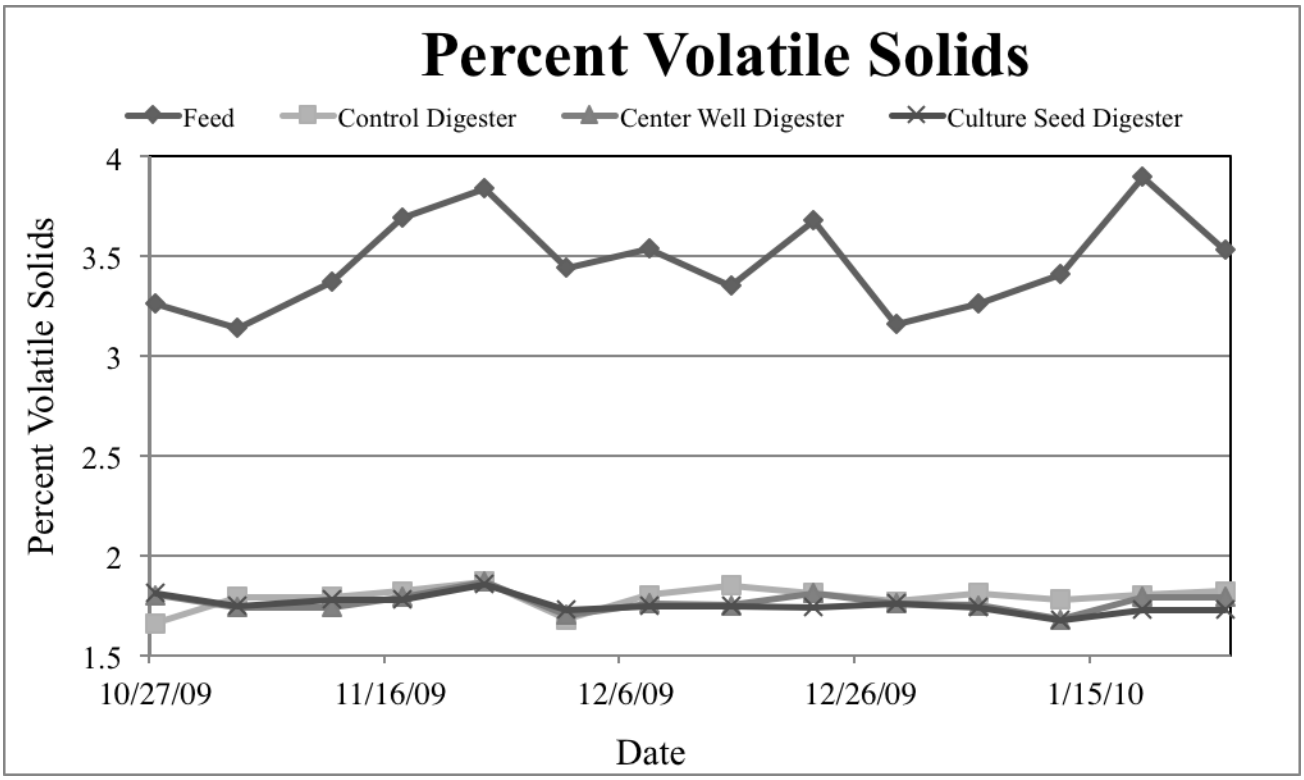


Figure 23: Phase 1 Percent Volatile Solids

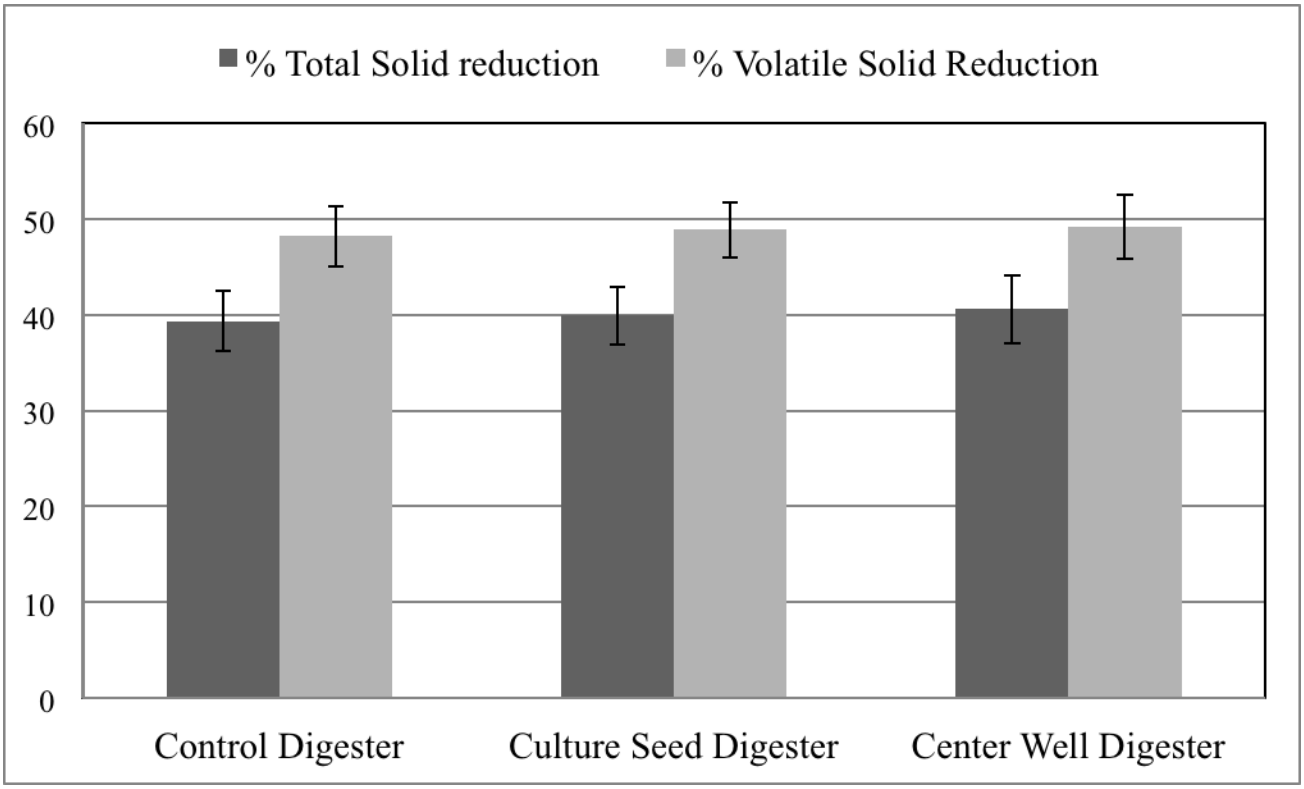


Figure 24: Phase 1 Volatile Solids Reduction and Total Solids Reduction

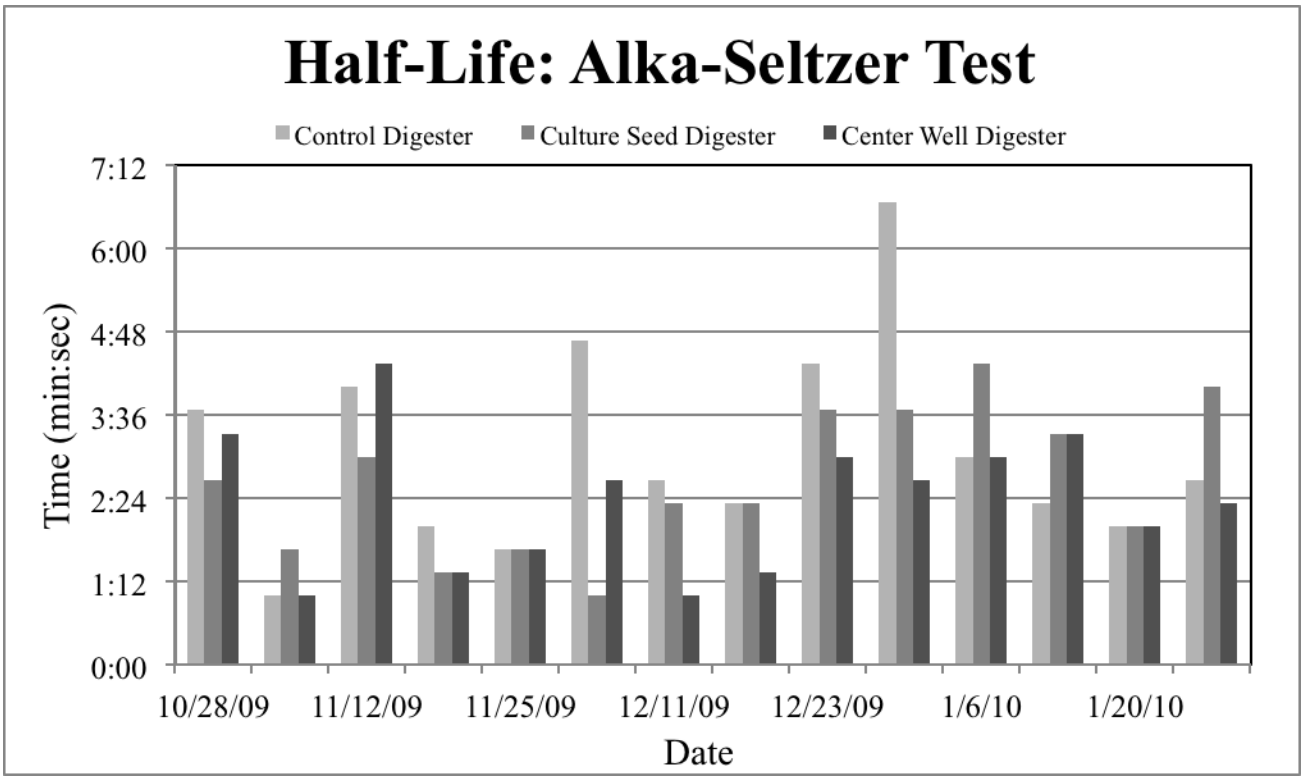


Figure 25: Phase 1 Half-Life: Alka-Seltzer Test

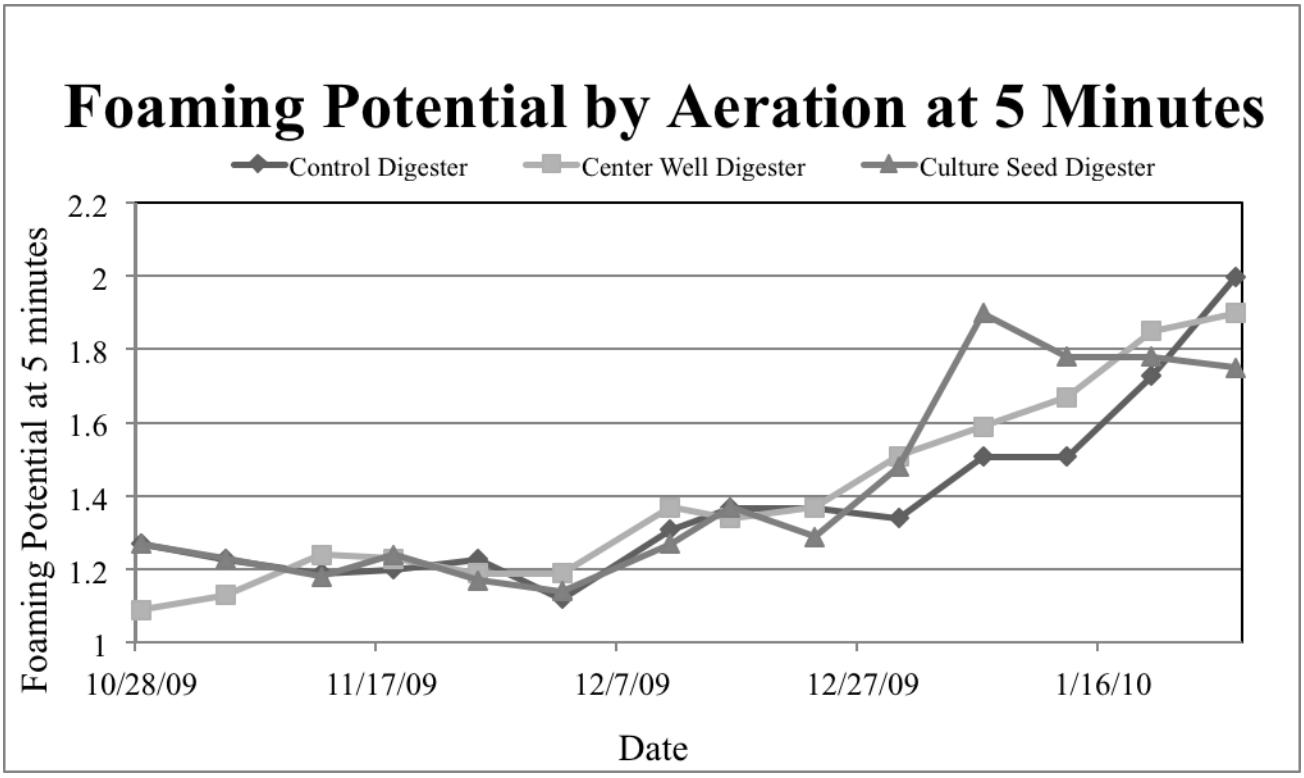


Figure 26: Phase 1 Foaming Potential by Aeration at 5 Minutes

Table 10: Phase 1 Summary of correlation coefficients

		Alkalinity	pH	TKN	TP	% Total Solids	VA-Acetic	VA-Iso-Butyric	VA-Iso-Valeric	VA-N Butyric	VA-N-Valeric	VA-Propionic	VA-Sec Valeric	Total VFA	% Volatile Solids	Alka-Seltzer	Aeration (5 min)	Filament Count	Numerical Rating
Alkalinity (ppm)		1																	
pH	r -square	0.9191	1																
	p-value	<0.01																	
TKN (ppm)	r -square	0.8286	0.7859	1															
	p-value	<0.01	<0.01																
TP (ppm)	r -square	0.9029	0.8600	0.9756	1														
	p-value	<0.01	<0.01	<0.01															
% Total Solids	r -square	0.8844	0.8800	0.8314	0.8955	1													
	p-value	<0.01	<0.01	<0.01	<0.01														
VA-Acetic (mg/L)	r -square	0.2282	0.2736	0.1683	0.2190	0.2280	1												
	p-value	<0.01	<0.01	0.0271	0.0100	<0.01													
VA-Iso-Butyric (mg/L)	r -square	0.2000	0.1563	0.2390	0.2017	0.2028	0.0750	1											
	p-value	0.0150	0.0338	<0.01	0.0145	0.0142	0.1500												
VA-Iso-Valeric (mg/L)	r -square	0.0651	0.0596	0.0245	0.0457	0.0333	0.0007	0.0053	1										
	p-value	0.1820	0.2020	0.4170	0.2660	0.3430	0.8880	0.7060											
VA-N Butyric (mg/L)	r -square	0.0703	0.0799	0.0007	0.0172	0.0396	0.3990	0.1863	0.1395	1									
	p-value	0.1650	0.1370	0.8960	0.4980	0.3000	<0.01	0.0194	0.0460										
VA-N-Valeric (mg/L)	r -square	0.1222	0.1095	0.0316	0.0562	0.0735	0.0409	0.0081	0.6257	0.3011	1								
	p-value	0.0630	0.0670	0.3560	0.2160	0.1550	0.2940	0.6430	0.8260	0.1340									
VA-Propionic (mg/L)	r -square	0.4159	0.4044	0.2230	0.3151	0.3959	0.6096	0.0171	0.0988	0.5940	0.3143	1							
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.4980	0.0970	<0.01	<0.01								
VA-Sec Valeric (mg/L)	r -square	0.0315	0.0186	0.0062	0.0111	0.0089	0.031	0.0193	0.9428	0.0905	0.6016	0.0348	1						
	p-value	0.3570	0.3960	0.6520	0.5250	0.6250	0.3610	0.4720	<0.01	0.1130	<0.01	0.3330							
Total VFA (mg/L)	r -square	0.3117	0.3402	0.1790	0.2544	0.2918	0.8864	0.0692	0.0451	0.6200	0.2017	0.8757	0.0046	1					
	p-value	<0.01	<0.01	0.0222	<0.01	<0.01	<0.01	0.1680	0.2690	<0.01	0.0145	<0.01	0.7270						
% Volatile Solids	r -square	0.8940	0.8832	0.8390	0.8945	0.9960	0.2108	0.2266	0.0265	0.0309	0.0742	0.3823	0.0070	0.2726	1				
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0142	0.3430	0.3000	0.1550	<0.01	0.6250	<0.01					
Foaming Potential Alka-Seltzer		0.7235	0.7244	0.8750	0.8627	0.8160	0.1491	0.3206	0.0023	0.0217	0.0106	0.0106	0.0013	0.1582	0.8221	1			
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.0424	<0.01	0.8067	0.4543	0.6027	<0.01	0.8529	0.0361	<0.01				
Foaming Potential 5 min	r -square	0.5583	0.3771	0.0073	0.1351	0.0473	0.0255	0.0054	0.0764	0.2894	0.0764	0.0206	0.0793	0.0559	0.0139	0.0113	1		
	p-value	<0.01	<0.01	0.6067	0.1479	0.1344	0.8233	0.6964	0.0980	0.0113	0.0980	0.7077	0.0939	0.5247	0.3179	0.5030			
Filament Count	r -square	0.7641	0.7217	0.6836	0.7014	0.8380	0.1128	0.3115	0.0161	0.0004	0.0016	0.2819	0.0230	0.1427	0.8470	0.6805	0.0314		
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.0806	<0.01	0.5198	0.9167	0.8421	<0.01	0.4410	0.0475	<0.01	<0.01	0.4424	1	
Numerical Rating	r -square	0.1210	0.1511	0.2990	0.0025	0.0962	0.1390	0.1326	0.0680	0.2573	0.0077	0.2088	0.0173	0.2200	0.0856	0.0657	0.1627	0.0606	
	p-value	0.0086	<0.01	0.5469	0.2523	0.0200	0.0507	0.0568	0.1803	<0.01	0.6568	0.0145	0.5046	<0.01	0.0287	0.0564	0.0698	0.0674	1

Table 11: Phase 1 Summary of ANOVA results
Geometric Mean (Average Deviation)

Parameter	No Foam	Weak Foam	Fast Collapse Foam	Weak Foam but Stable	p-value
Alkalinity (ppm)	672 (144)	2743 (1023)	3472 (317)	3196 (745)	0.161
pH	6.49 (0.1)	7.14 (0.28)	7.27 (0.11)	7.20 (0.16)	<0.01
TKN (ppm)	2783 (491)	1134 (480)	1055 (175)	** 973 -	<0.01
TP (ppm)	1326 (206)	425 (286)	371 (59)	** 315 -	<0.01
%TS	4.14 (0.18)	2.96 (0.67)	2.45 (0.31)	2.91 (0.71)	<0.01
%VS	4.24 (0.22)	2.77 (0.64)	2.58 (0.065)	2.57 (0.075)	<0.01
Alka-Seltzer (mL)	64 (18)	258 (90)	288 (39)	345 (59)	<0.01
Aeration (mL)	-	53 (44)	37 (36)	53 (57)	0.374
Total VFA (mg/L)	-	210 (116)	219 (88)	** 169 -	0.823
Filament Count (#/uL)	19.11 (8.98)	1.16 (9.61)	1.04 (0.50)	1.11 (1.02)	<0.01
Numerical Rating	3.41 (0.80)	3.77 (0.59)	3.87 (0.38)	4.24 (0.35)	0.048

FP – foaming potential
** Only one value was in column
Blanks indicate measurement was not conducted

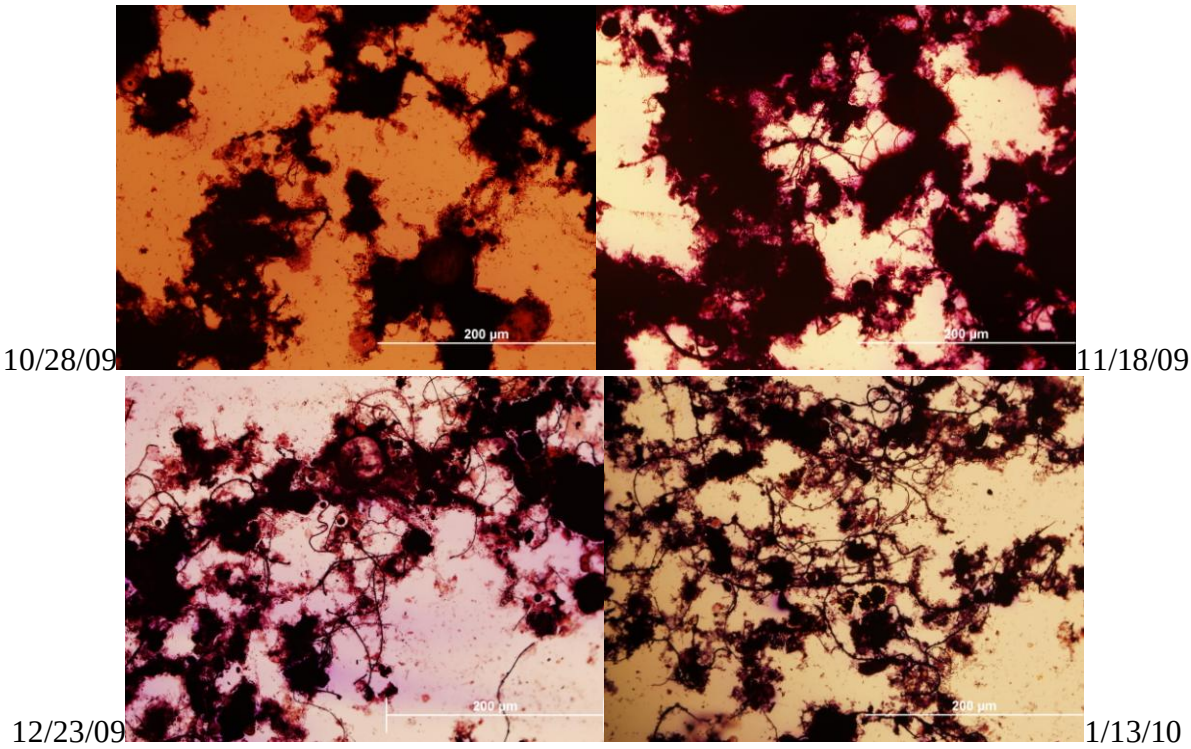


Figure 27: Phase 1 Gram Stain Analysis of Feed (50/50 WAS/Primary Sludge)

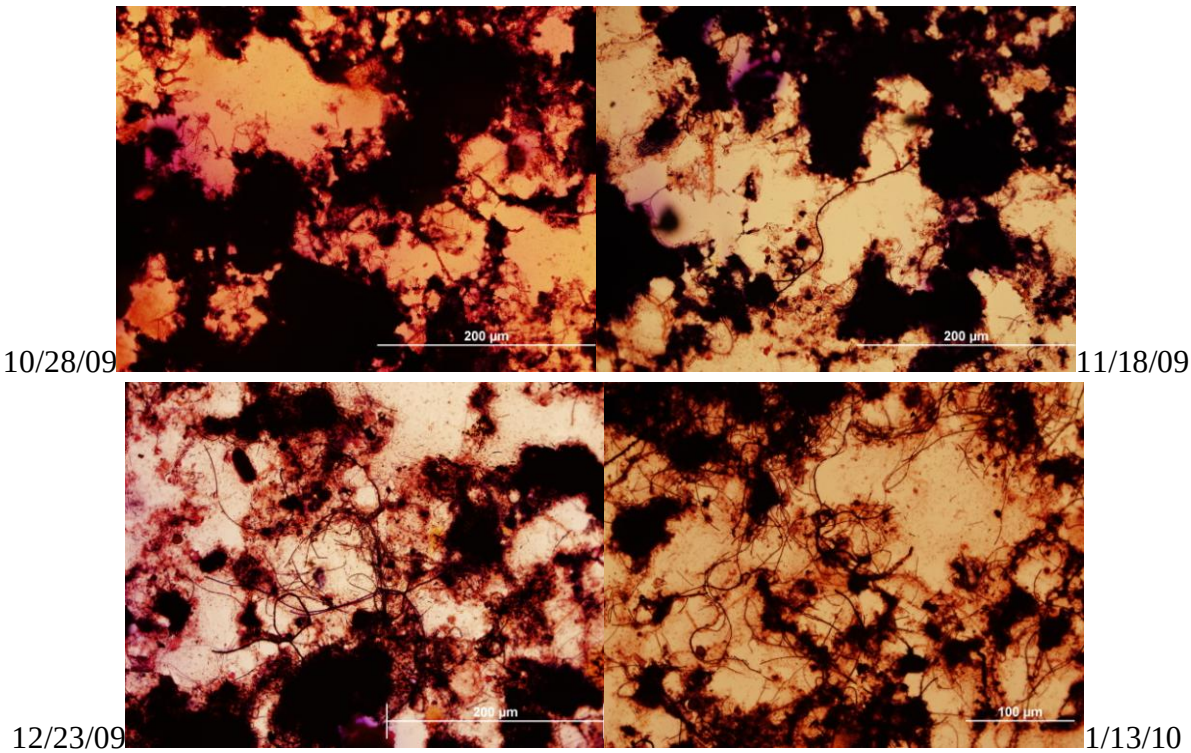
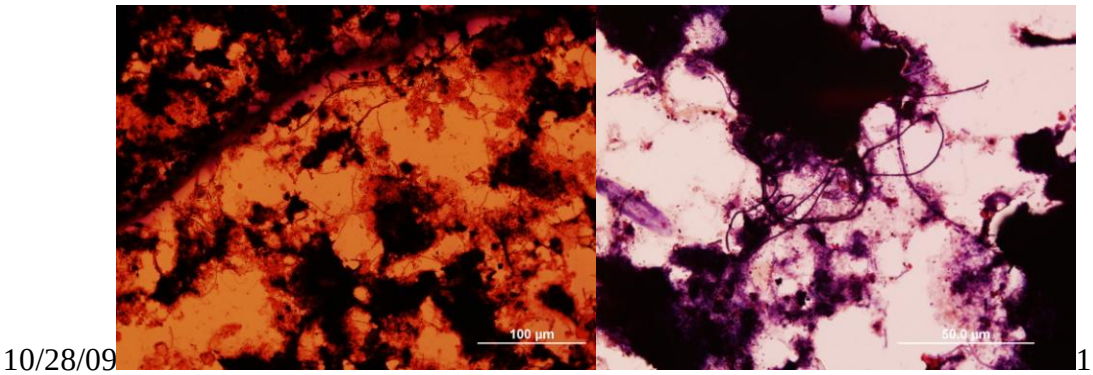
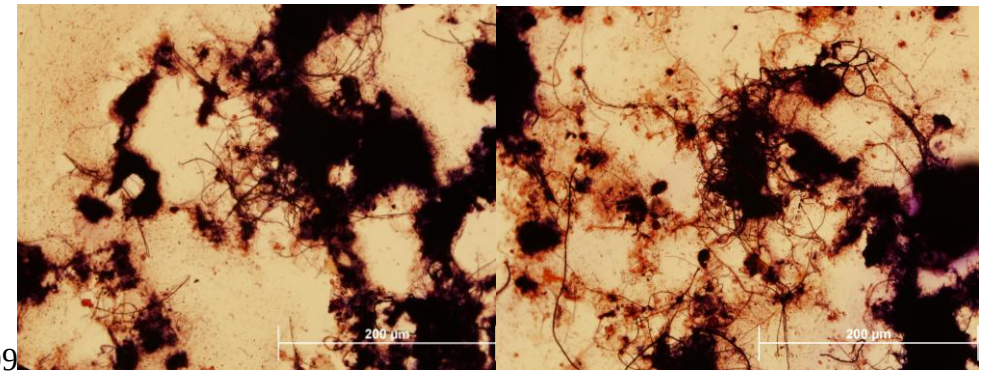


Figure 28: Phase 1 Gram Stain Photos of Control Digester (mesophilic)

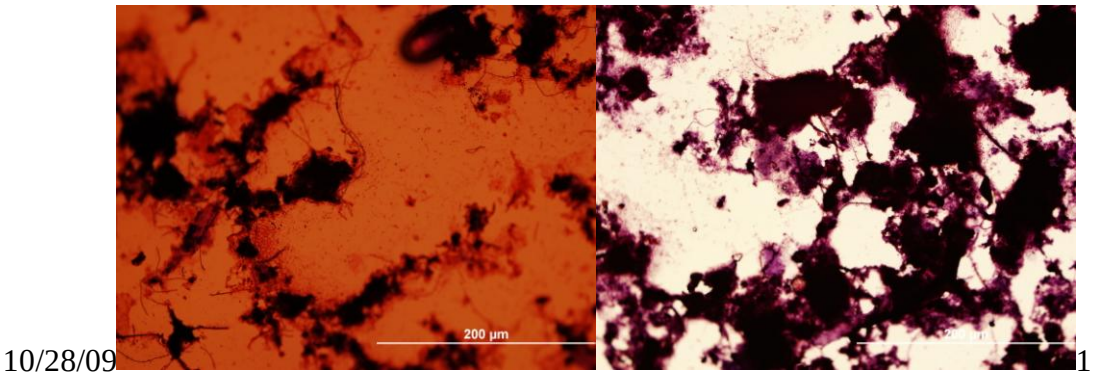


10/28/09 11/18/09

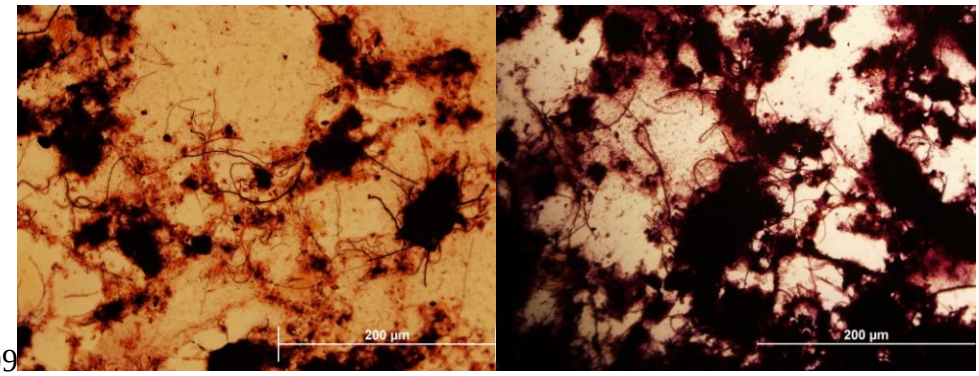


12/23/09 1/13/10

Figure 29: Phase 1 Gram Stain Photos of Culture Seed Digester



10/28/09 11/18/09



12/23/09 1/13/10

Figure 30: Phase 1 Gram Stain Photos of Center Well Digester

APPENDIX B: Phase 2

Table 12: Phase 2 Summary Table of Foaming Results Over Time

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
3/3/10				
Feed	50	3:00	-	-
Acid Feed	115	3:00	-	-
Dig Control	240	1:40	48	68
Dig Thermo	240	3:40	51	71
Dig Acid phase-Thermo	380	0:40	66	91
3/11/10				
Feed	55	2:20	-	-
Acid Feed	120	2:20	-	-
Dig Control	230	2:00	48	73
Dig Thermo	350	1:00	52	67
Dig Acid phase-Thermo	380	0:20	50	75
3/16/10				
Feed	60	2:40	-	-
Acid Feed	130	2:20	-	-
Dig Control	280	2:00	65	85
Dig Thermo	220	1:00	65	70
Dig Acid phase-Thermo	450	0:40	40	55
3/24/10				
Feed	65	5:00	-	-
Acid Feed	130	4:00	-	-
Dig Control	250	3:00	39	59
Dig Thermo	230	2:40	70	90
Dig Acid phase-Thermo	170	2:00	59	94
3/31/10				
Feed	65	2:40	-	-
Acid Feed	210	1:40	-	-
Dig Control	290	2:40	67	102
Dig Thermo	270	2:20	85	100
Dig Acid phase-Thermo	300	1:40	60	90

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
4/8/10				
Feed	50	4:20	-	-
Acid Feed	200	1:40	-	-
Dig Control	300	2:20	45	75
Dig Thermo	290	1:00	50	85
Dig Acid phase-Thermo	340	1:00	47	82
4/14/10				
Feed	55	0:20	-	-
Acid Feed	180	2:40	-	-
Dig Control	270	2:40	51	86
Dig Thermo	230	2:20	44	79
Dig Acid phase-Thermo	450	1:00	60	90
4/22/10				
Feed	75	3:00	-	-
Acid Feed	230	4:00	-	-
Dig Control	360	1:20	50	60
Dig Thermo	270	3:00	63	78
Dig Acid phase-Thermo	420	0:40	55	85
4/28/10				
Feed	40	6:20	-	-
Acid Feed	190	10:00	-	-
Dig Control	270	1:40	31	41
Dig Thermo	300	1:00	55	65
Dig Acid phase-Thermo	300	2:00	55	60
5/4/10				
Feed	50	1:40	-	-
Acid Feed	230	6:20	-	-
Dig Control	280	1:40	30	45
Dig Thermo	330	1:40	55	70
Dig Acid phase-Thermo	350	1:20	45	65
5/11/10				
Feed	45	6:00	-	-
Acid Feed	220	1:40	-	-
Dig Control	310	1:20	30	45
Dig Thermo	200	4:00	55	70
Dig Acid phase-Thermo	350	0:40	45	65

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
5/18/10				
Feed	55	5:20	-	-
Acid Feed	220	7:20	-	-
Dig Control	210	2:20	30	50
Dig Thermo	300	2:20	50	70
Dig Acid phase-Thermo	300	2:00	35	65
5/25/10				
Feed	50	2:00	-	-
Acid Feed	280	4:00	-	-
Dig Control	250	2:20	40	50
Dig Thermo	220	2:40	51	66
Dig Acid phase-Thermo	490	0:40	40	65

Table 13: Phase 2 Summary Table of Sample Chemistry Over Time

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
3/3/10						
Feed	694	6.7	2250	1020	3.5	226.88
Acid Feed	3050	5.7	755	664	2.9	5123
Dig Control	3900	7.4	878	259	1.8	111.16
Dig Thermo	5020	7.7	1410	315	1.43	518.57
Dig Acid phase-Thermo	4770	7.3	1030	266	1.49	770.3
3/11/10						
Feed	594	6.3	-	-	3.58	-
Acid Feed	3440	5.6	-	-	2.74	-
Dig Control	3740	7.4	-	-	1.87	-
Dig Thermo	4970	7.7	-	-	1.3	-
Dig Acid phase-Thermo	4430	7.6	-	-	1.31	-

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
3/16/10						
Feed	320	6.3	2710	1140	3.53	169.86
Acid Feed	3220	5.5	865	621	2.89	6556
Dig Control	3770	7.3	1110	281	1.7	275.09
Dig Thermo	4510	7.6	1390	291	1.42	435.91
Dig Acid phase-Thermo	4330	7.6	1470	346	1.14	657.86
3/24/10						
Feed	780	6.6	-	-	3.39	-
Acid Feed	2840	5.4	-	-	2.76	-
Dig Control	3770	7.3	-	-	1.87	-
Dig Thermo	5360	7.6	-	-	1.63	-
Dig Acid phase-Thermo	5340	7.8	-	-	1.76	-
3/31/10						
Feed	1050	6.2	2560	978	3.19	862.3
Acid Feed	3040	5.5	163	338	2.68	5954
Dig Control	3930	7.2	1070	276	1.83	266.54
Dig Thermo	5230	7.7	1360	273	1.73	388.8
Dig Acid phase-Thermo	5220	7.6	1470	290	1.65	349.28
4/8/10						
Feed	826	6.2	-	-	3.42	-
Acid Feed	3112	5.6	-	-	2.55	-
Dig Control	4210	7.3	-	-	1.59	-
Dig Thermo	4910	7.5	-	-	1.54	-
Dig Acid phase-Thermo	5330	7.7	-	-	1.72	-
4/14/10						
Feed	641	6.6	2409	891	3.14	205.1
Acid Feed	2670	5.5	841	592	2.62	5225
Dig Control	4040	7.4	1140	292	1.72	176.78
Dig Thermo	4840	7.6	1370	282	1.53	273.53
Dig Acid phase-Thermo	5340	7.7	1570	307	1.3	318.41
4/22/10						
Feed	660	6.3	-	-	3.51	-
Acid Feed	2860	5.6	-	-	2.59	-
Dig Control	3930	7.3	-	-	1.64	-
Dig Thermo	5120	7.7	-	-	1.37	-
Dig Acid phase-Thermo	5360	7.8	-	-	1.5	-

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
4/28/10						
Feed	710	6.6	2340	1040	3.6	210.7
Acid Feed	2970	5.5	769	609	2.9	5651
Dig Control	3800	7.2	1160	277	1.7	35
Dig Thermo	4940	7.7	1340	273	1.8	313.43
Dig Acid phase-Thermo	4870	7.7	1380	283	1.5	226.82
5/4/10						
Feed	720	6.5	-	-	3.37	-
Acid Feed	3010	5.4	-	-	2.93	-
Dig Control	3950	7.2	-	-	1.78	-
Dig Thermo	4840	7.6	-	-	1.82	-
Dig Acid phase-Thermo	5040	7.6	-	-	1.6	-
5/11/10						
Feed	967	6.3	2820	1270	3.3	392.88
Acid Feed	2900	5.3	737	708	2.51	7225
Dig Control	4060	7.1	1070	295	1.67	206.67
Dig Thermo	4750	7.5	1290	312	1.72	465.8
Dig Acid phase-Thermo	5160	7.6	1270	286	1.58	338.86
5/18/10						
Feed	783	6.3	-	-	3.33	-
Acid Feed	3210	5.6	-	-	2.5	-
Dig Control	4060	7.5	-	-	1.68	-
Dig Thermo	4780	7.6	-	-	1.67	-
Dig Acid phase-Thermo	5330	7.9	-	-	1.57	-
5/25/10						
Feed	912	6.3	2310	949	3.18	158.13
Acid Feed	2710	5.4	709	663	2.73	5440
Dig Control	4040	7.4	1070	330	1.81	60.69
Dig Thermo	4660	7.6	1100	283	1.78	48.86
Dig Acid phase-Thermo	5020	7.8	1410	353	1.7	108.33

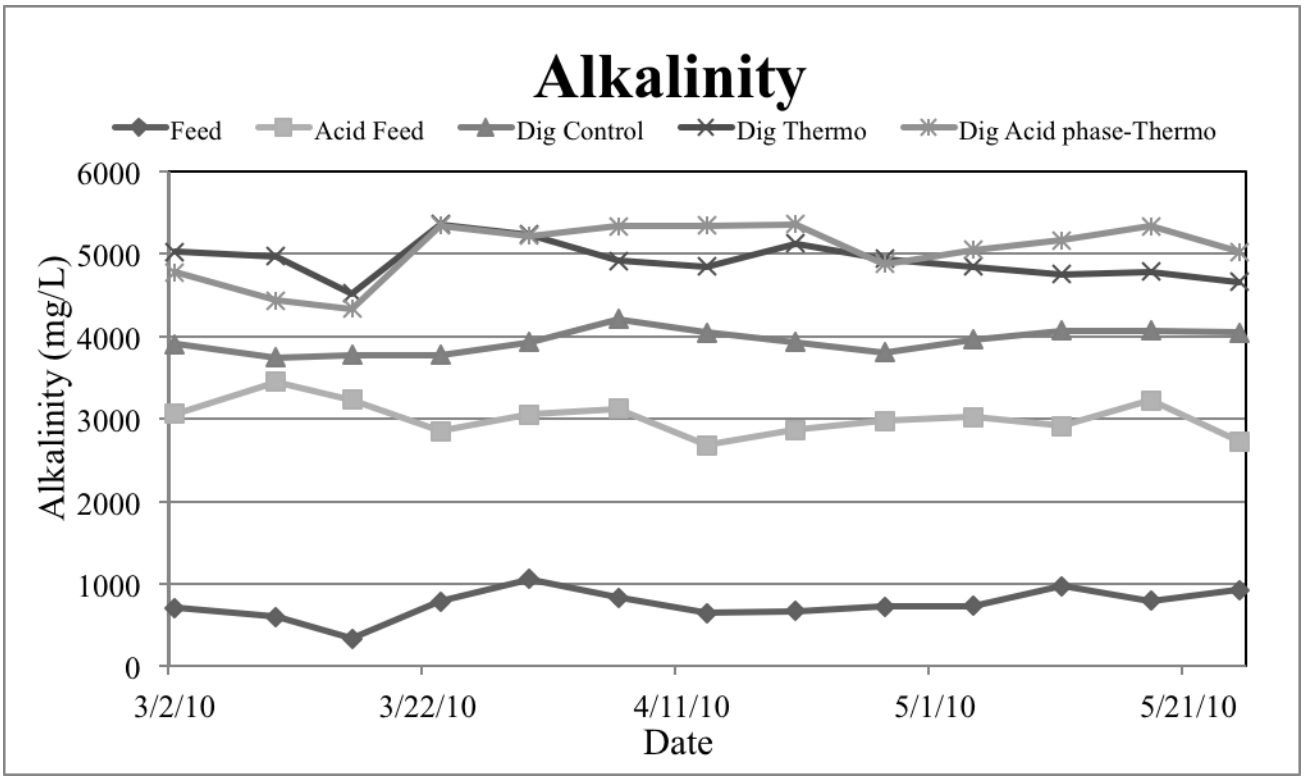


Figure 31: Phase 2 Alkalinity

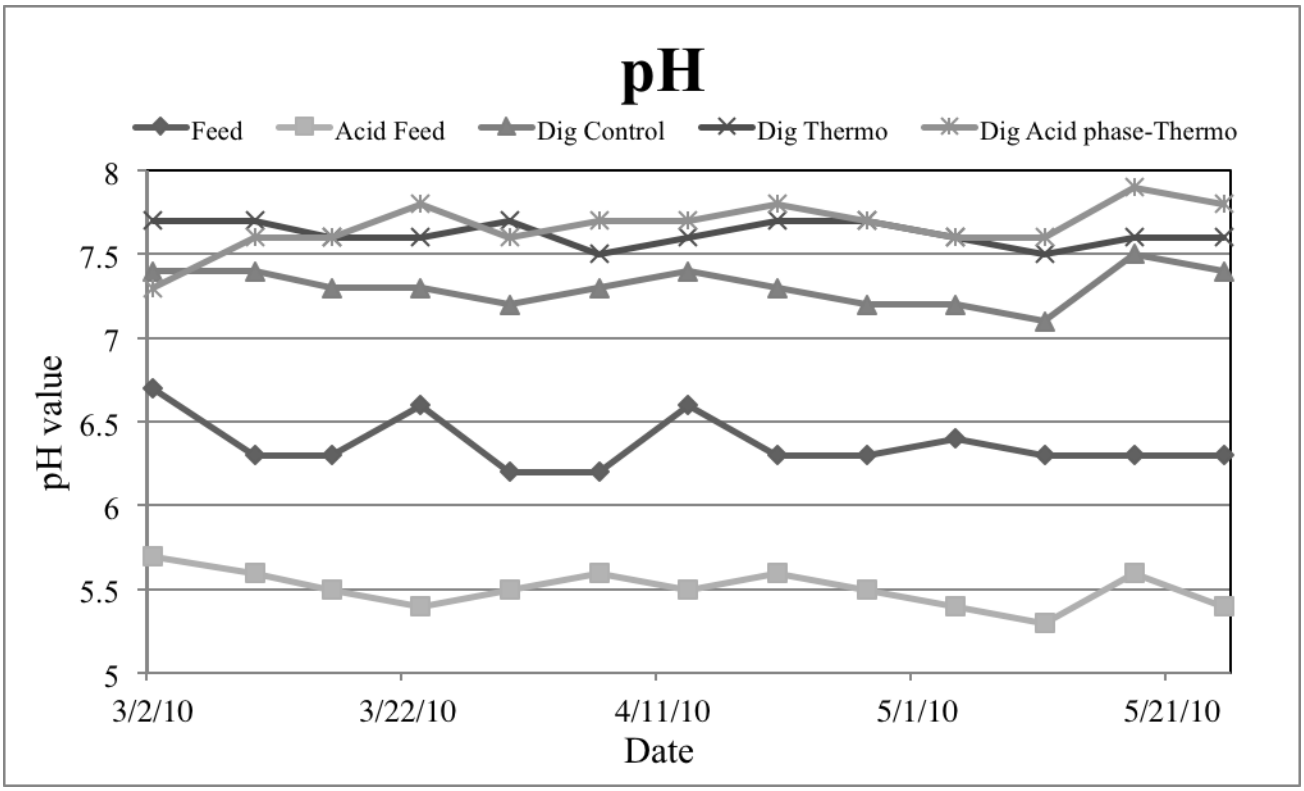


Figure 32: Phase 2 pH

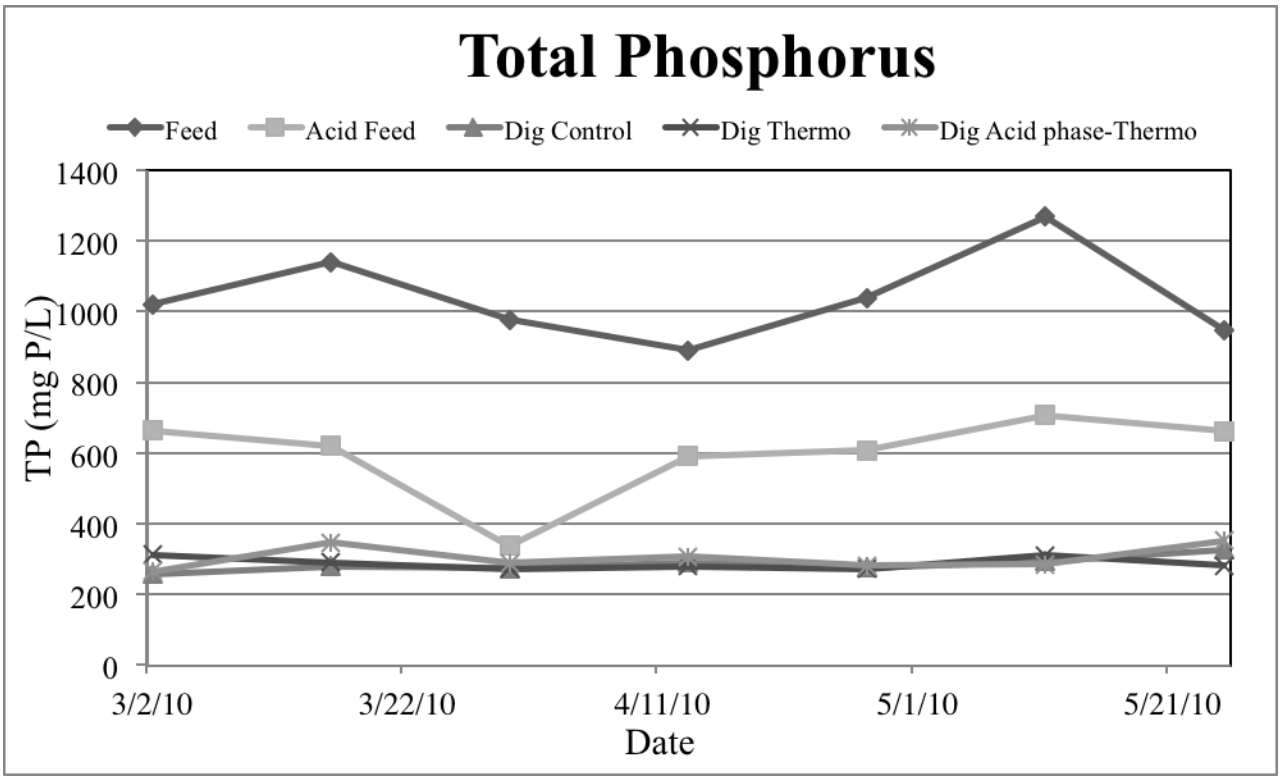


Figure 33: Phase 2 Total Phosphorus

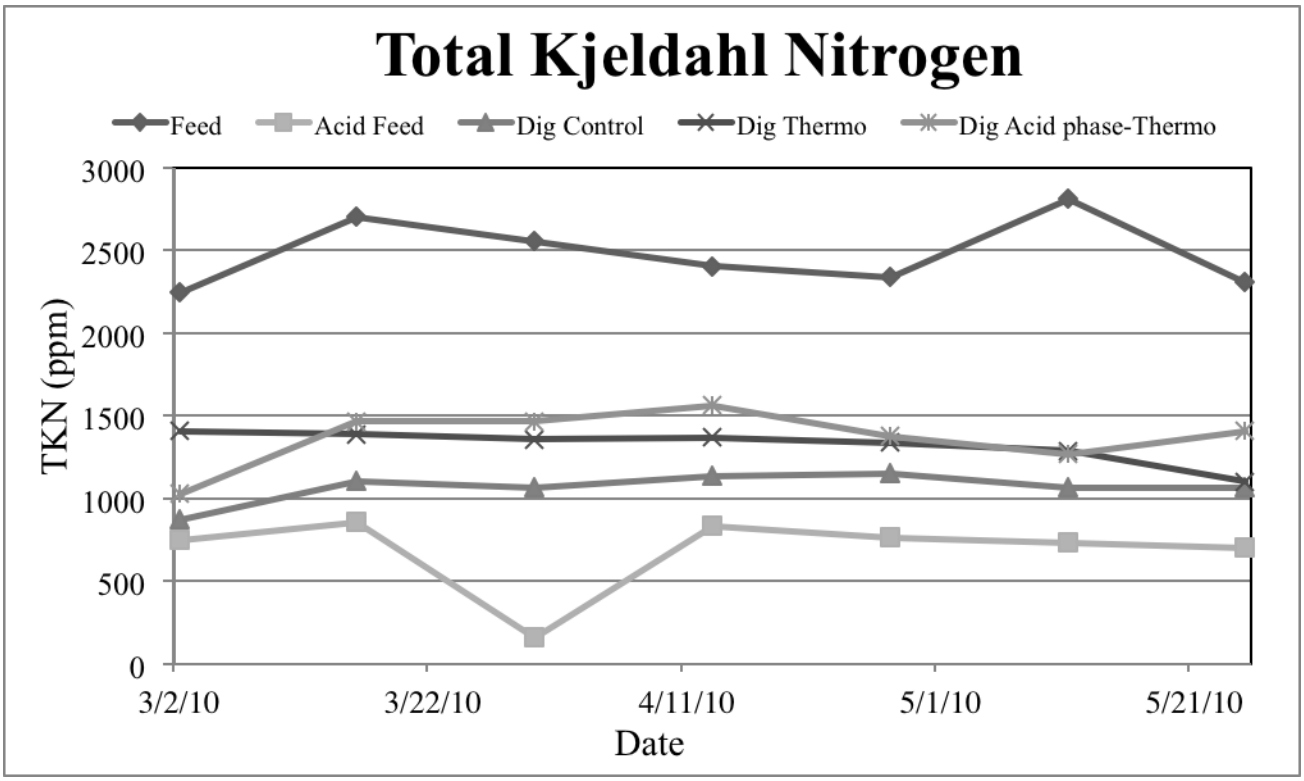


Figure 34: Phase 2 Total Kjeldahl Nitrogen

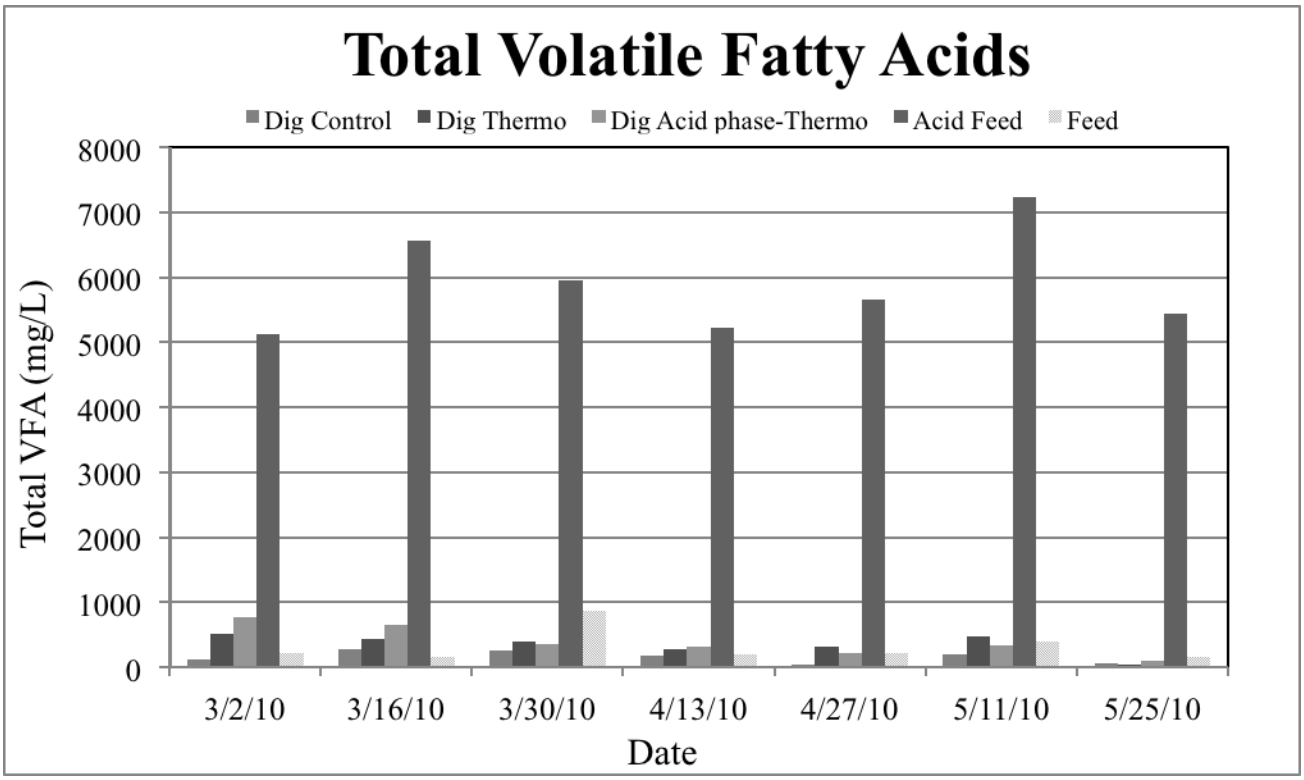


Figure 35: Phase 2 Total Volatile Fatty Acids

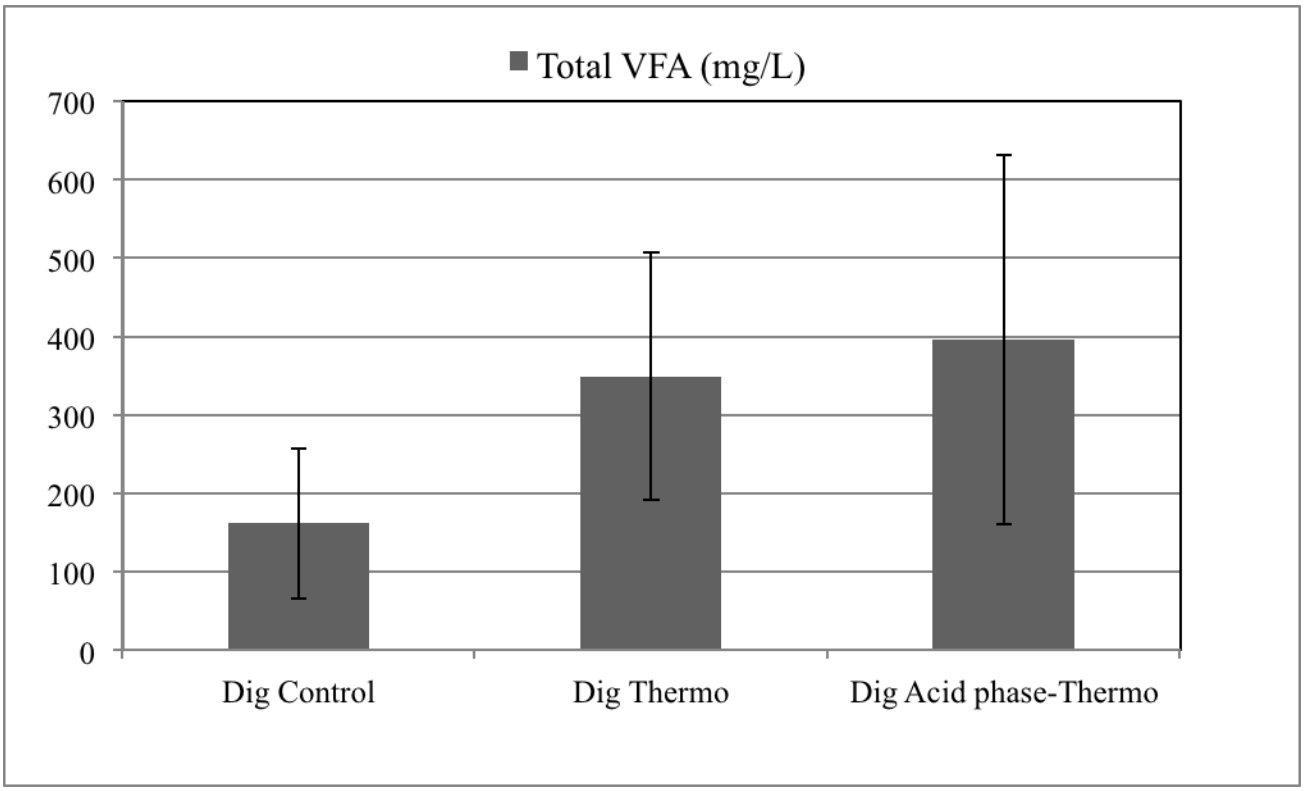


Figure 36: Phase 2 Total Volatile Fatty Acids

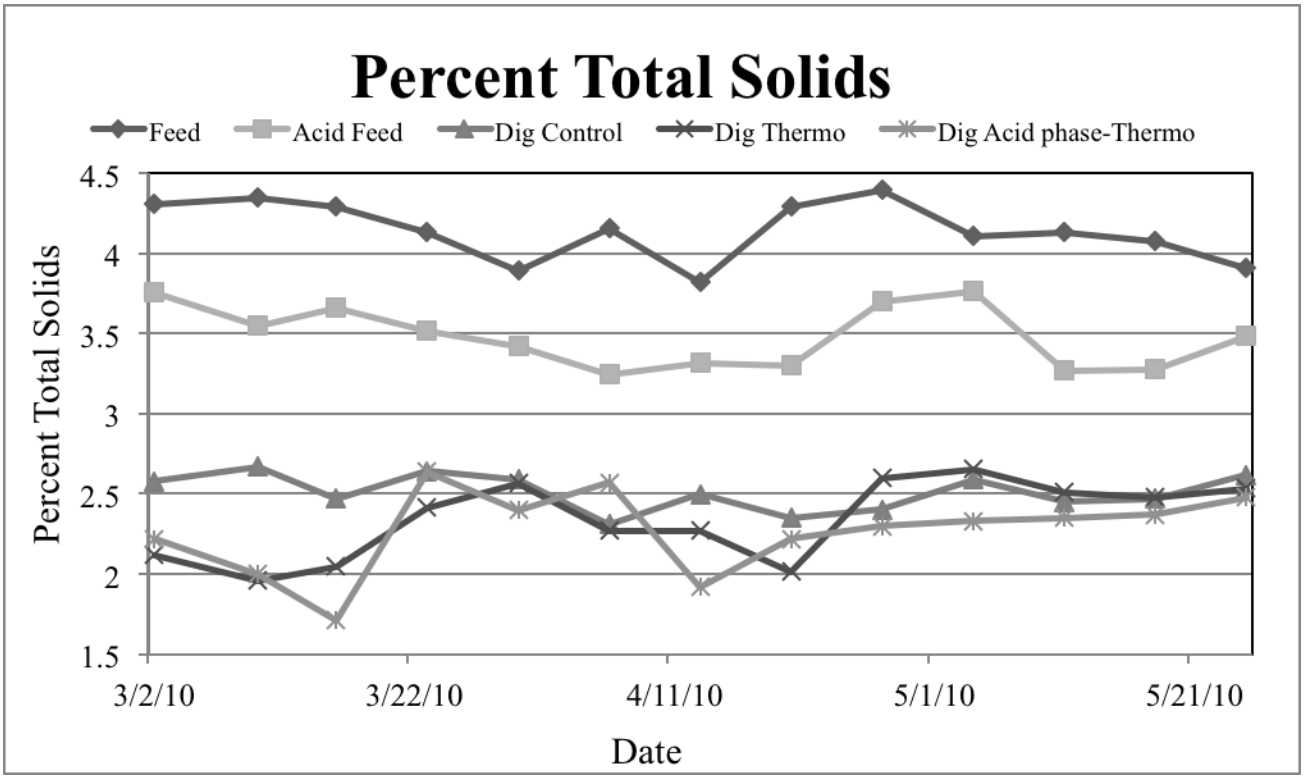


Figure 37: Phase 2 Percent Total Solids

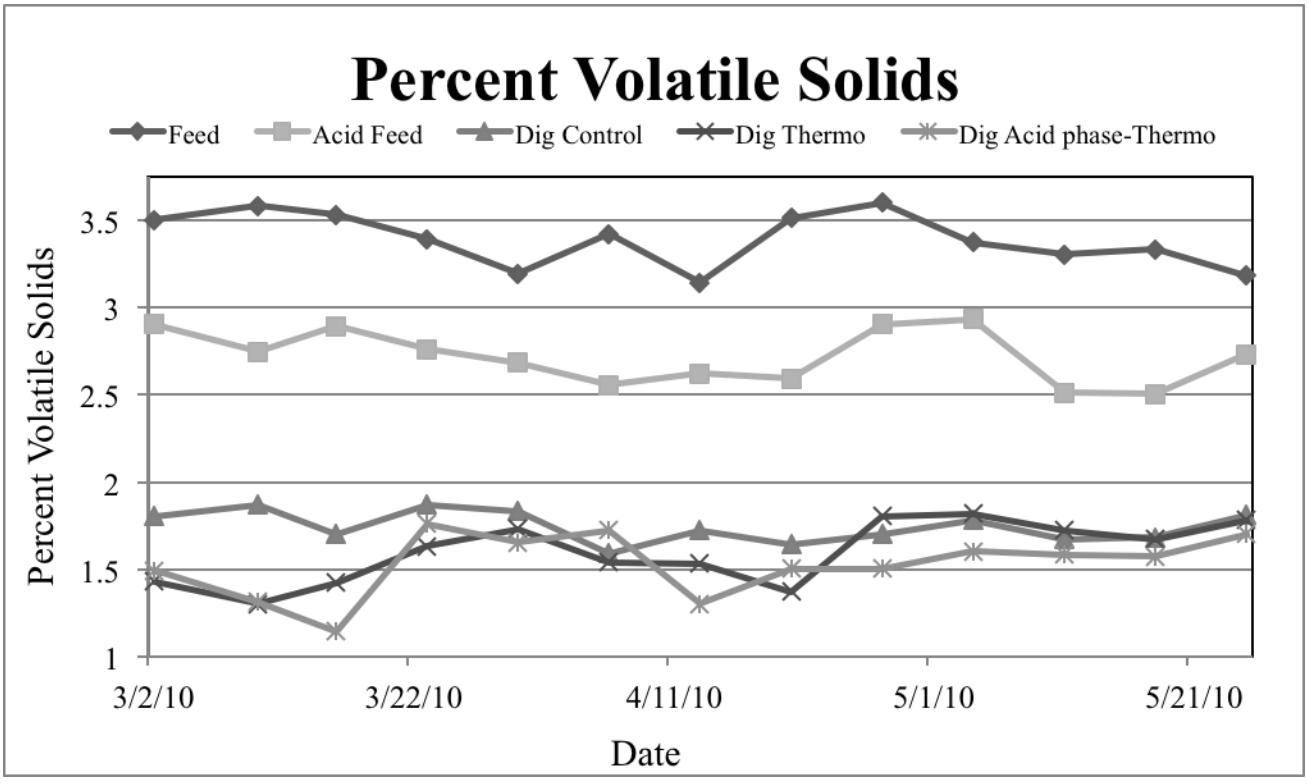


Figure 38: Phase 2 Percent Volatile Solids

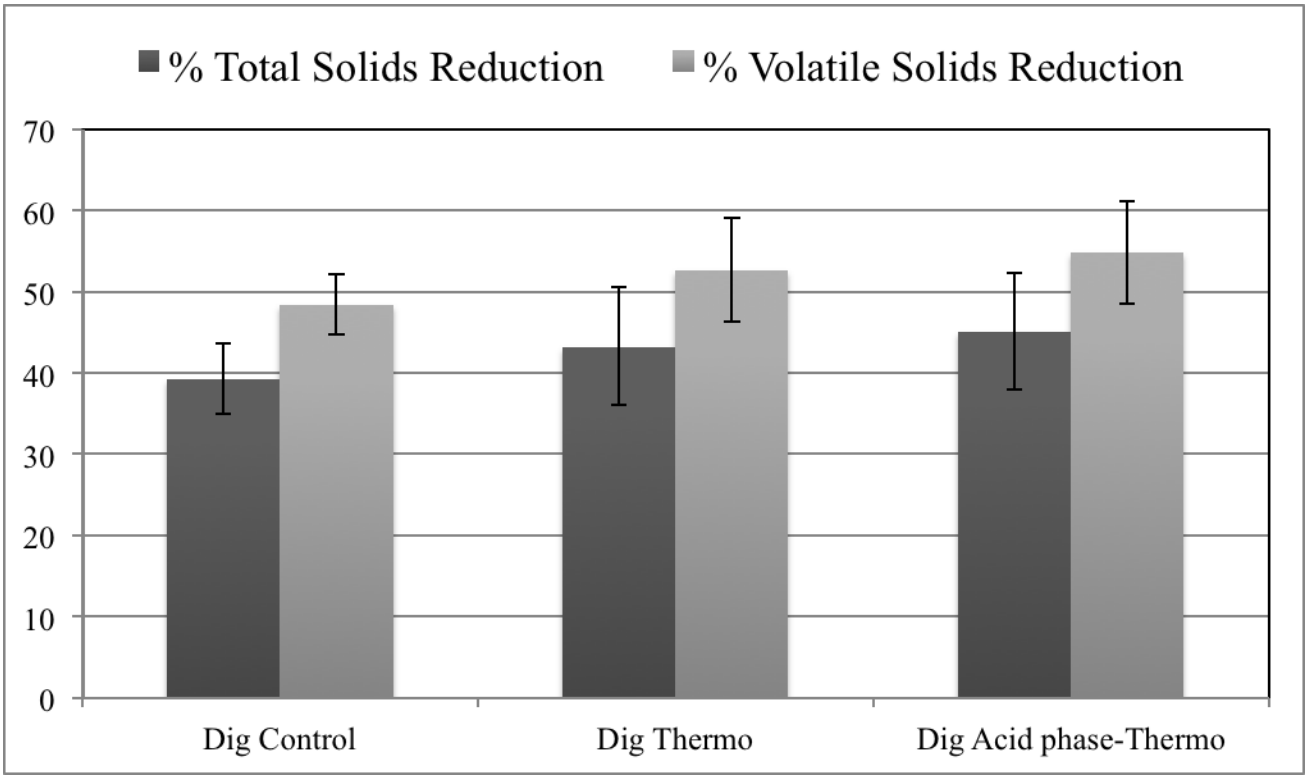


Figure 39: Phase 2 Volatile Solids Reduction and Total Solids Reduction

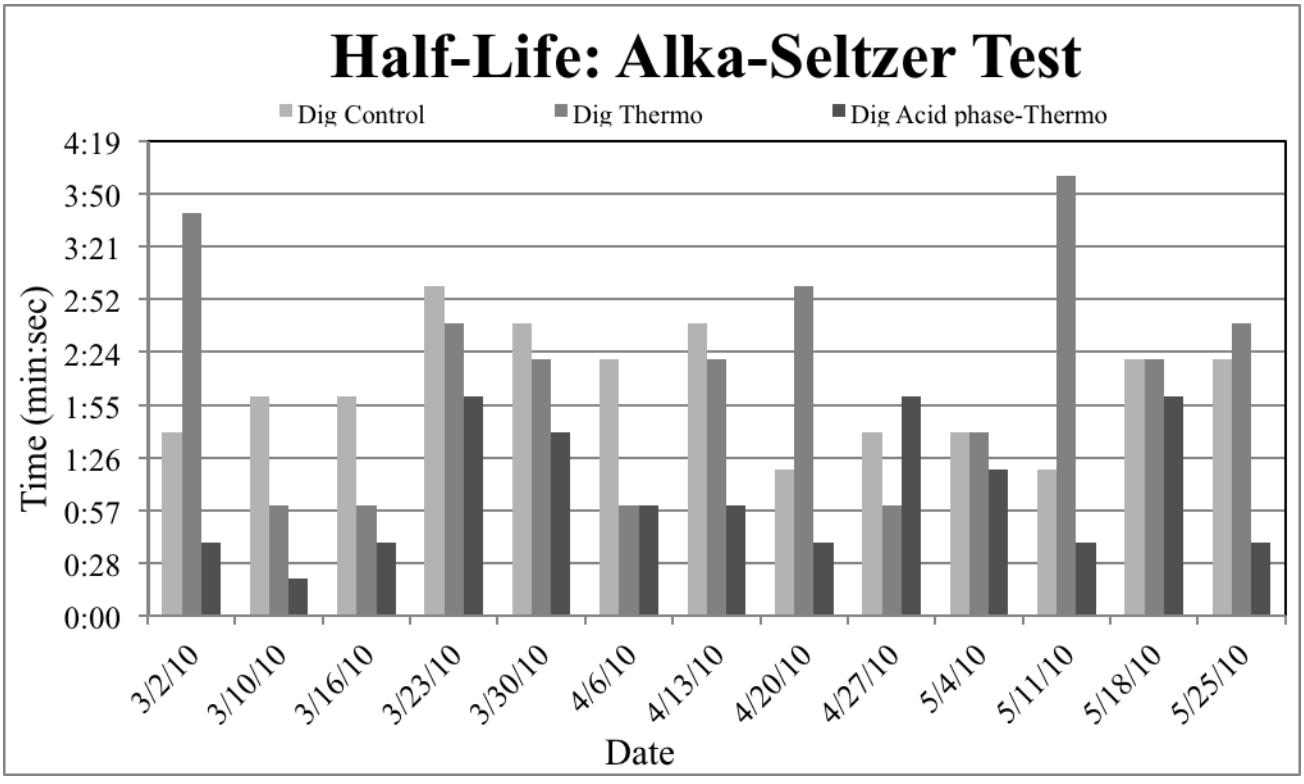


Figure 40: Phase 2 Half-Life: Alka-Seltzer Test

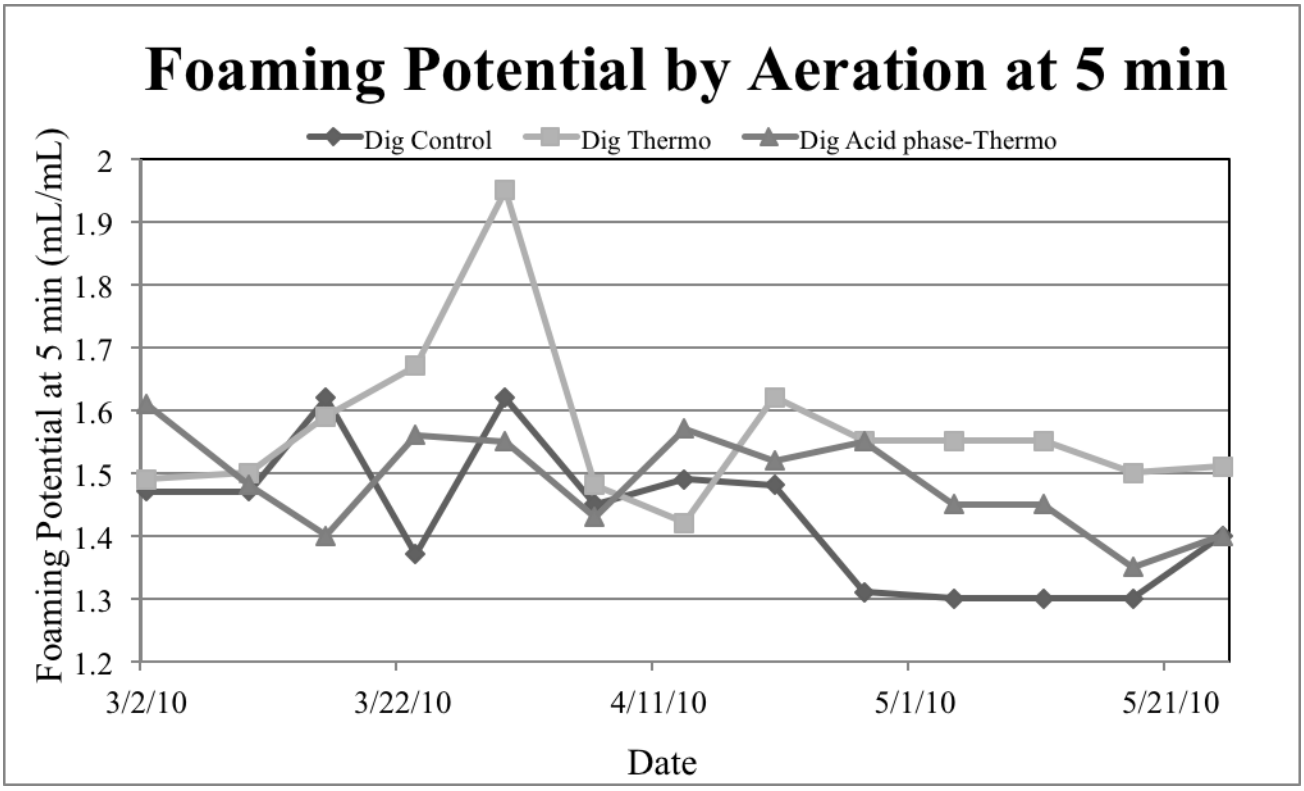


Figure 41: Phase 2 Foaming Potential by Aeration at 5 minutes

Table 14: Phase 2 Summary of Correlation Coefficients

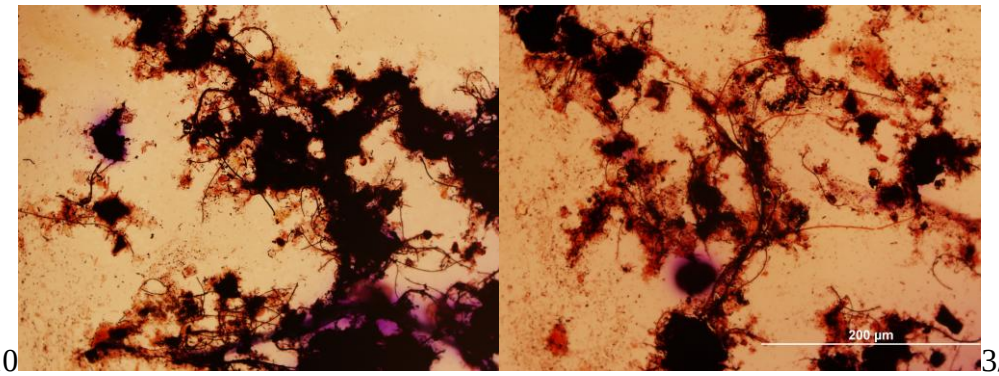
Phase 2		Alkalinity	pH	TKN-D	TP-D	% Total Solids	VA-Acetic	VA-Iso-Butryic	VA-Iso-Valeric	VA-N Butyric	VA-N Valeric	VA-Propionic	VA-Sec Valeric	Total VFA	% Volatile Solids	Alka Seltzer	Aeration	Filament Count	Numerical Rating
Alkalinity (ppm)		1																	
pH	r-square	0.0001	1																
	p-value	<0.01																	
TKN (ppm)	r-square	0.7235	0.7143	1															
	p-value	<0.01	<0.01																
TP (ppm)	r-square	0.5861	0.7865	0.3186	1														
	p-value	<0.01	<0.01	0.0018															
% Total Solids	r-square	0.8500	0.6011	0.6663	0.6704	1													
	p-value	<0.01	<0.01	<0.01	<0.01														
VA-Acetic (mg/L)	r-square	0.0244	0.6672	0.5806	0.8070	0.1143	1												
	p-value	0.3697	<0.01	<0.01	<0.01	0.0470													
VA-Iso-Butyric (mg/L)	r-square	0.0213	0.6582	0.5975	0.8161	0.1203	0.9766	1											
	p-value	0.4024	<0.01	<0.01	<0.01	0.0413	<0.01												
VA-Iso-Valeric (mg/L)	r-square	0.0277	0.6791	0.6023	0.8139	0.1319	0.9780	0.9960	1										
	p-value	0.3395	<0.01	<0.01	<0.01	0.0320	<0.01	<0.01											
VA-N Butyric (mg/L)	r-square	0.0258	0.6757	0.6062	0.8233	0.1259	0.9874	0.9947	0.9929	1									
	p-value	0.3562	<0.01	<0.01	<0.01	0.0365	<0.01	<0.01	<0.01										
VA-N-Valeric (mg/	r-square	0.0260	0.6768	0.6050	0.8300	0.1280	0.9860	0.9918	0.9926	0.9975	1								
	p-value	0.3546	<0.01	<0.01	<0.01	0.0348	<0.01	<0.01	<0.01	<0.01									
VA-Propionic (mg/L)	r-square	0.0358	0.6903	0.5682	0.8559	0.1562	0.9503	0.9774	0.9665	0.9745	0.9740	1							
	p-value	0.2761	<0.01	<0.01	<0.01	0.0188	<0.01	<0.01	<0.01	<0.01	<0.01								
VA-Sec Valeric (mg/L)	r-square	0.0273	0.6762	0.6026	0.8203	0.1280	0.9801	0.9978	0.9975	0.9957	0.9934	0.9704	1						
	p-value	0.3433	<0.01	<0.01	<0.01	0.0348	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
Total VFA (mg/L)	r-square	0.1294	0.0235	0.1105	0.0043	0.3595	0.9195	0.8224	0.6029	0.8621	0.6565	0.8567	0.6756	1					
	p-value	0.1093	0.5069	0.1409	0.7780	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01						
% Volatile Solids	r-square	0.8814	0.6142	0.6812	0.7157	0.9944	0.1163	0.1198	0.1320	0.1258	0.1277	0.1546	0.1285	0.3996	1				
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.0450	0.0417	0.0319	<0.01	0.0351	0.0195	0.0345	<0.01					
alka seltzer (mL)	r-square	0.7372	0.3807	0.3012	0.1816	0.7313	0.0161	0.0195	0.0215	0.0205	0.0200	0.0327	0.0228	0.0210	0.7398	1			
	p-value	<0.01	<0.01	0.0025	0.0237	<0.01	0.4669	0.4234	0.4010	0.4114	0.4180	0.2988	0.3870	0.4064	<0.01				
Aeration (mL)	r-square	0.1102	0.0199	0.0335	0.0939	0.0001	0.0725	0.0542	0.0147	0.1299	0.1285	0.0137	0.0001	0.0990	0.0990	0.0279	1		
	p-value	0.1415	0.5418	0.4274	0.1767	0.9918	0.2378	0.3096	0.6008	0.1905	0.1106	0.6136	0.9620	0.1647	0.1647	0.4695			
Filament Count	r-square	0.4010	0.5945	0.3277	0.5938	0.4648	0.3899	0.3542	0.3660	0.3868	0.3647	0.3465	0.3767	0.1098	0.4777	0.3332	0.0724	1	
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.1424	<0.01	<0.01	0.3693		
Numerical Rating	r-square	0.0645	0.0050	0.0001	0.1064	0.0077	0.0849	0.0762	0.0835	0.0797	0.0834	0.0602	0.0800	0.0784	0.0094	0.0556	0.0142	0.0318	1
	p-value	0.0412	0.8658	0.9860	0.0903	0.4856	0.0895	0.1085	0.0923	0.1003	0.0924	0.1556	0.0761	0.1032	0.4434	0.0587	0.4443	0.3506	

Table 15: Phase 2 Summary of ANOVA results
Geometric Mean (Average Deviation)

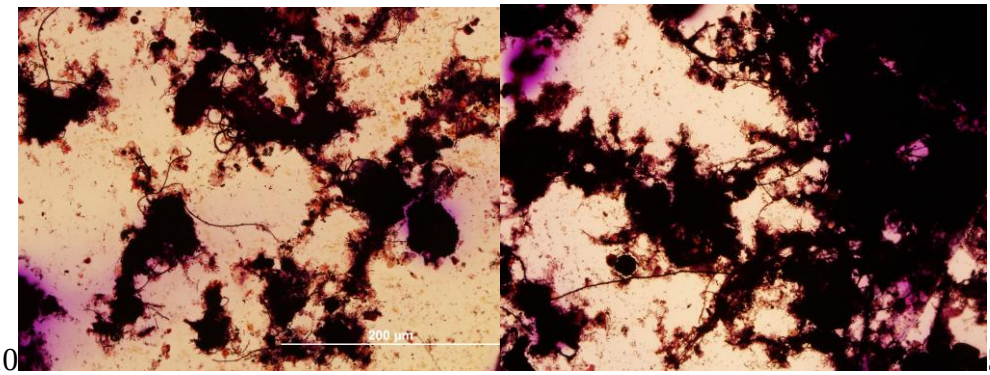
Parameter	No Foam	Weak Foam	Fast Collapse Foam	Weak Foam but Stable	p-value
Alkalinity	717.4 (183.8)	3666.6 (972.8)	4459.9 (642.4)	4122.7 (1182.6)	<0.01
pH	6.37 (0.16)	6.46 ± (1.07)	7.36 (0.54)	6.65 (0.54)	<0.01
TKN	-	1021.6 (331.9)	1091.3 (354.7)	1025.1 (296.2)	0.6046
TP	-	418 (190.2)	300 (27.4)	353.6 (196)	0.0243
%TS	4.14 (0.18)	2.96 (0.67)	2.45 (0.31)	2.91 (0.71)	<0.01
%VS	3.38 (0.15)	2.03 (0.62)	1.69 (0.29)	2.08 (0.70)	<0.01
Alka-Seltzer (mL)	54.3 (9.4)	218 (85.2)	292 (71.5)	270 (108.1)	<0.01
Aeration (mL)	-	76.0 (12.9)	65.0 (16.64)	94.9 (4.6)	<0.01
Total VFA	-	615.5 (2789)	811.7 (2944)	2221.5 (3437)	0.864
Filament Count	21.67 (8.2)	1.83 (9.6)	4.00 (16.6)	1.08 (0.6)	<0.01
Numerical Rating	3.64 (0.4)	3.18 (0.9)	3.11 (0.7)	3.48 (1.1)	0.1026

**Only one value

Blanks indicate measurement was not conducted

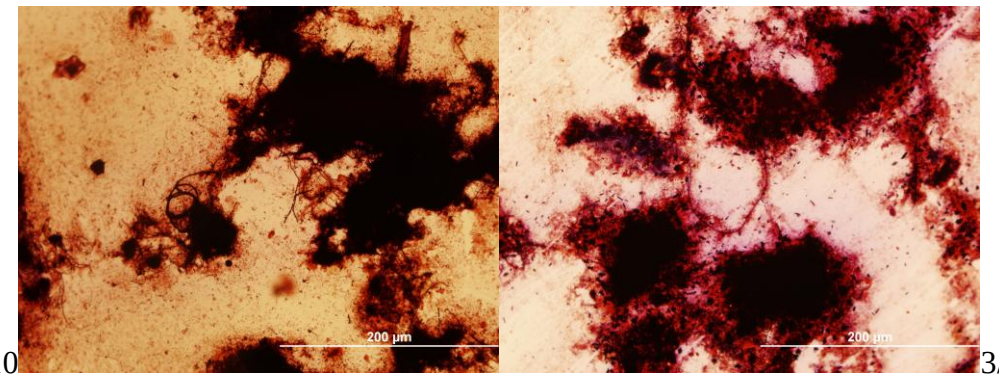


3/11/10 3/24/10

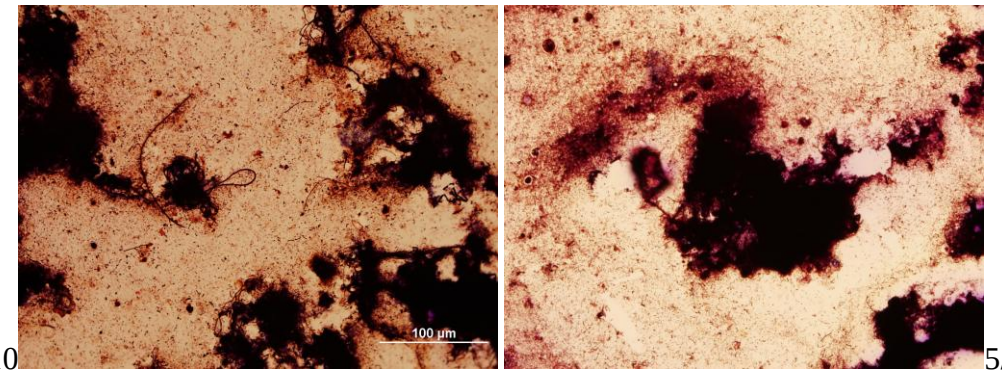


4/14/10 5/19/10

Figure 42: Phase 2 Gram Stain Analysis of Feed (50/50 WAS/Primary Sludge)



3/11/10 3/24/10



4/14/10 5/19/10

Figure 43: Phase 2 Gram Stain Analysis of Acid Phase Mesophilic Digester

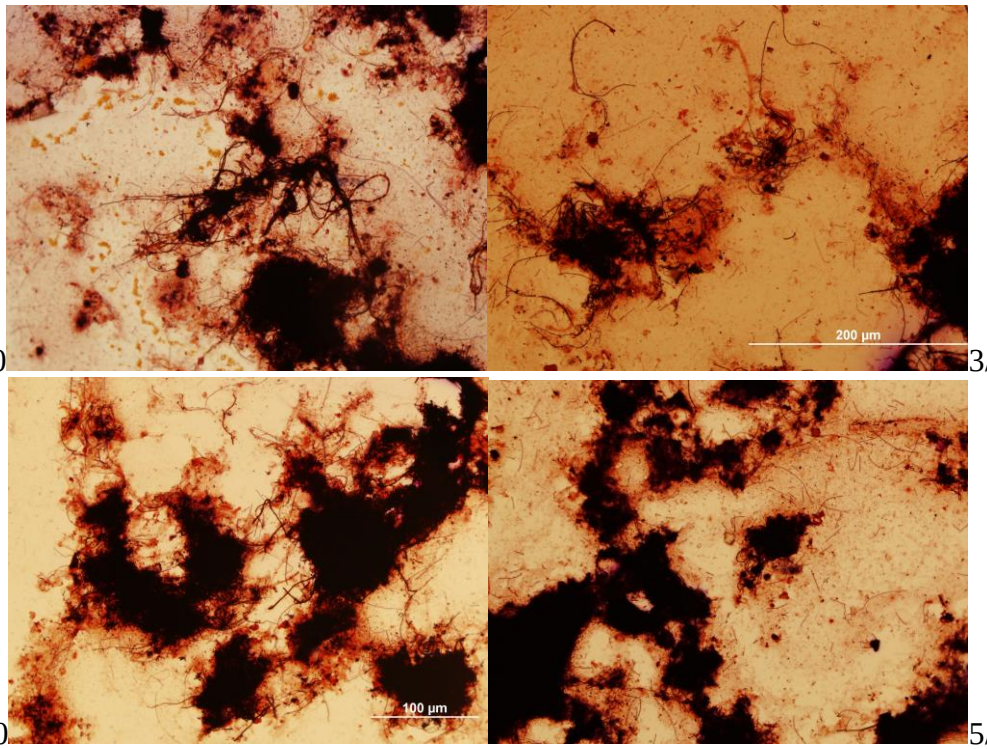


Figure 44: Phase 2 Gram Stain Analysis of Control Digester (Mesophilic)

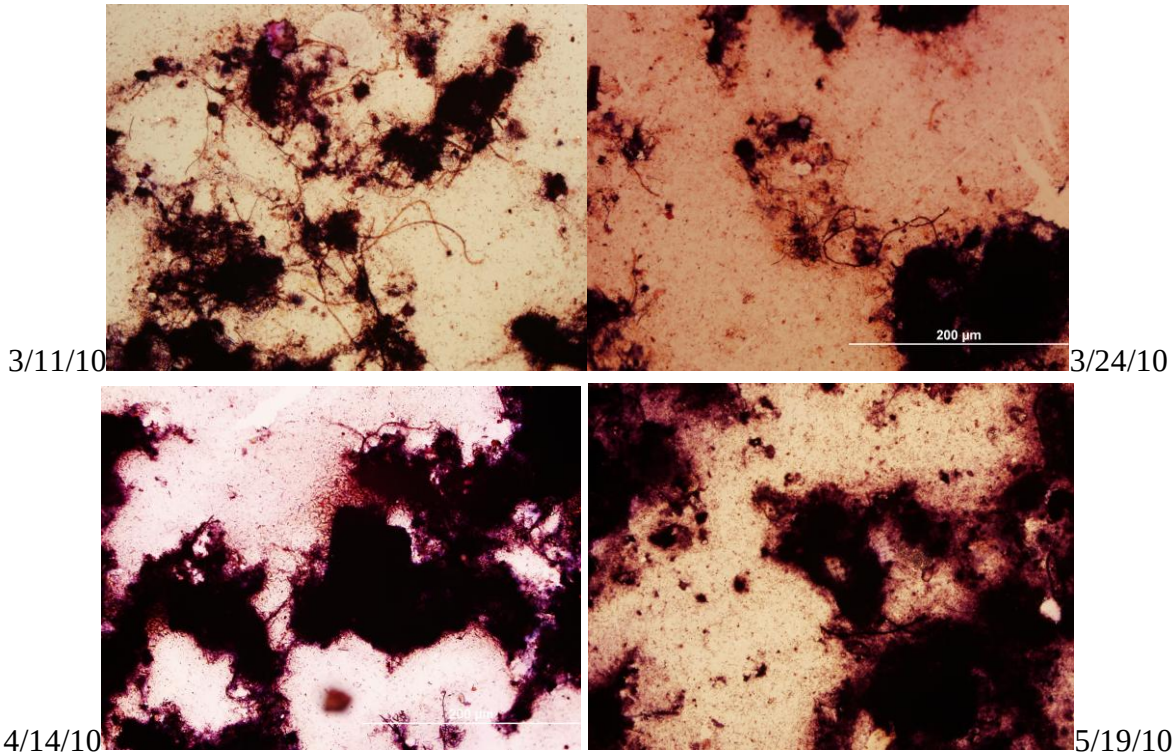


Figure 45: Phase 2 Gram Stain Analysis of Thermophilic Digester

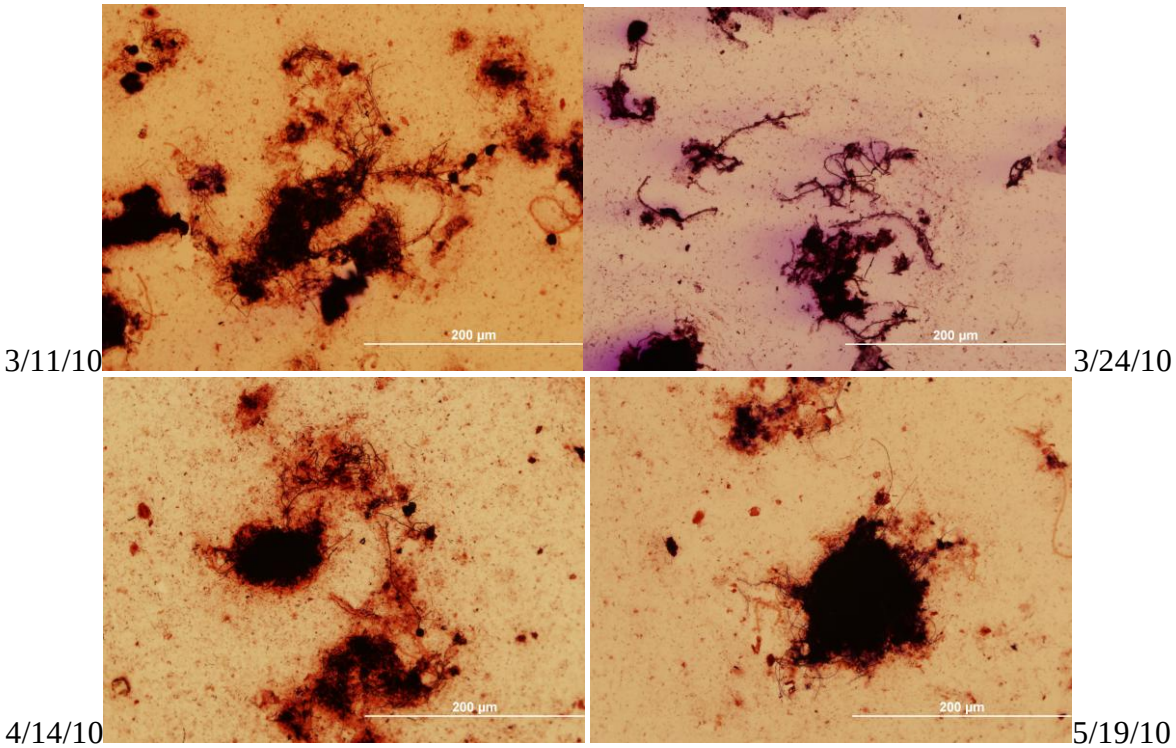


Figure 46: Phase 2 Gram Stain Analysis of Acid phase-Thermophilic Digester

APPENDIX C: Phase 3

Table 16: Phase 3 Summary Table of Foaming Results Over Time

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
11/23/10				
Feed	100	4:00	-	-
Acid Phase dig (37)	480	3:20	-	-
Acid phase dig (46)	460	2:40	-	-
Dig Control	380	0:40	30	40
Dig Acid phase (37)-Thermo	460	1:20	45	80
Dig Acid phase (46)-Thermo	600	0:40	55	90
11/30/10				
Feed	125	2:00	-	-
Acid Phase dig (37)	260	6:40	-	-
Acid phase dig (46)	200	2:20	-	-
Dig Control	270	1:20	25	45
Dig Acid phase (37)-Thermo	490	1:00	70	85
Dig Acid phase (46)-Thermo	490	0:40	45	75
12/7/10				
Feed	60	2:00	-	-
Acid Phase dig (37)	320	4:00	-	-
Acid phase dig (46)	320	3:20	-	-
Dig Control	380	0:40	40	55
Dig Acid phase (37)-Thermo	390	1:00	40	95
Dig Acid phase (46)-Thermo	440	0:40	50	90
12/14/10				
Feed	90	3:20	-	-
Acid Phase dig (37)	180	4:20	-	-
Acid phase dig (46)	290	6:20	-	-
Dig Control	240	1:40	35	70
Dig Acid phase (37)-Thermo	390	1:40	50	95
Dig Acid phase (46)-Thermo	450	1:20	50	95
12/21/10				
Feed	125	3:00	-	-
Acid Phase dig (37)	190	3:40	-	-
Acid phase dig (46)	190	3:40	-	-
Dig Control	440	1:00	30	50
Dig Acid phase (37)-Thermo	300	1:00	40	60
Dig Acid phase (46)-Thermo	430	0:40	65	90

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
12/28/10				
Feed	65	3:40	-	-
Acid Phase dig (37)	250	6:40	-	-
Acid phase dig (46)	280	4:20	-	-
Dig Control	370	1:00	35	55
Dig Acid phase (37)-Thermo	310	1:40	45	70
Dig Acid phase (46)-Thermo	380	0:40	55	90
1/4/11				
Feed	75	3:00	-	-
Acid Phase dig (37)	250	3:40	-	-
Acid phase dig (46)	250	4:00	-	-
Dig Control	380	1:40	75	100
Dig Acid phase (37)-Thermo	310	2:00	65	100
Dig Acid phase (46)-Thermo	350	1:20	65	105
1/11/11				
Feed	70	12:00	-	-
Acid Phase dig (37)	104	11:40	-	-
Acid phase dig (46)	240	3:00	-	-
Dig Control	170	0:20	80	100
Dig Acid phase (37)-Thermo	380	1:20	90	130
Dig Acid phase (46)-Thermo	380	1:40	90	130
1/18/11				
Feed	65	4:40	-	-
Acid Phase dig (37)	210	3:40	-	-
Acid phase dig (46)	230	2:40	-	-
Dig Control	250	1:20	70	120
Dig Acid phase (37)-Thermo	300	1:40	90	155
Dig Acid phase (46)-Thermo	480	0:40	60	90
1/25/11				
Feed	50	3:00	-	-
Acid Phase dig (37)	160	3:00	-	-
Acid phase dig (46)	270	4:40	-	-
Dig Control	450	0:40	60	125
Dig Acid phase (37)-Thermo	250	3:00	125	145
Dig Acid phase (46)-Thermo	220	2:20	170	205

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
2/1/11				
Feed	70	7:20	-	-
Acid Phase dig (37)	160	2:00	-	-
Acid phase dig (46)	280	6:40	-	-
Dig Control	220	1:40	135	170
Dig Acid phase (37)-Thermo	340	1:00	90	140
Dig Acid phase (46)-Thermo	400	1:20	50	130
2/8/11				
Feed	100	4:20	-	-
Acid Phase dig (37)	200	1:40	-	-
Acid phase dig (46)	330	7:40	-	-
Dig Control	350	2:00	100	160
Dig Acid phase (37)-Thermo	350	1:40	90	150
Dig Acid phase (46)-Thermo	250	5:20	140	215
2/15/11				
Feed	50	8:20	-	-
Acid Phase dig (37)	190	2:20	-	-
Acid phase dig (46)	280	4:00	-	-
Dig Control	280	1:40	75	90
Dig Acid phase (37)-Thermo	400	2:00	90	140
Dig Acid phase (46)-Thermo	440	1:20	95	160
2/22/11				
Feed	50	1:40	-	-
Acid Phase dig (37)	200	7:40	-	-
Acid phase dig (46)	260	5:00	-	-
Dig Control	310	1:40	100	175
Dig Acid phase (37)-Thermo	310	3:40	140	200
Dig Acid phase (46)-Thermo	460	1:20	75	150

Table 17: Phase 3 Summary Table of Chemistry Over Time

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
11/18/10						
Feed	767	6.6	-	-	3.11	-
Acid Phase dig (37)	2580	5.2	-	-	2.52	-
Acid phase dig (46)	2920	5.2	-	-	2.29	-
Dig Control	2780	7	-	-	1.45	-
Dig Acid phase (37)-Thermo	3170	6.9	-	-	1.05	-
Dig Acid phase (46)-Thermo	3530	6.9	-	-	1.06	-
11/23/10						
Feed	847	6.3	2570	1320	2.96	324
Acid Phase dig (37)	2660	5.5	662	860	2.33	4644
Acid phase dig (46)	3090	5.4	725	753	2.12	4280
Dig Control	2560	7	617	167	1.35	104
Dig Acid phase (37)-Thermo	3430	6.7	985	248	0.94	3171
Dig Acid phase (46)-Thermo	3610	6.7	826	203	0.89	2672
11/30/10						
Feed	686	6.6	-	-	2.88	-
Acid Phase dig (37)	2690	5.6	-	-	2.29	-
Acid phase dig (46)	3330	5.7	-	-	1.86	-
Dig Control	2860	7.2	-	-	1.53	-
Dig Acid phase (37)-Thermo	3460	7.4	-	-	0.88	-
Dig Acid phase (46)-Thermo	3710	7.3	-	-	0.77	-
12/7/10						
Feed	767	6.4	2590	1240	3.04	348
Acid Phase dig (37)	2960	5.6	758	754	2.5	4674
Acid phase dig (46)	3100	5.6	914	793	2.42	5383
Dig Control	2880	7.1	811	289	1.54	162
Dig Acid phase (37)-Thermo	4180	7.5	1190	323	1.53	480
Dig Acid phase (46)-Thermo	4270	7.7	1270	310	1.34	413
12/14/10						
Feed	791	6.4	-	-	3.52	-
Acid Phase dig (37)	3060	5.6	-	-	2.98	-
Acid phase dig (46)	3260	5.6	-	-	1.93	-
Dig Control	3080	7.2	-	-	1.51	-
Dig Acid phase (37)-Thermo	4830	7.6	-	-	1.49	-
Dig Acid phase (46)-Thermo	5540	7.6	-	-	1.17	-

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
12/21/10						
Feed	767	6.2	2830	1200	3.61	408
Acid Phase dig (37)	2980	5.5	700	621	2.74	4800
Acid phase dig (46)	3340	5.4	922	697	2.58	5864
Dig Control	3300	7.3	939	302	1.47	54
Dig Acid phase (37)-Thermo	4480	7.7	1230	294	1.12	42
Dig Acid phase (46)-Thermo	4730	7.8	1380	319	1.09	65
12/28/10						
Feed	789	6.3	-	-	3.6	-
Acid Phase dig (37)	3220	5.6	-	-	2.66	-
Acid phase dig (46)	3440	5.6	-	-	2.69	-
Dig Control	3040	7.4	-	-	1.39	-
Dig Acid phase (37)-Thermo	4670	7.7	-	-	0.62	-
Dig Acid phase (46)-Thermo	5100	7.7	-	-	0.98	-
1/4/11						
Feed	1020	6.5	2330	1060	3.54	530
Acid Phase dig (37)	3300	5.7	862	714	2.9	5545
Acid phase dig (46)	3700	5.7	1020	722	2.78	5551
Dig Control	3510	7.2	1030	289	1.7	66
Dig Acid phase (37)-Thermo	4720	7.6	1280	296	0.59	392
Dig Acid phase (46)-Thermo	5070	7.6	1430	345	0.93	549
1/11/11						
Feed	937	6.4	-	-	3.5	-
Acid Phase dig (37)	3460	5.5	-	-	2.57	-
Acid phase dig (46)	3610	5.5	-	-	2.75	-
Dig Control	3850	7.3	-	-	1.55	-
Dig Acid phase (37)-Thermo	4980	7.5	-	-	0.69	-
Dig Acid phase (46)-Thermo	5090	7.6	-	-	0.96	-
1/18/11						
Feed	906	6.3	2710	1120	3.66	41
Acid Phase dig (37)	3550	5.5	915	717	2.88	3237
Acid phase dig (46)	3750	5.6	976	673	2.63	3177
Dig Control	3940	7.5	1040	268	1.71	53
Dig Acid phase (37)-Thermo	5060	7.5	1310	266	2.02	84
Dig Acid phase (46)-Thermo	5050	7.6	1370	294	1.23	99

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
1/25/11						
Feed	783	6.3	-	-	3.85	-
Acid Phase dig (37)	3600	5.4	-	-	2.85	-
Acid phase dig (46)	3670	5.4	-	-	2.96	-
Dig Control	3940	7.2	-	-	0.53	-
Dig Acid phase (37)-Thermo	5340	7.4	-	-	1.4	-
Dig Acid phase (46)-Thermo	5570	7.6	-	-	0.81	-
2/1/11						
Feed	768	6.3	2280	979	3.33	434
Acid Phase dig (37)	3010	5.4	726	667	2.85	5583
Acid phase dig (46)	3340	5.6	875	664	2.96	5742
Dig Control	4060	7.6	1080	277	0.53	100
Dig Acid phase (37)-Thermo	5230	7.6	1460	321	1.4	767
Dig Acid phase (46)-Thermo	5510	7.7	1270	269	0.81	287
2/8/11						
Feed	575	6.3	-	-	3.65	-
Acid Phase dig (37)	2990	5.3	-	-	2.87	-
Acid phase dig (46)	3070	5.4	-	-	2.75	-
Dig Control	4150	7.7	-	-	1.54	-
Dig Acid phase (37)-Thermo	5350	7.6	-	-	1.48	-
Dig Acid phase (46)-Thermo	5610	7.8	-	-	1.41	-
2/15/11						
Feed	905	6.2	2250	927	3.57	435
Acid Phase dig (37)	3050	5.4	803	664	2.7	4525
Acid phase dig (46)	3250	5.7	858	604	2.71	4720
Dig Control	4030	7.4	1010	251	1.11	5300
Dig Acid phase (37)-Thermo	5240	7.6	1510	321	1.4	7080
Dig Acid phase (46)-Thermo	5120	7.5	1370	287	1.56	6786
2/22/11						
Feed	917	6.2	-	-	3.62	-
Acid Phase dig (37)	3150	5.6	-	-	3.07	-
Acid phase dig (46)	3230	5.5	-	-	3.16	-
Dig Control	4240	7.1	-	-	1.92	-
Dig Acid phase (37)-Thermo	5140	7.5	-	-	1.53	-
Dig Acid phase (46)-Thermo	5260	7.5	-	-	1.48	-

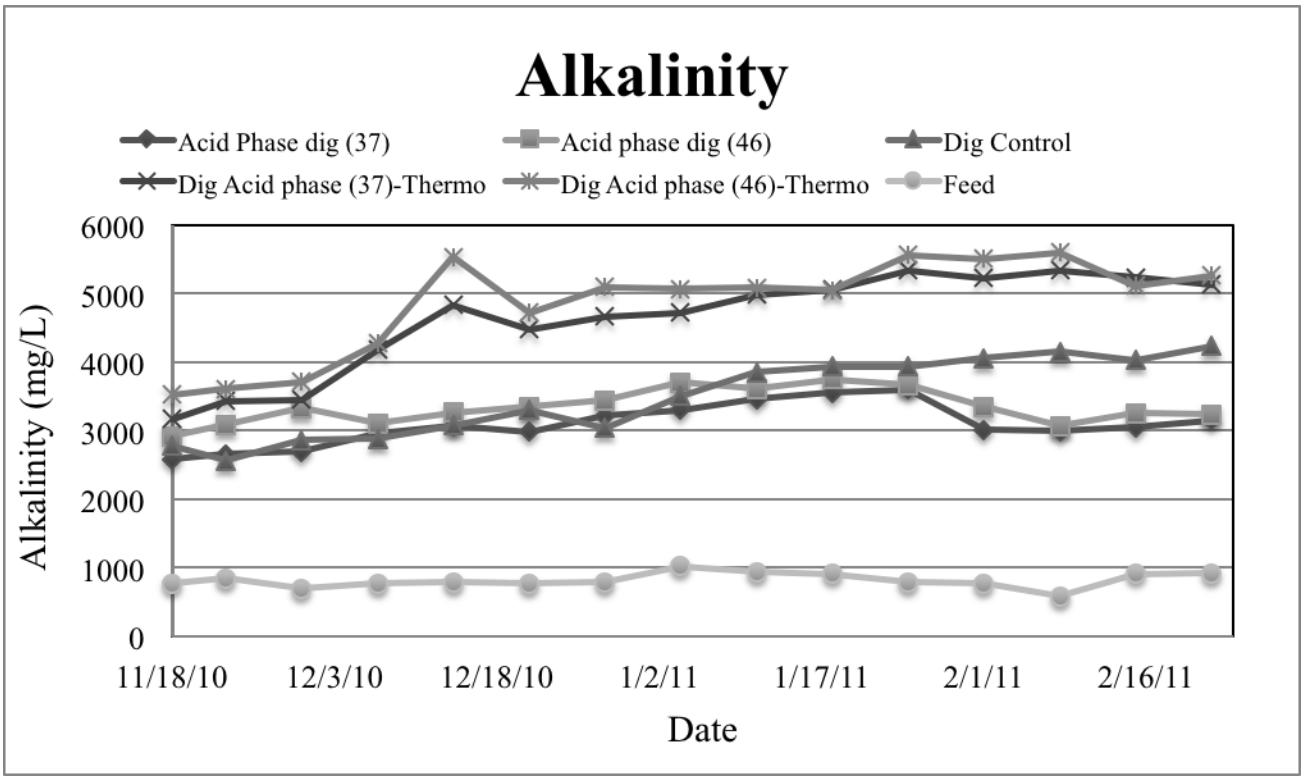


Figure 47: Phase 3 Alkalinity

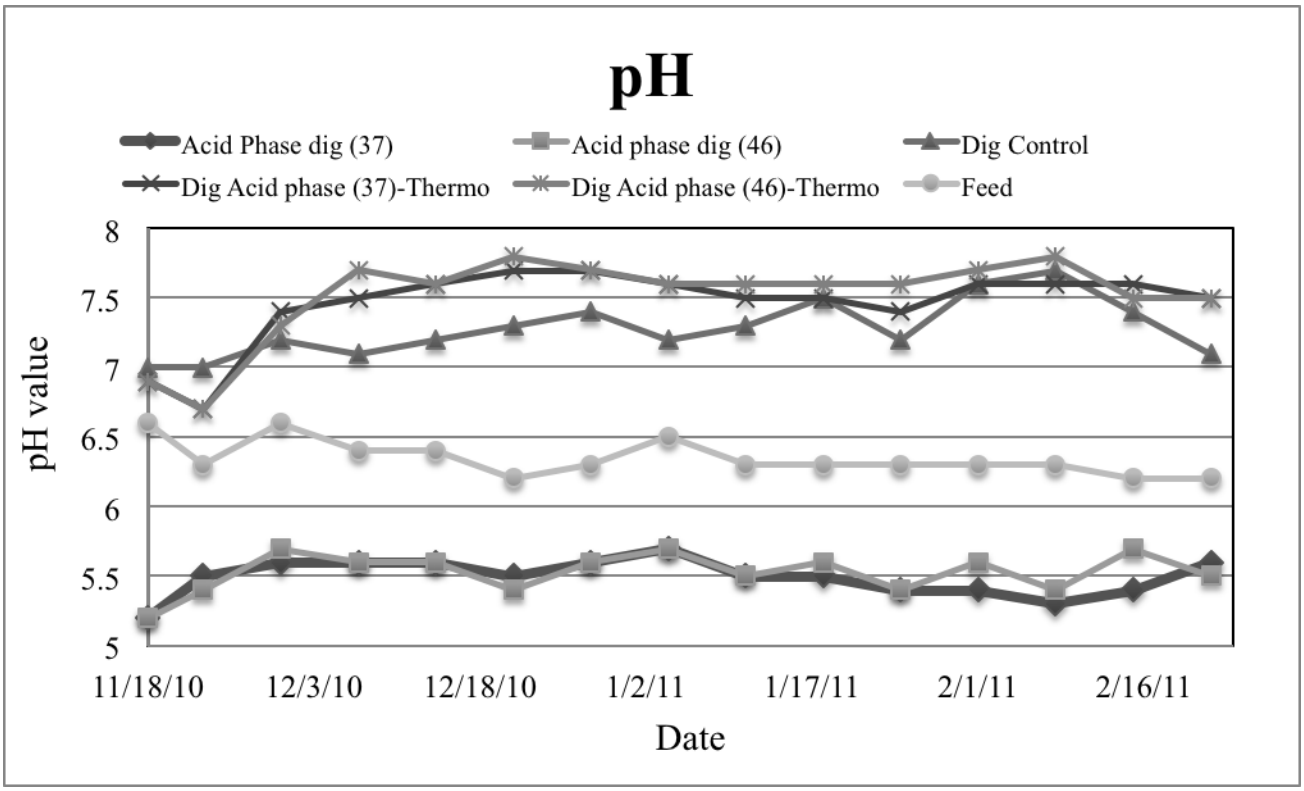


Figure 48: Phase 3 pH

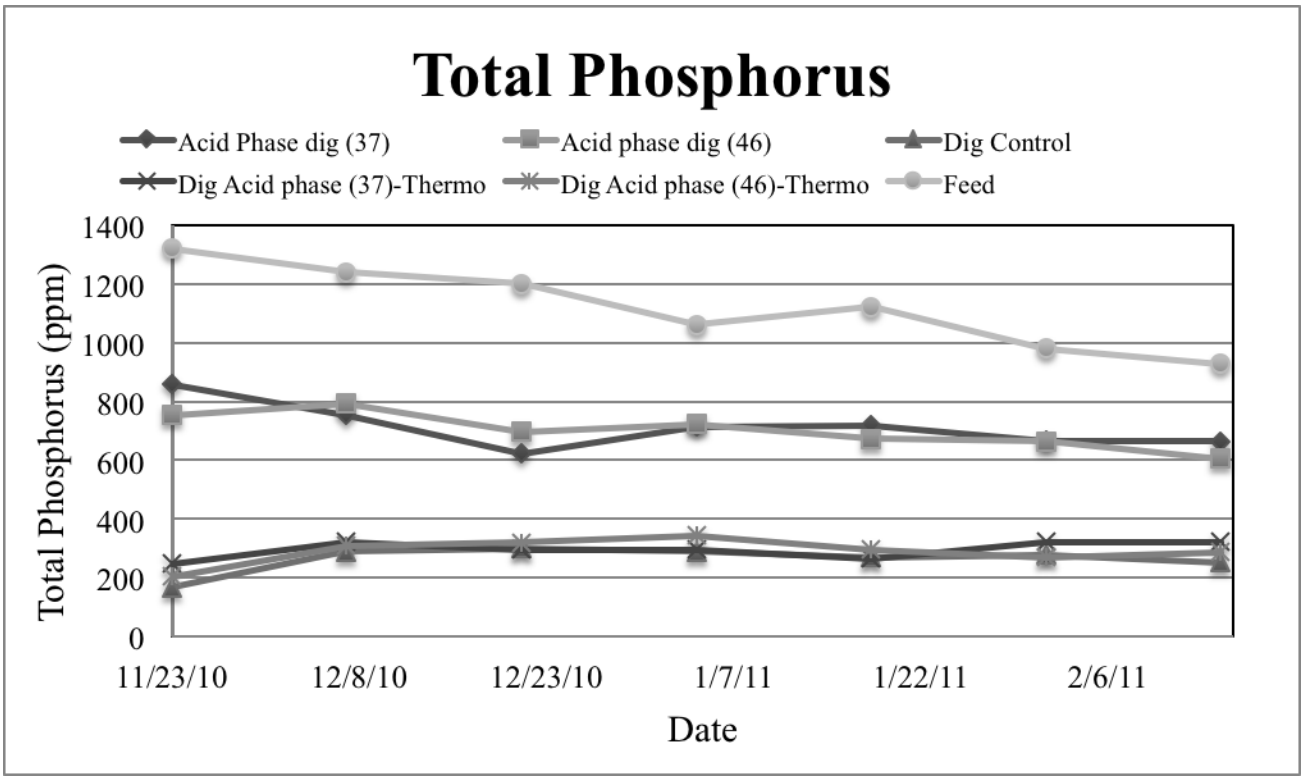


Figure 49: Phase 3 Total Phosphorus

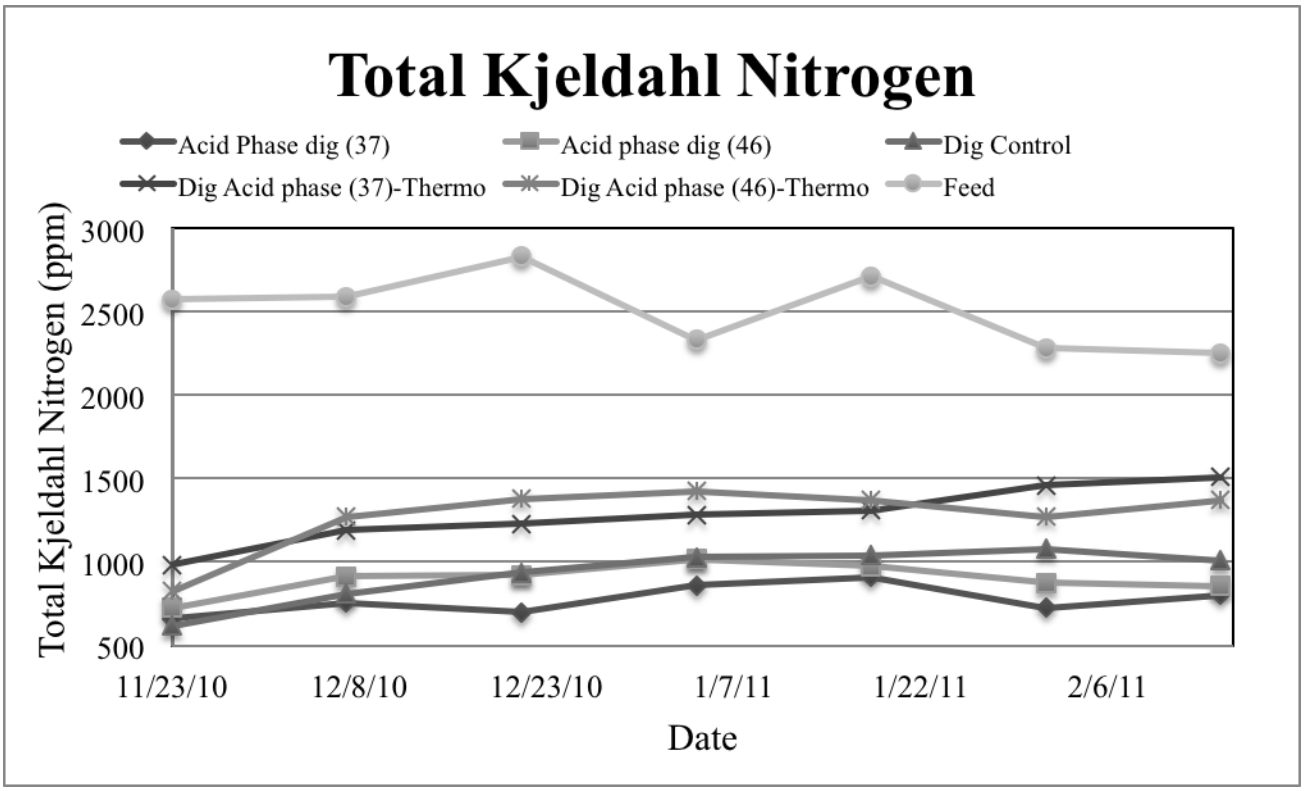


Figure 50: Phase 3 Total Kjeldahl Nitrogen

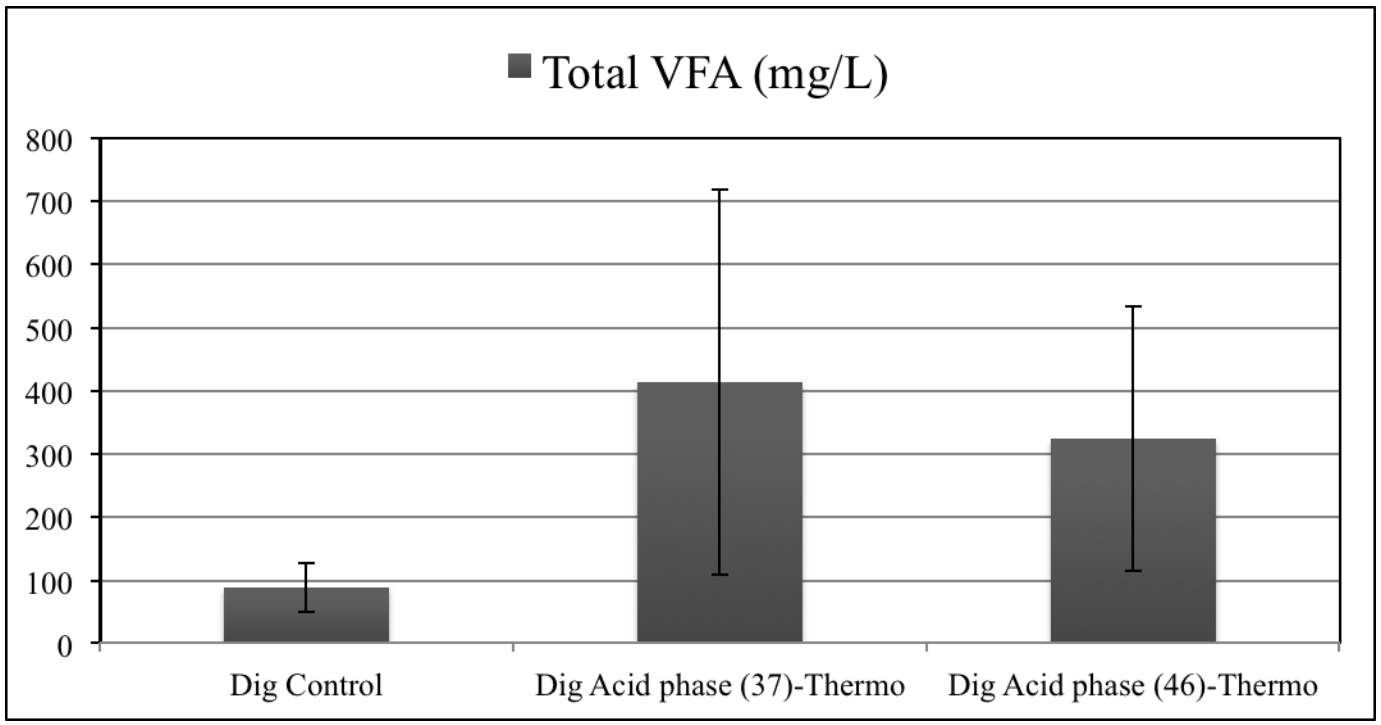


Figure 51: Phase 3 Total VFA

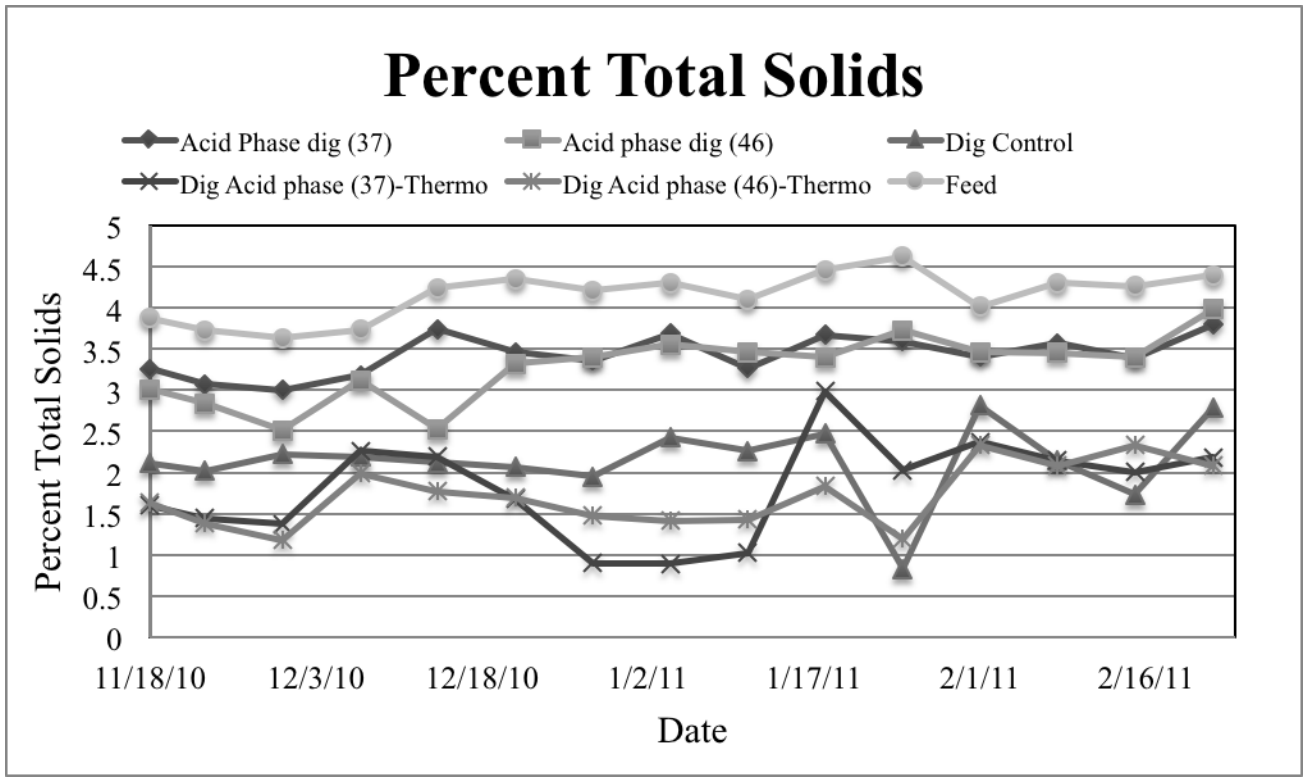


Figure 52: Phase 3 Percent Total Solids

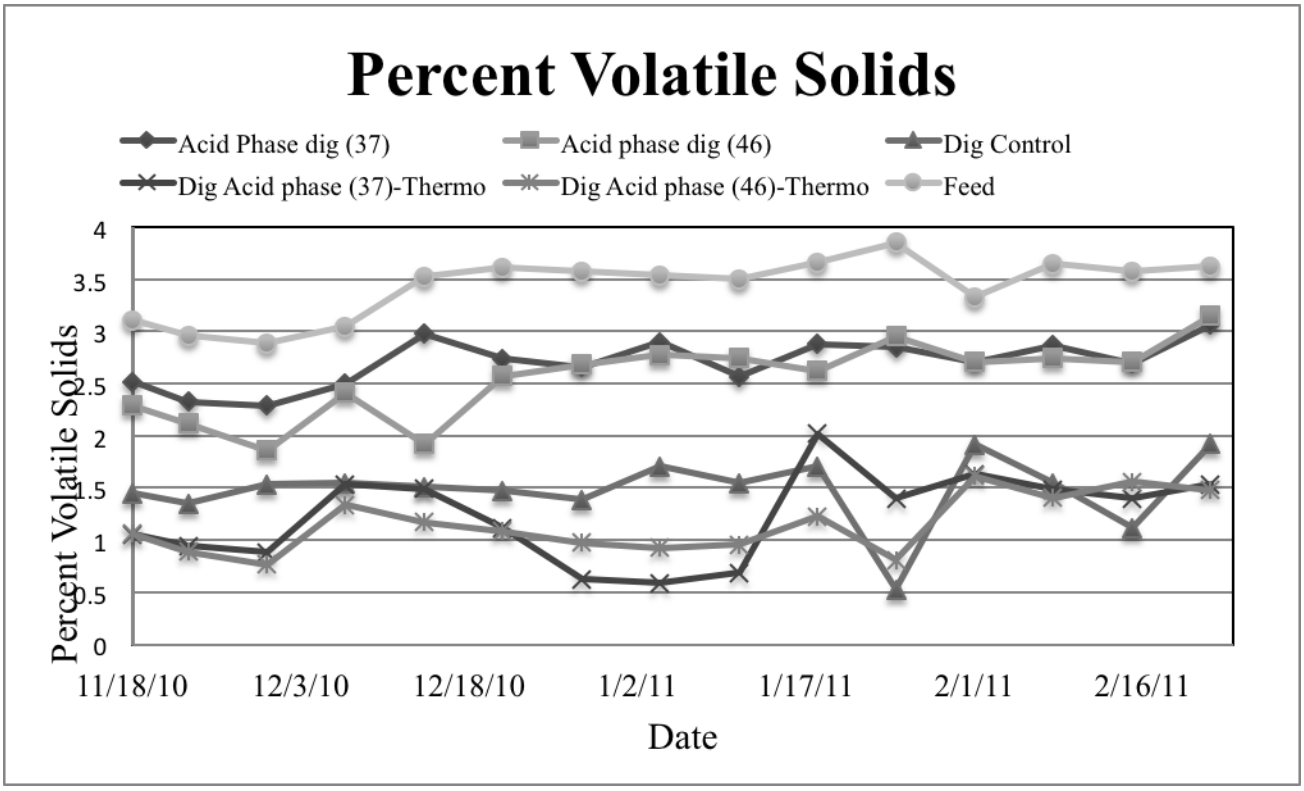


Figure 53: Phase 3 Percent Volatile Solids

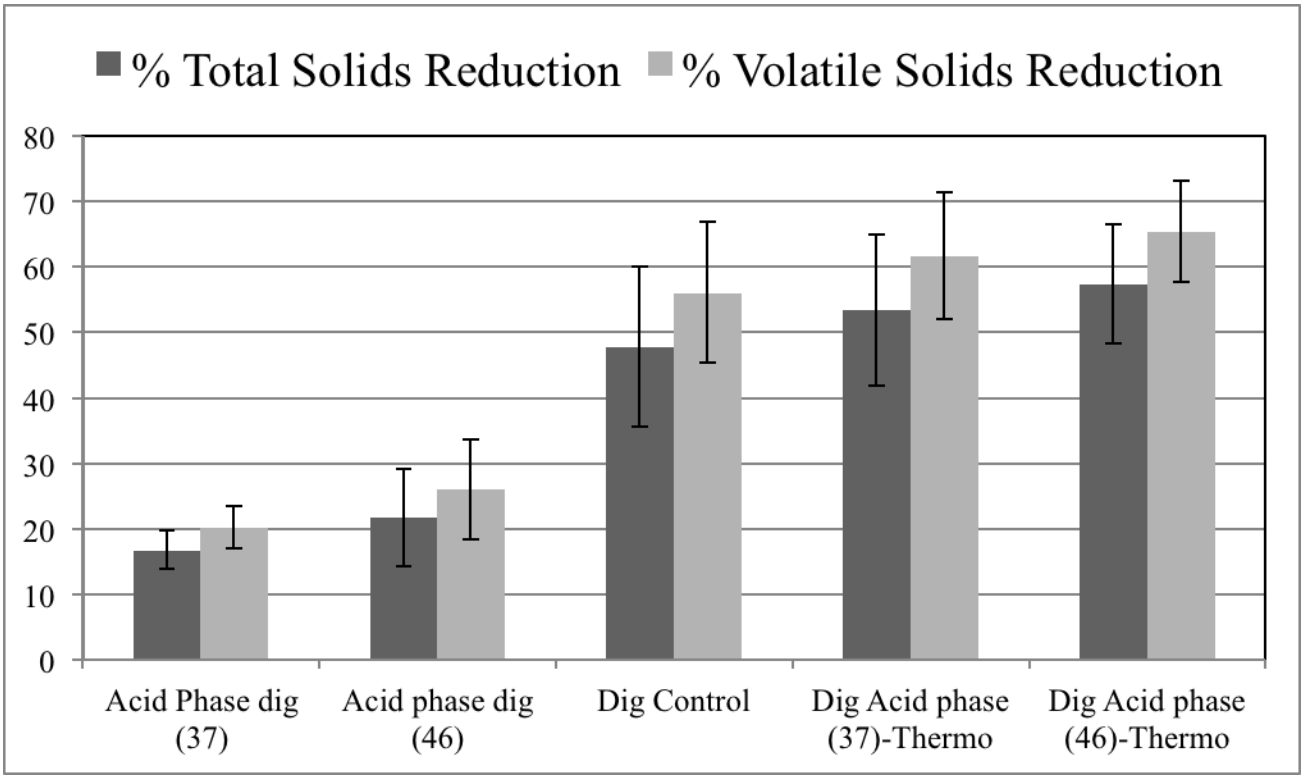


Figure 54: Phase 3 Volatile Solids Reduction and Total Solids Reduction

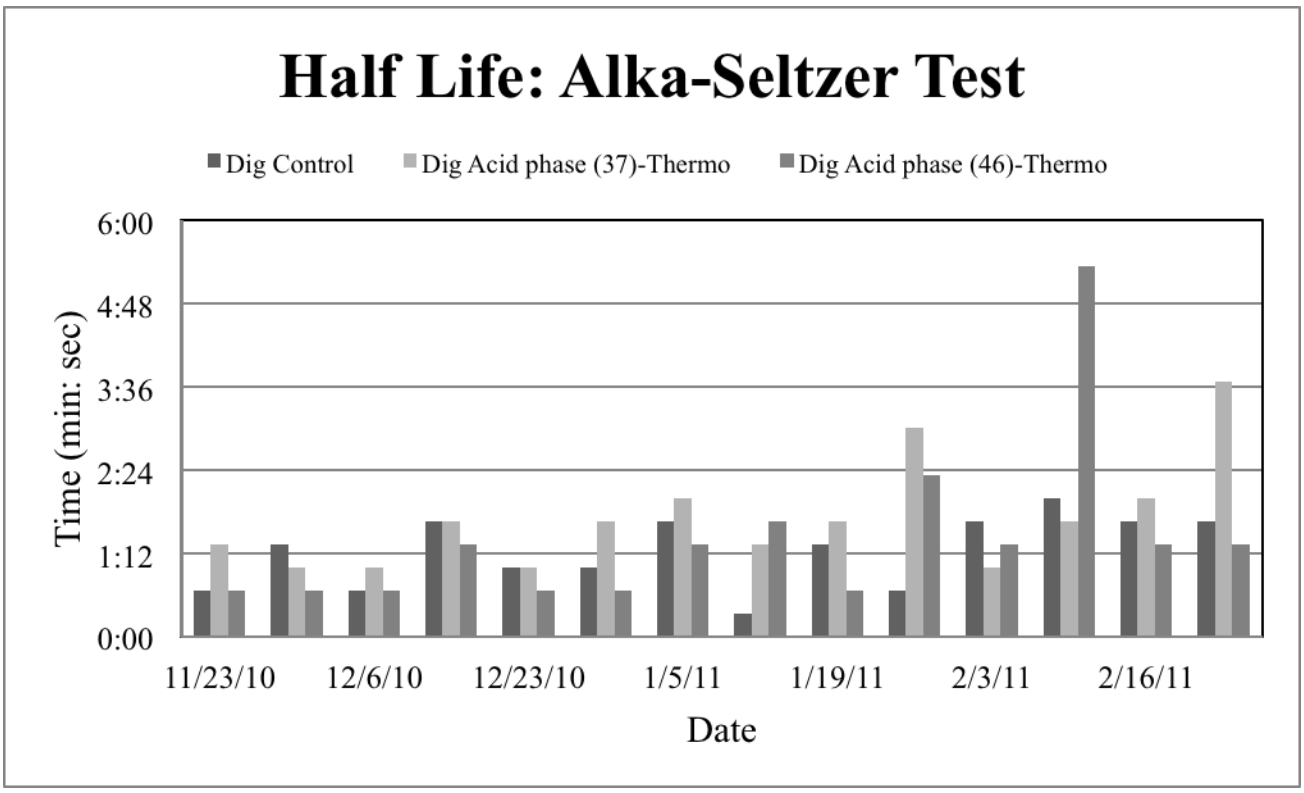


Figure 55: Phase 3 Half Life: Alka-Seltzer Foaming Potential Test

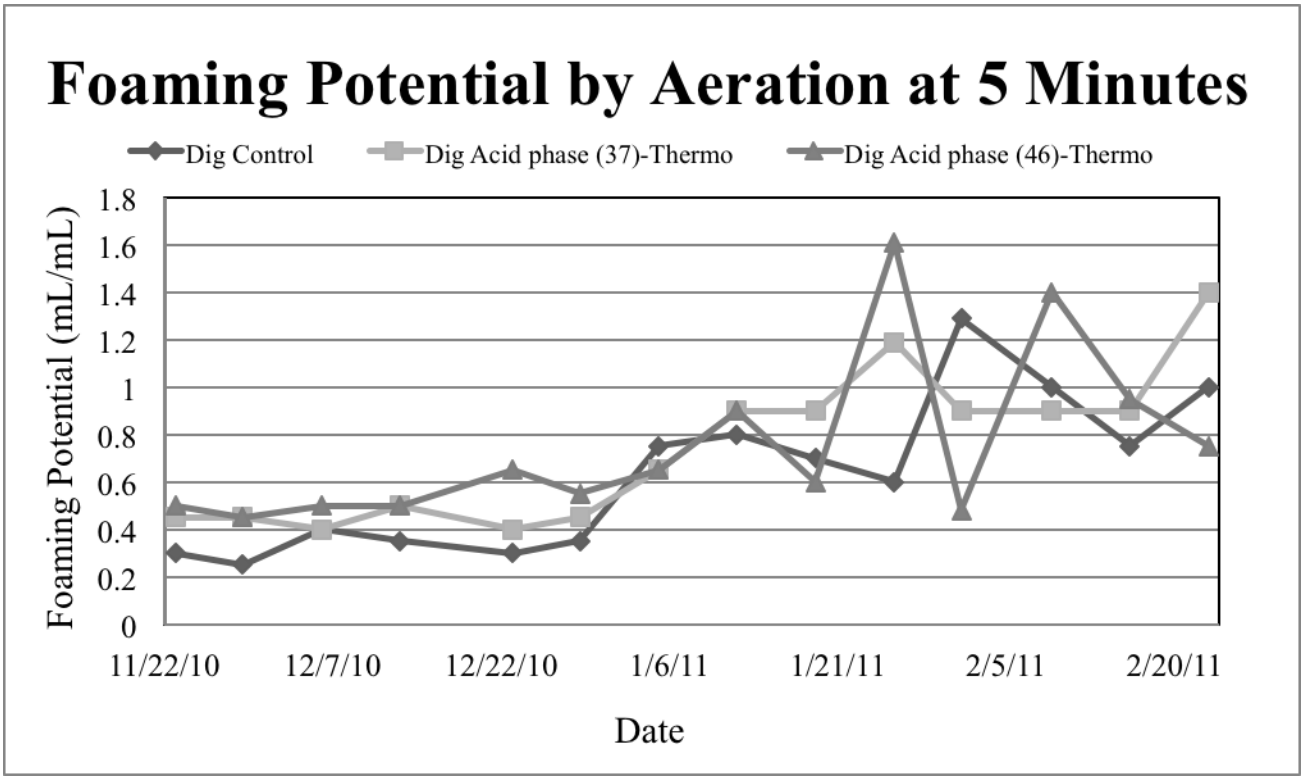


Figure 56: Phase 3 Foaming Potential by Aeration

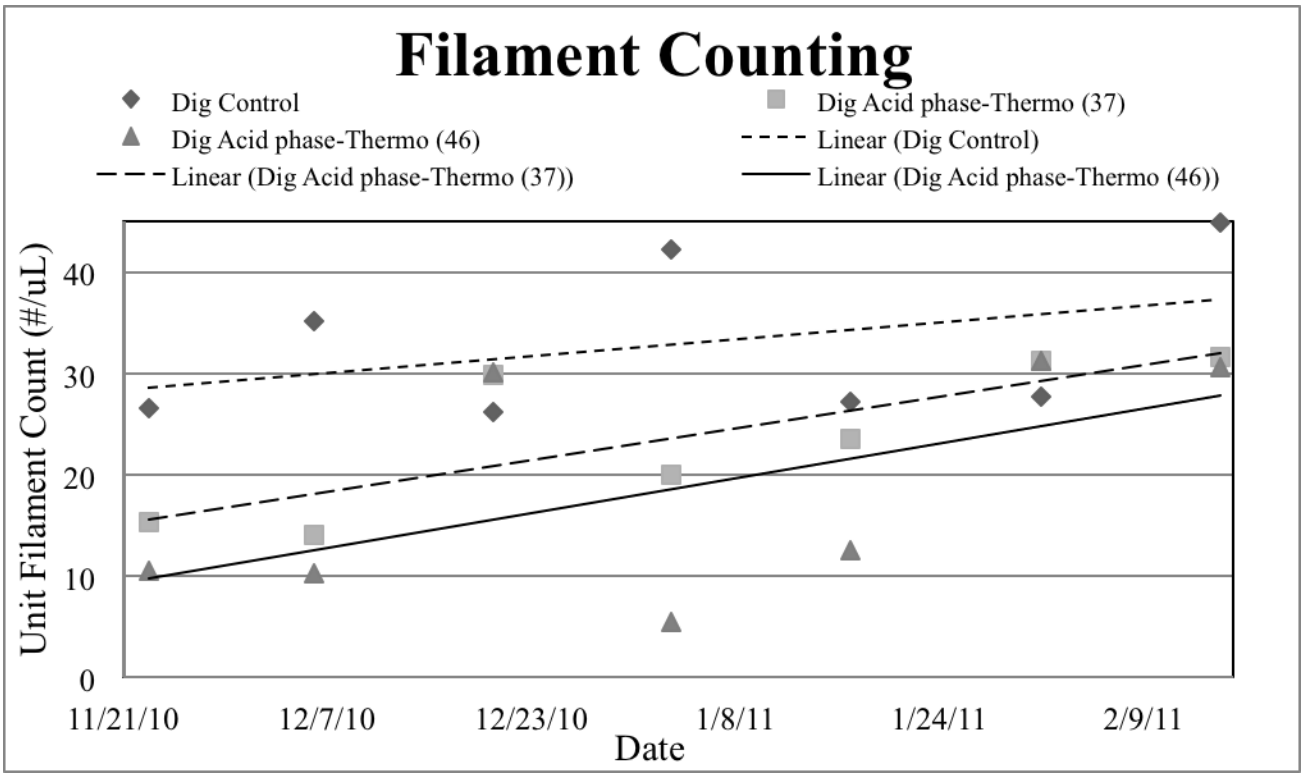


Figure 57: Phase 3 Filament Counting

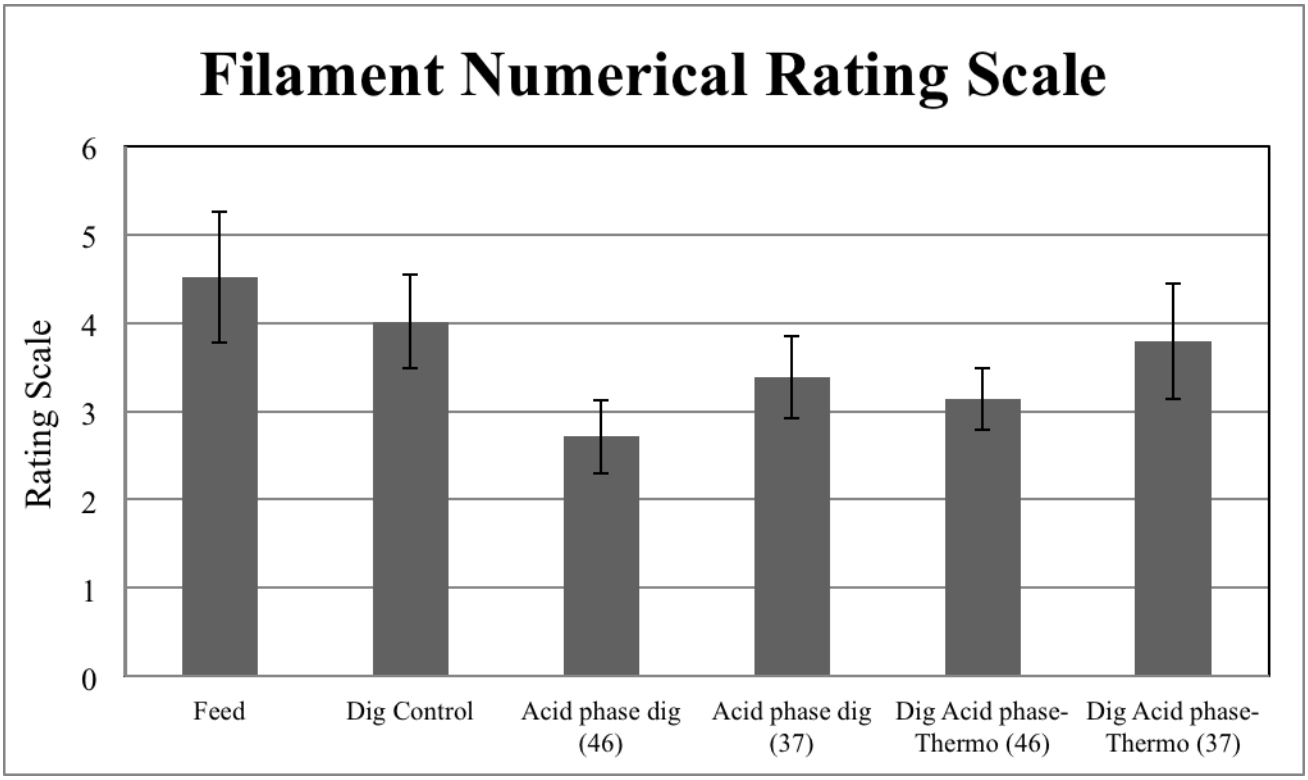


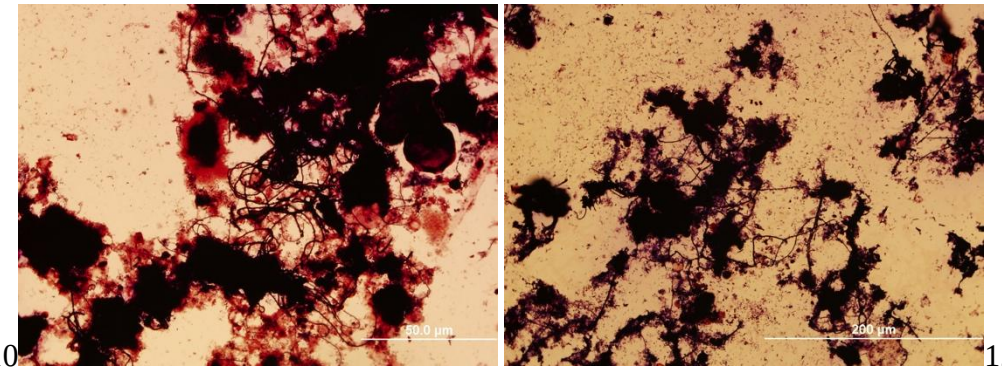
Figure 58: Phase 3 Filament Numerical Rating Scale

Table 18: Phase 3 Summary of Correlation Coefficients

Phase 3		Alkalinity	pH	TKN	TP	% Total Solids	VA-Acetic	VA-Iso-Butryic	VA-Iso-Valeric	VA-N Butyric	VA-N Valeric	VA-Propionic	VA-Sec Valeric	Total VFA	% Volatile Solids	Alka Seltzer	Half-life	VFA/Alkalinity	FP (max)	FP (5 min)	Filament Count	Numerical Rating
Alkalinity (ppm)	r^2-value	1																				
	p-value																					
pH	r^2-value	0.27	1																			
	p-value	<0.01																				
TKN	r^2-value	0.30	0.02	1																		
	p-value	<0.01	0.35																			
TP (ppm)	r^2-value	0.64	0.44	0.36	1																	
	p-value	<0.01	<0.01	<0.01																		
% Total Solids	r^2-value	0.53	0.51	0.19	0.7300	1																
	p-value	<0.01	<0.01	<0.01	<0.01																	
VA-Acetic (mg/L)	r^2-value	0.00	0.59	0.04	0.05	0.06	1															
	p-value	0.73	<0.01	<0.01	0.19	0.13																
VA-Iso-Butyric (mg/L)	r^2-value	0.00	0.28	0.29	0.52	0.01	0.8100	1														
	p-value	0.86	<0.01	<0.01	0.15	0.04	<0.01															
VA-Iso-Valeric (mg/L)	r^2-value	0.00	0.68	0.28	0.05	0.10	0.82	0.99	1													
	p-value	0.85	<0.01	<0.01	0.14	0.04	<0.01	<0.01														
VA-N Butyric (mg/L)	r^2-value	0.00	0.73	0.27	0.08	0.16	0.77	0.95	0.9500	1												
	p-value	0.71	<0.01	<0.01	0.06	<0.01	<0.01	<0.01	<0.01													
VA-N-Valeric (mg/L)	r^2-value	0.00	0.72	0.28	0.08	0.15	0.77	0.94	0.93	0.99	1											
	p-value	0.75	<0.01	<0.01	0.08	<0.01	<0.01	<0.01	<0.01	<0.01												
VA-Propionic (mg/L)	r^2-value	0.01	0.71	0.23	0.09	0.15	0.90	0.86	0.85	0.90	0.9100	1										
	p-value	0.45	<0.01	<0.01	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01											
VA-Sec Valeric (mg/L)	r^2-value	0.00	0.68	0.26	0.07	0.13	0.77	0.97	0.96	0.96	0.9400	0.8400	1									
	p-value	0.84	<0.01	<0.01	0.10	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01										
Total VFA (mg/L)	r^2-value	0.00	0.70	0.27	0.07	0.11	0.95	0.93	0.93	0.93	0.93	0.97	0.91	1								
	p-value	0.67	<0.01	<0.01	0.10	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01									
% Volatile Solids	r^2-value	0.57	0.52	0.21	0.78	0.99	0.06	0.11	0.11	0.16	0.1500	0.1600	0.1400	0.1200	1							
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.11	0.04	0.03	<0.01	<0.01	<0.01	0.02	0.03								
Foaming potential alka seltzer		0.44	0.20	0.36	0.53	0.61	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.61	1						
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.73	0.81	0.82	0.48	0.48	0.48	0.55	0.81	<0.01							
Half-life	r^2-value	0.18	0.20	0.07	0.47	0.58	0.11	0.12	0.12	0.16	0.16	0.19	0.14	0.15	0.38	0.3100	1					
	p-value	<0.01	<0.01	0.09	<0.01	<0.01	0.03	0.02	0.02	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01						
VFA/Alkalinity	r^2-value	0.10	0.80	0.16	0.17	0.20	0.90	0.84	0.84	0.88	0.88	0.96	0.82	0.95	0.22	0.02	0.26	1				
	p-value	0.13	<0.01	<0.01	<0.01	<0.01	0.67	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.34	<0.01					
Foaming Potential Aeration (max)		0.45	0.12	0.34	0.07	0.06	0.00	0.12	0.01	0.00	0.01	0.01	0.15	0.00	0.05	0.09	0.43	0.01	1			
	p-value	<0.01	0.02	<0.01	0.26	0.11	0.83	0.13	0.63	0.84	0.92	0.66	0.08	0.84	0.17	0.05	<0.01	0.65				
Foaming Potential Aeration (5 min)		0.32	0.10	0.18	0.04	0.03	0.02	0.08	0.03	0.01	0.00	0.04	0.09	0.02	0.03	0.19	0.45	0.03	0.84			
	p-value	<0.01	0.04	0.05	0.41	0.31	0.57	0.22	0.48	0.61	0.82	0.41	0.18	0.58	0.27	<0.01	<0.01	0.47	<0.01	1		
Filament Count	r2-value	0.01	0.01	0.02	0.01	0.14	0.20	0.00	0.15	0.16	0.09	0.23	0.01	0.19	0.18	0.17	0.10	0.17	0.01	0.06	1	
	p-value	0.64	0.70	0.55	0.71	0.10	0.04	0.80	0.09	0.07	0.17	0.03	0.76	0.05	0.05	0.06	0.16	0.06	0.70	0.27		
Numerical Rating	r2-value	0.10	0.06	0.35	0.05	0.03	0.24	0.31	0.33	0.25	0.25	0.19	0.29	0.25	0.03	0.21	0.00	0.17	0.01	0.00		1
	p-value	<0.01	0.02	<0.01	0.15	0.12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.09	<0.01	0.90	<0.01	0.92	0.77		

Table 19: Phase 3 Summary of ANOVA results
Geometric Mean (Average Deviation)

Parameter	Weak Foam	Fast Collapse Foam	Weak Foam but Stable	p-value
Alkalinity (mg/L)	4271 (985)	4306 (863)	3565 (816)	0.01
pH	7.59 (0.22)	7.31 (0.33)	6.21 (0.99)	<0.01
TKN (ppm)	1095 (263)	1249 (265)	943 (260)	0.147
TP (ppm)	279 (45)	277 (38)	447 (231)	<0.01
%TS	1.90 (0.43)	1.67 (0.76)	2.54 (0.91)	<0.01
%VS	1.43 (1.08)	1.44 (2.69)	1.90 (0.84)	<0.01
Alka-Seltzer (mL)	337 (88)	395 (80)	257 (114)	<0.01
Aeration (mL)	100 (50)	125 (45)	100 (30)	0.540
Total VFA (mg/L)	187 (240)	574 (1631)	955 (2431)	<0.01
Filament Count	26.2 (9.4)	22.6 (8.1)	17.5 (12.9)	0.408
Numerical Rating	3.63 (0.6)	3.40 (0.5)	3.49 (0.5)	0.500
Half-Life (min:sec)	1.22 (1.01)	1.47 (0.98)	2.17 (2.30)	0.024

11/29/10  12/13/10

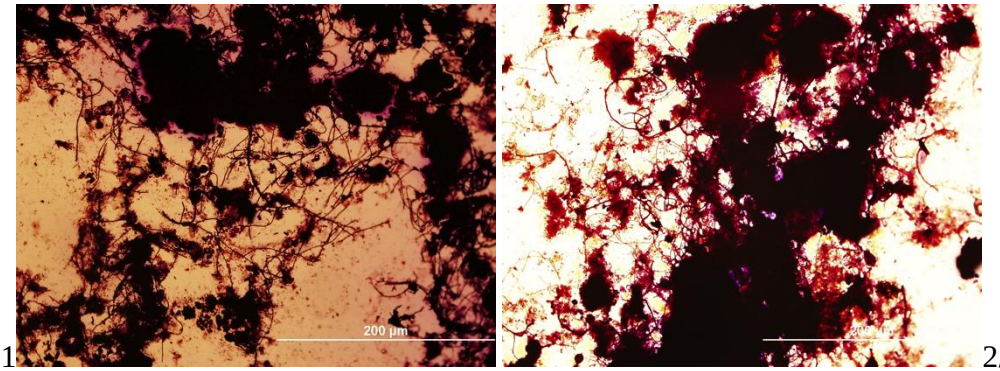
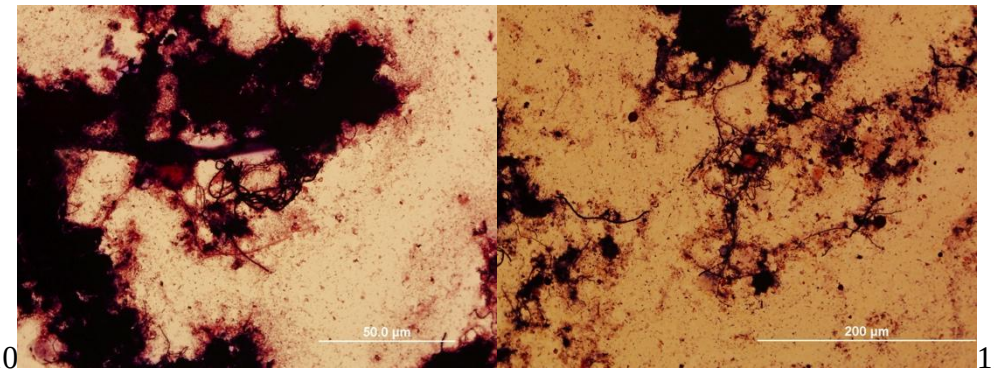
1/19/11  2/16/11

Figure 59: Phase 3 Gram Stain Analysis of Feed (50/50 WAS/Primary Sludge)

11/29/10  12/13/10

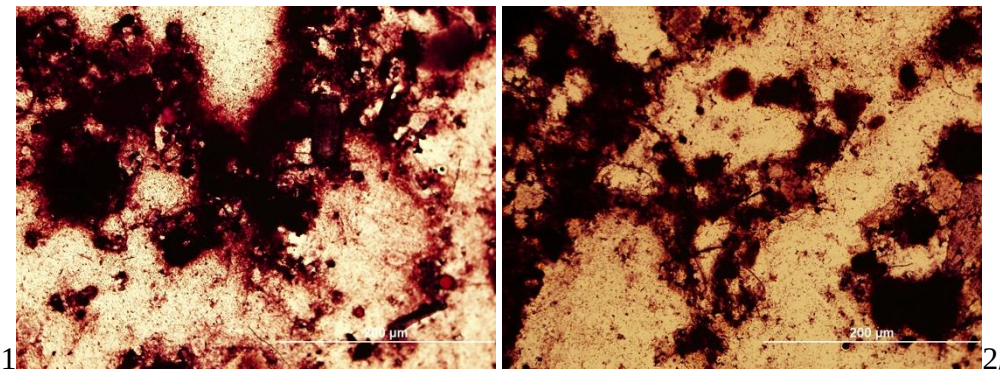
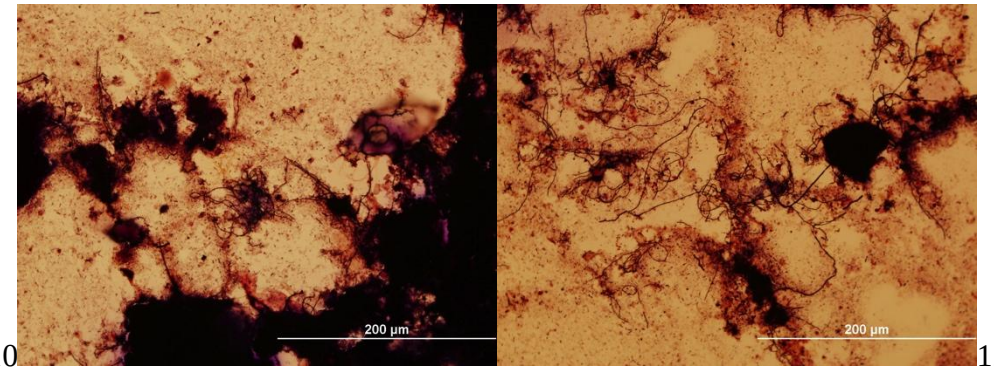
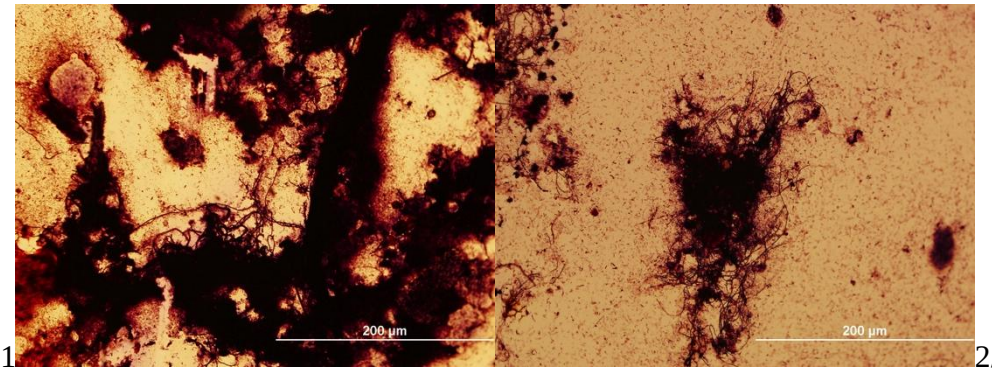
1/19/11  2/16/11

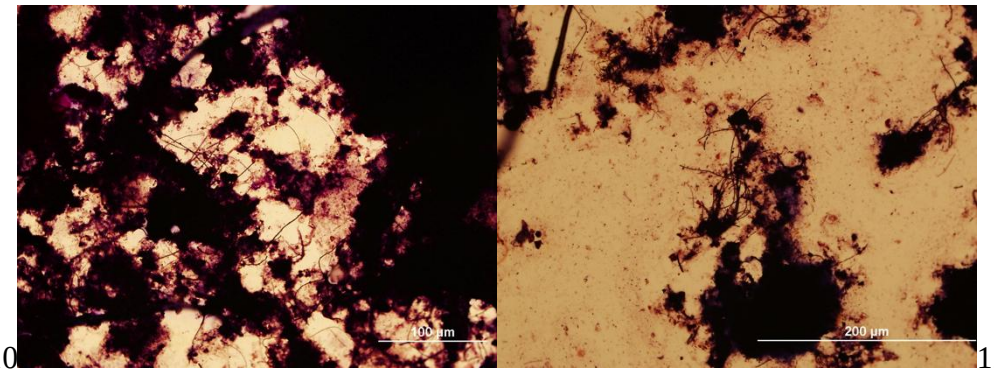
Figure 60: Phase 3 Gram Stain Analysis of Acid Phase Mesophilic Digester

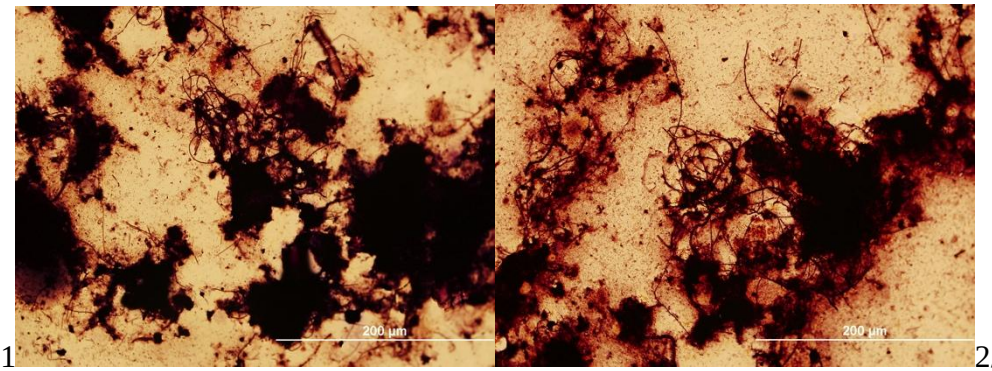
11/29/10  12/13/10



1/19/11 2/16/11

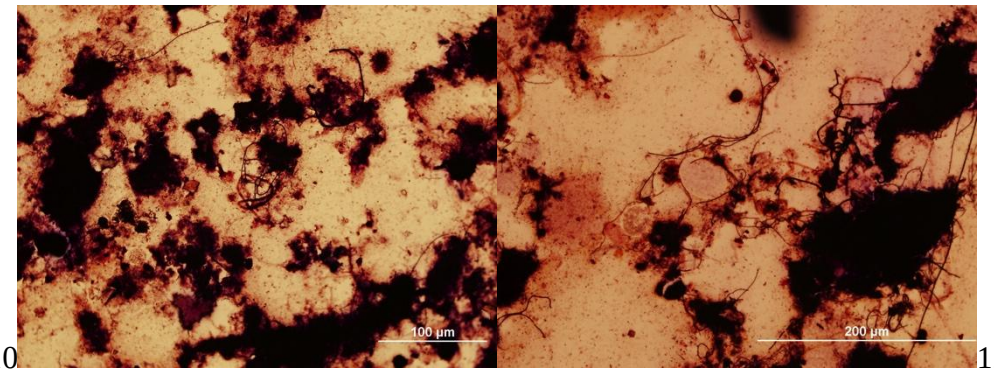
Figure 61: Phase 3 Gram Stain Analysis of Acid Phase Digester at 46ºC

11/29/10  12/13/10

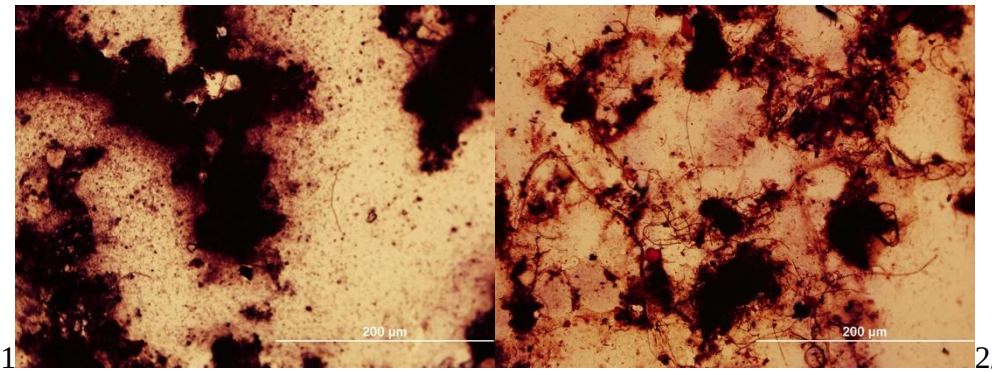


1/19/11 2/16/11

Figure 62: Phase 3 Gram Stain Analysis of Control Digester (Mesophilic)

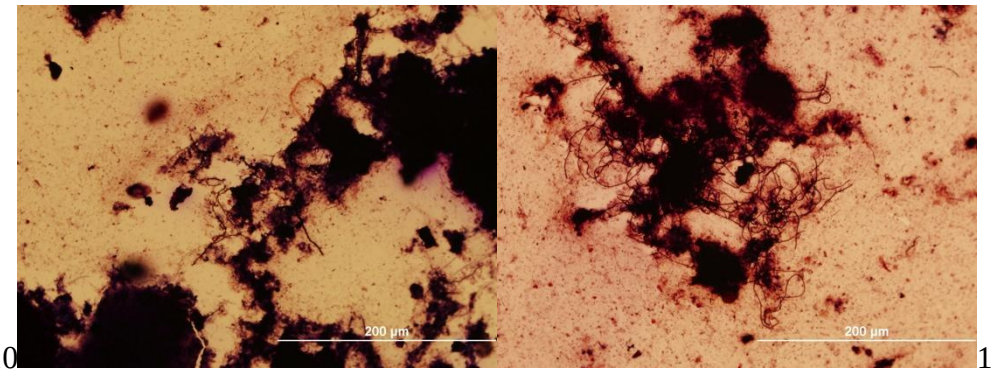
11/29/10 

12/13/10

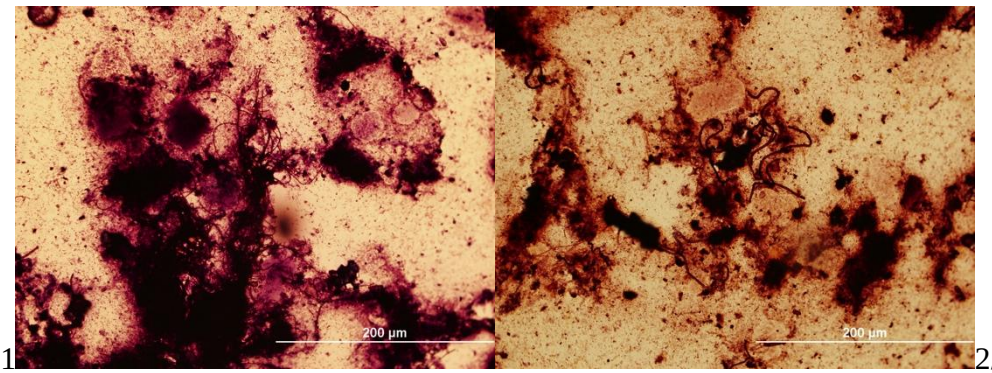
1/19/11 

2/16/11

Figure 63: Phase 3 Gram Stain Analysis of Mesophilic Acid Phase-Thermophilic Digester

11/29/10 

12/13/10

1/19/11 

2/16/11

Figure 64: Phase 3 Gram Stain Analysis of Acid Phase (46ºC)-Thermophilic Digester

APPENDIX D: Phase 4

Table 20: Phase 4 Summary Table of Foaming Results Over Time

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
3/4/11				
Feed (50/50 WAS/Primary)	50	1:20	-	-
Feed (50/50 p-released WAS/Primary)	95	3:00	-	-
Acid Phase dig	150	5:00	-	-
Acid phase dig (p-released WAS)	200	3:00	-	-
Dig Control	280	1:20	95	155
Dig Acid phase-Thermo	350	1:20	80	115
Dig Acid phase (p-released WAS)-Thermo	400	2:00	-	-
3/9/11				
Feed (50/50 WAS/Primary)	50	3:00	-	-
Feed (50/50 p-released WAS/Primary)	90	2:00	-	-
Acid Phase dig	160	4:00	-	-
Acid phase dig (p-released WAS)	180	6:20	-	-
Dig Control	280	1:20	70	130
Dig Acid phase-Thermo	340	1:40	90	140
Dig Acid phase (p-released WAS)-Thermo	400	2:40	110	175
3/16/11				
Feed (50/50 WAS/Primary)	55	3:40	-	-
Feed (50/50 p-released WAS/Primary)	95	5:00	-	-
Acid Phase dig	160	8:20	-	-
Acid phase dig (p-released WAS)	190	30:00	-	-
Dig Control	260	1:20	90	130
Dig Acid phase-Thermo	410	1:00	100	130
Dig Acid phase (p-released WAS)-Thermo	450	1:00	105	125
3/23/11				
Feed (50/50 WAS/Primary)	50	3:00	-	-
Feed (50/50 p-released WAS/Primary)	90	2:20	-	-
Acid Phase dig	180	8:00	-	-
Acid phase dig (p-released WAS)	410	3:40	-	-
Dig Control	190	1:40	55	85
Dig Acid phase-Thermo	380	1:20	100	130
Dig Acid phase (p-released WAS)-Thermo	450	2:20	105	150
3/30/11				
Feed (50/50 WAS/Primary)	60	2:00	-	-
Feed (50/50 p-released WAS/Primary)	95	2:00	-	-
Acid Phase dig	250	7:00	-	-
Acid phase dig (p-released WAS)	350	2:00	-	-
Dig Control	310	2:00	70	95
Dig Acid phase-Thermo	350	2:00	90	125
Dig Acid phase (p-released WAS)-Thermo	390	2:00	100	160

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
4/6/11				
Feed (50/50 WAS/Primary)	80	2.67	-	-
Feed (50/50 p-released WAS/Primary)	120	2	-	-
Acid Phase dig	160	8.33	-	-
Acid phase dig (p-released WAS)	360	9.33	-	-
Dig Control	260	2	60	90
Dig Acid phase-Thermo	460	0.67	85	115
Dig Acid phase (p-released WAS)-Thermo	460	1.67	100	175
4/13/11				
Feed (50/50 WAS/Primary)	60	3.67	-	-
Feed (50/50 p-released WAS/Primary)	100	4	-	-
Acid Phase dig	190	5.67	-	-
Acid phase dig (p-released WAS)	190	7	-	-
Dig Control	290	1.33	50	65
Dig Acid phase-Thermo	410	1	90	125
Dig Acid phase (p-released WAS)-Thermo	440	1	100	115
4/17/11				
Feed (50/50 WAS/Primary)	60	3	-	-
Feed (50/50 p-released WAS/Primary)	95	2	-	-
Acid Phase dig	110	6	-	-
Acid phase dig (p-released WAS)	200	5	-	-
Dig Control	320	1.67	95	110
Dig Acid phase-Thermo	500	0.67	75	100
Dig Acid phase (p-released WAS)-Thermo	590	0.67	105	135
4/21/11				
Feed (50/50 WAS/Primary)	70	3	-	-
Feed (50/50 p-released WAS/Primary)	100	2.33	-	-
Acid Phase dig	240	4.33	-	-
Acid phase dig (p-released WAS)	320	6.67	-	-
Dig Control	370	1	80	100
Dig Acid phase-Thermo	460	1.33	90	135
Dig Acid phase (p-released WAS)-Thermo	530	1.33	90	130
4/27/11				
Feed (50/50 WAS/Primary)	75	2.67	-	-
Feed (50/50 p-released WAS/Primary)	85	2.33	-	-
Acid Phase dig	160	7	-	-
Acid phase dig (p-released WAS)	230	5.67	-	-
Dig Control	280	1.33	50	70
Dig Acid phase-Thermo	450	0.67	60	100
Dig Acid phase (p-released WAS)-Thermo	510	0.67	85	105

Sample	Foam Volume Max. Alka-Seltzer (mL)	Foam Half life Alka-Seltzer (min:sec)	Foam Aeration V5-Vo (mL)	Foam Aeration Vmax-Vo (mL)
5/4/11				
Feed (50/50 WAS/Primary)	75	3	-	-
Feed (50/50 p-released WAS/Primary)	90	2	-	-
Acid Phase dig	200	6	-	-
Acid phase dig (p-released WAS)	370	5	-	-
Dig Control	460	1	55	90
Dig Acid phase-Thermo	250	4.67	110	140
Dig Acid phase (p-released WAS)-Thermo	560	2.67	160	200

Table 21: Phase 4 Summary Table of Chemistry Results Over Time

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
3/9/11						
Feed (50/50 WAS/Primary)	960	6	-	-	3.64	-
Feed (50/50 p-released WAS/Primary)	-	-	-	-	-	-
Acid Phase dig	2760	5.5	-	-	3.09	-
Acid phase dig (p-released WAS)	-	-	-	-	-	-
Dig Control	4080	7.4	-	-	1.81	-
Dig Acid phase-Thermo	5130	7.7	-	-	1.23	-
Dig Acid phase (p-released WAS)-Thermo	-	-	-	-	-	-
3/16/11						
Feed (50/50 WAS/Primary)	823	6.2	2320	885	3.78	576.5
Feed (50/50 p-released WAS/Primary)	960	5.9	2080	707	3.62	721.5
Acid Phase dig	2780	5.5	720	561	2.94	5288.3
Acid phase dig (p-released WAS)	2470	5.5	564	367	3.21	4427.7
Dig Control	4120	7.5	2230	514	1.74	321.3
Dig Acid phase-Thermo	5080	7.8	1400	271	1.62	825.0
Dig Acid phase (p-released WAS)-Thermo	5090	7.7	1300	210	1.56	645.1

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
3/30/11						
Feed (50/50 WAS/Primary)	666	6.5	1990	818	2.68	354.1
Feed (50/50 p-released WAS/Primary)	794	6	1750	632	2.78	584.1
Acid Phase dig	2430	5.3	571	505	2.86	4758.0
Acid phase dig (p-released WAS)	2060	5.4	444	315	2.44	3608.1
Dig Control	3870	7.5	826	203	1.62	429.7
Dig Acid phase-Thermo	4950	7.6	1020	189	1.56	610.4
Dig Acid phase (p-released WAS)-Thermo	4640	7.7	930	128	1.67	534.3
4/6/11						
Feed (50/50 WAS/Primary)	829	6.3	-	-	4.01	-
Feed (50/50 p-released WAS/Primary)	926	6	-	-	3.95	-
Acid Phase dig	2540	5.1	-	-	3.19	-
Acid phase dig (p-released WAS)	2540	5.4	-	-	2.95	-
Dig Control	3880	7.3	-	-	1.52	-
Dig Acid phase-Thermo	4900	7.7	-	-	1.63	-
Dig Acid phase (p-released WAS)-Thermo	4430	7.6	-	-	1.55	-
4/13/11						
Feed (50/50 WAS/Primary)	790	6.2	2100	839	3.39	114.5
Feed (50/50 p-released WAS/Primary)	926	5.9	2120	749	3.38	372.3
Acid Phase dig	2660	5.5	1440	230	2.93	3014.7
Acid phase dig (p-released WAS)	2300	5.5	1070	123	2.72	255.3
Dig Control	3700	7.3	1040	226	1.57	332.8
Dig Acid phase-Thermo	5090	7.8	671	473	1.56	446.4
Dig Acid phase (p-released WAS)-Thermo	4510	7.8	575	377	1.49	476.0
4/21/11						
Feed (50/50 WAS/Primary)	878	6.1	-	-	3.73	-
Feed (50/50 p-released WAS/Primary)	1020	6	-	-	3.66	-
Acid Phase dig	2540	5.4	-	-	3.19	-
Acid phase dig (p-released WAS)	2350	5.5	-	-	2.92	-
Dig Control	3860	7.3	-	-	1.46	-
Dig Acid phase-Thermo	4760	7.6	-	-	1.29	-
Dig Acid phase (p-released WAS)-Thermo	4630	7.6	-	-	1.48	-

Sample	Alkalinity (ppm)	pH	TKN (ppm)	TP (ppm)	% VS	Total VFA (mg/L)
4/27/11						
Feed (50/50 WAS/Primary)	916	6.2	2150	829	3.49	123.9
Feed (50/50 p-released WAS/Primary)	1014	6	2280	786	3.82	129.3
Acid Phase dig	2680	5.4	624	429	3.17	4426.9
Acid phase dig (p-released WAS)	2630	5.6	567	326	2.98	2550.7
Dig Control	3660	7.3	934	188	1.52	320.0
Dig Acid phase-Thermo	4540	7.7	1230	182	1.35	485.7
Dig Acid phase (p-released WAS)-Thermo	4340	7.6	1020	112	1.46	545.7
5/4/11						
Feed (50/50 WAS/Primary)	840	6.2	-	-	3.12	-
Feed (50/50 p-released WAS/Primary)	1030	5.9	-	-	3.75	-
Acid Phase dig	2940	5.4	-	-	3.25	-
Acid phase dig (p-released WAS)	2890	5.5	-	-	3.13	-
Dig Control	4040	7.3	-	-	1.63	-
Dig Acid phase-Thermo	4910	7.6	-	-	1.74	-
Dig Acid phase (p-released WAS)-Thermo	4640	7.6	-	-	1.45	-

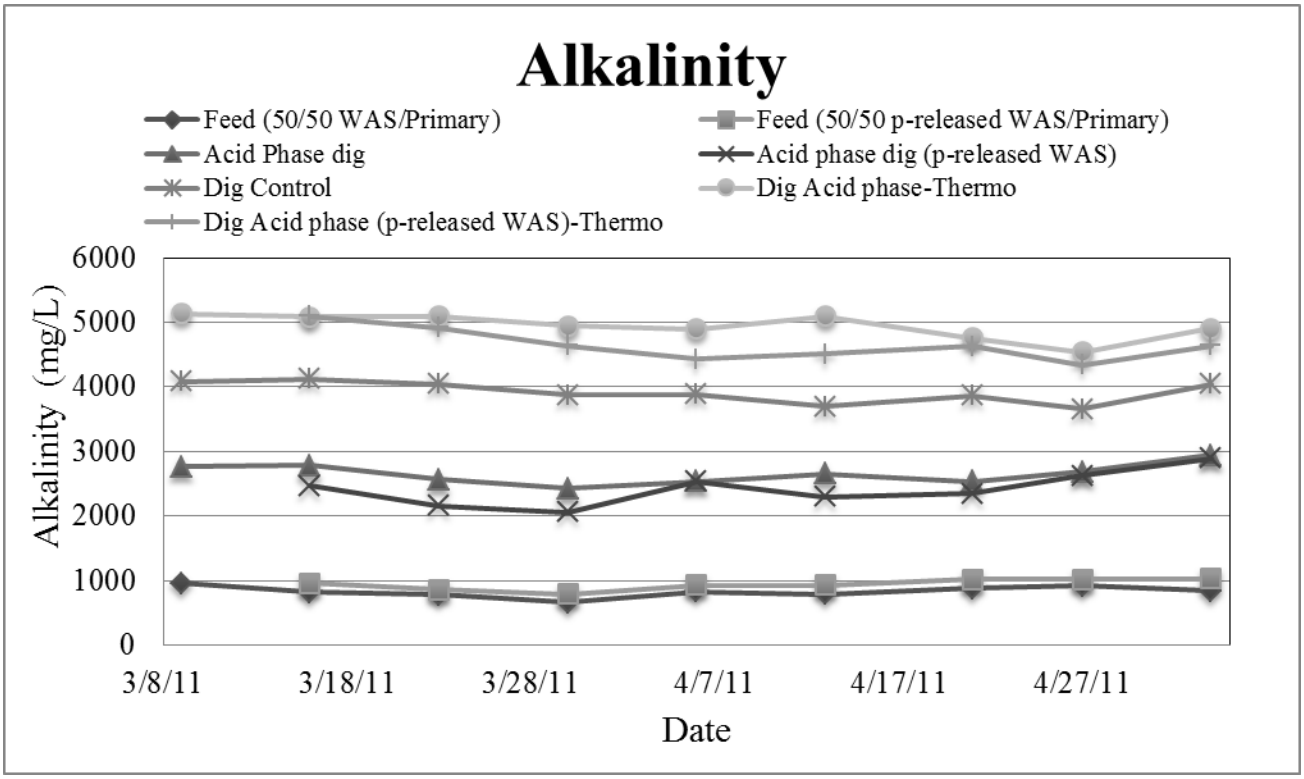


Figure 65: Phase 4 Alkalinity

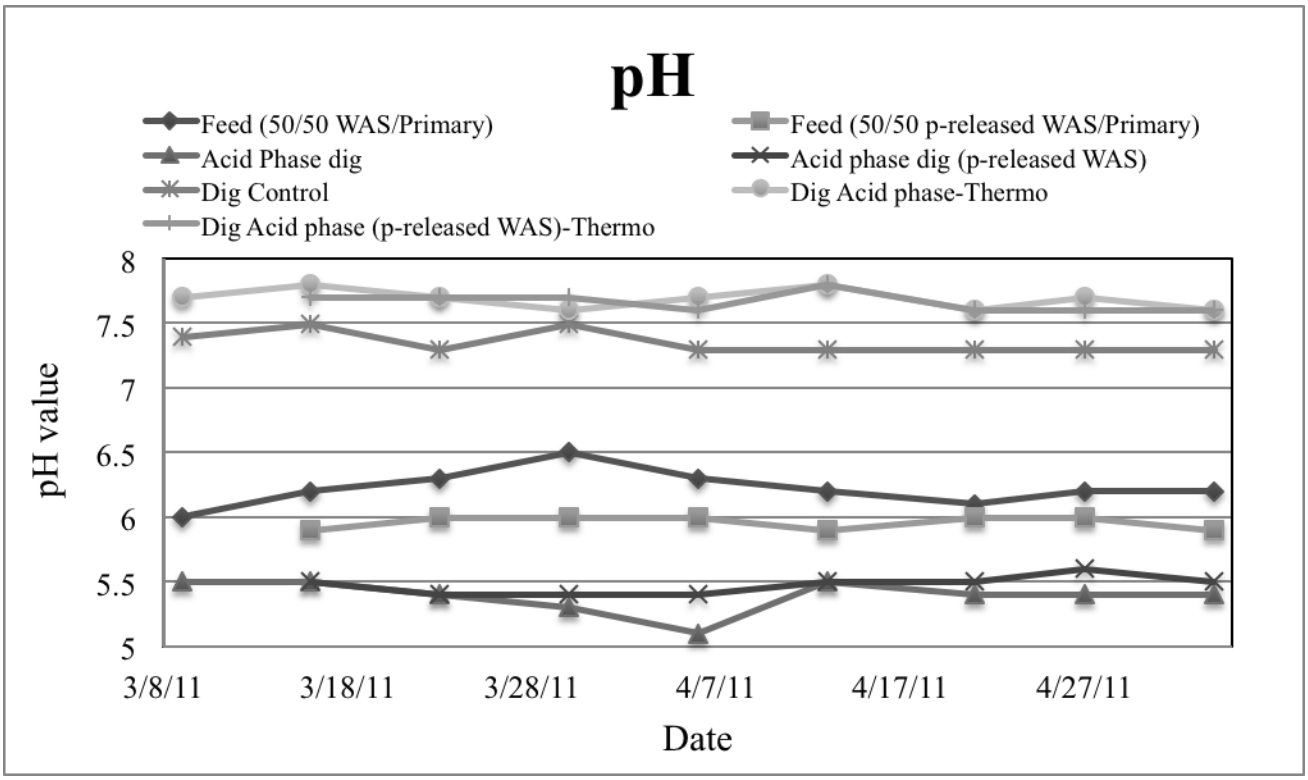


Figure 66: Phase 4 pH

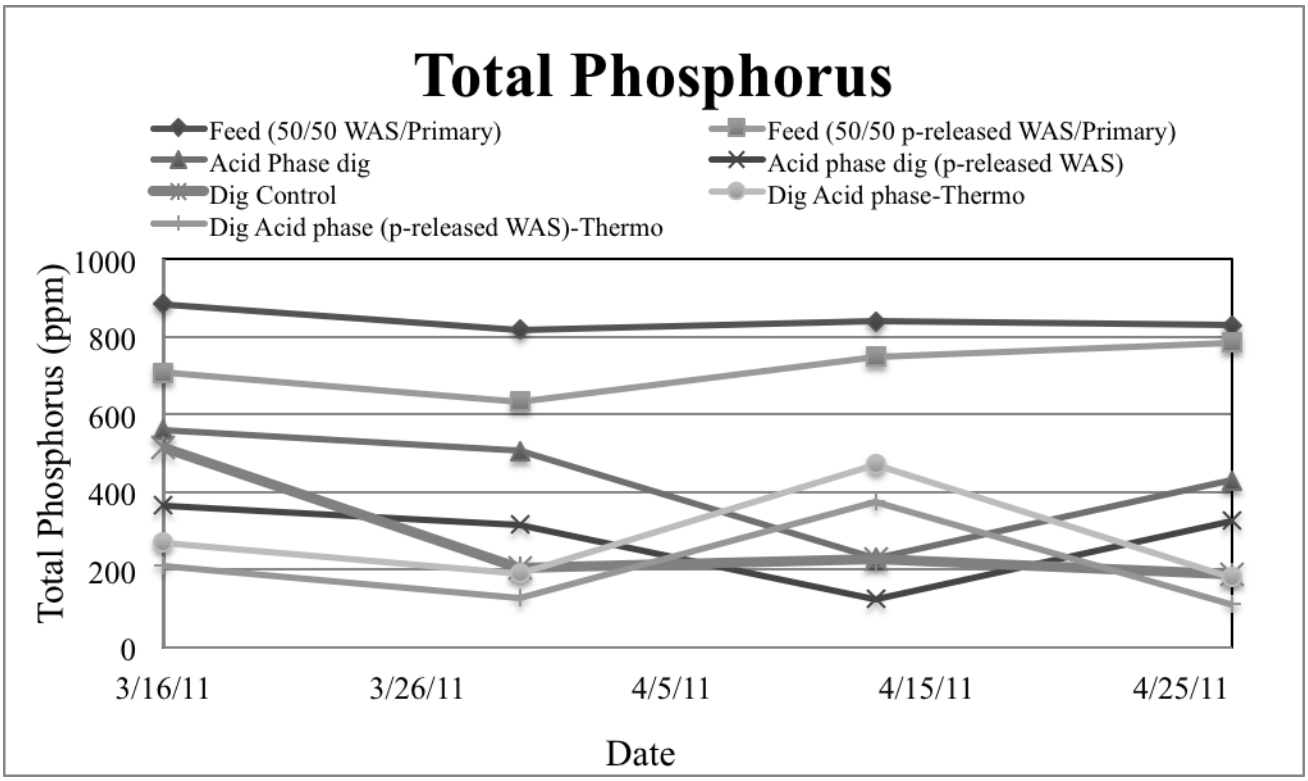


Figure 67: Phase 4 Total Phosphorus

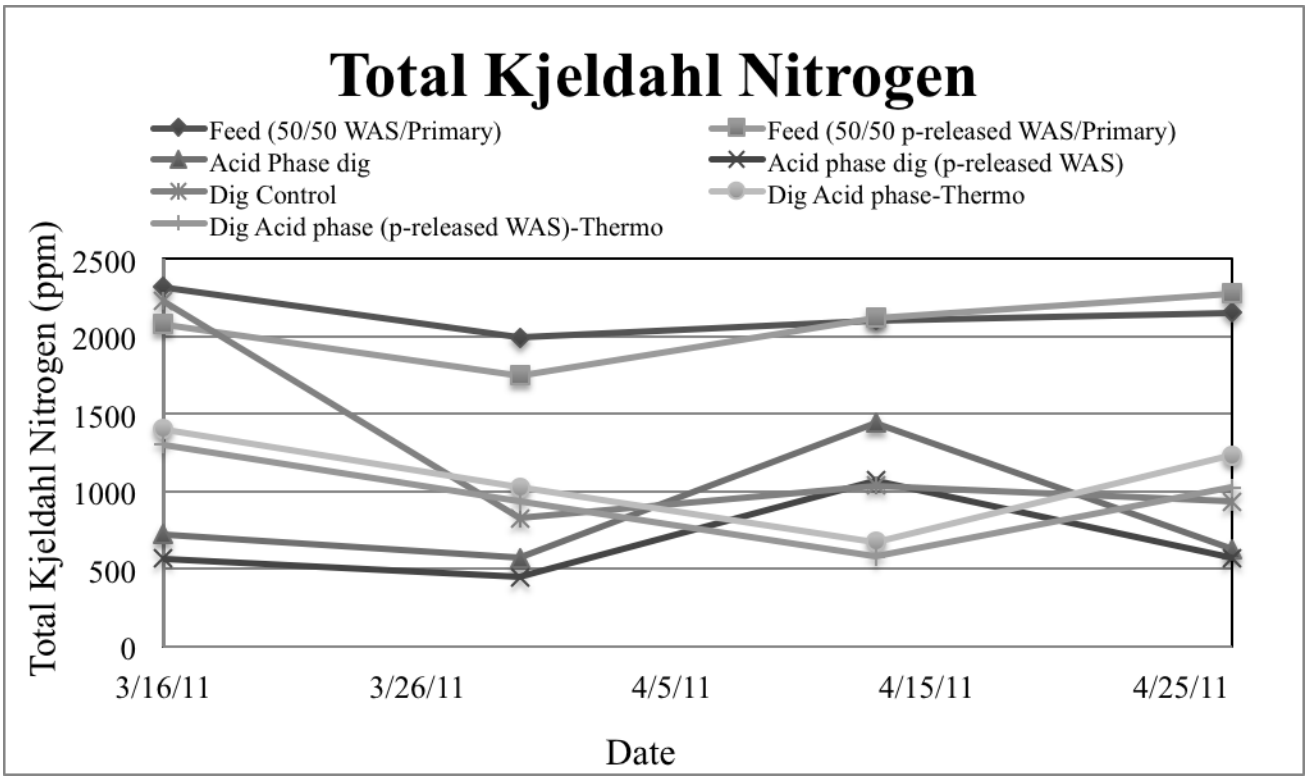


Figure 68: Phase 4 Total Kjeldahl Nitrogen

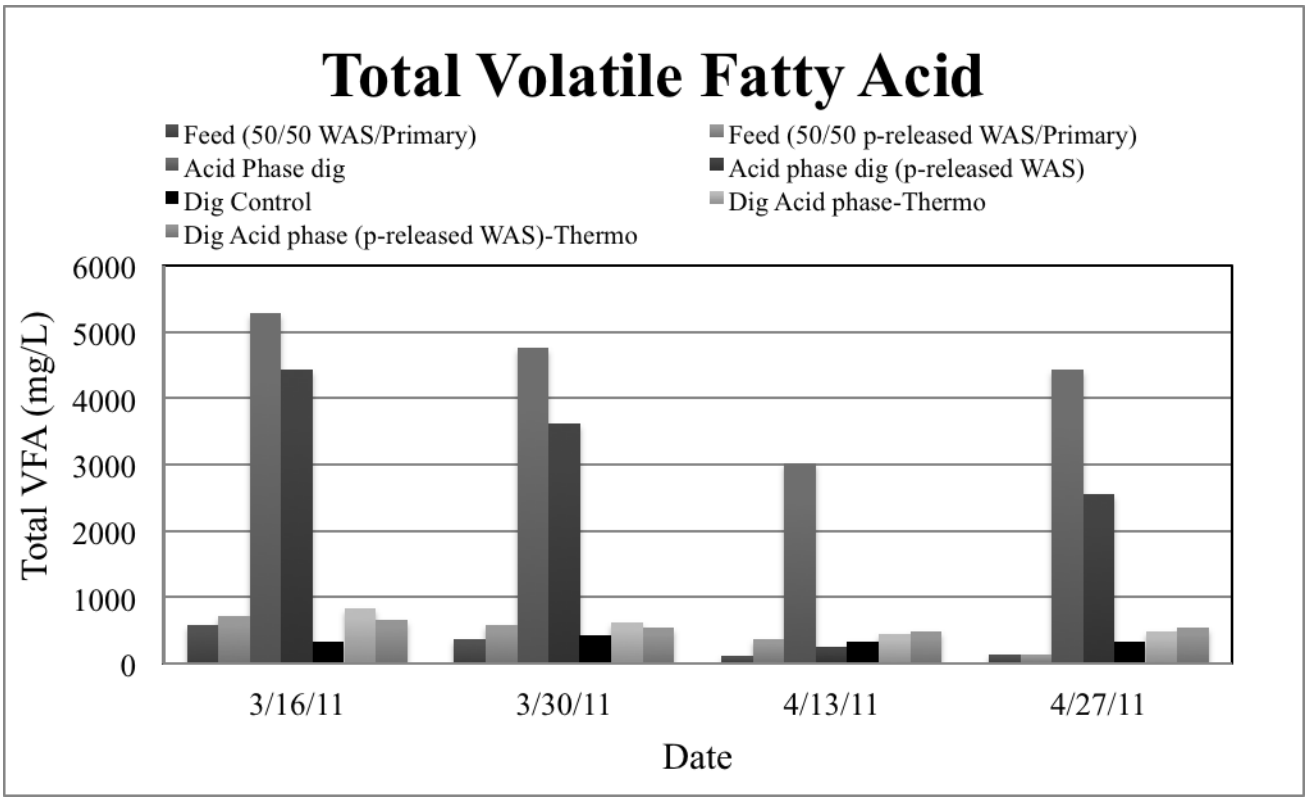


Figure 69: Phase 4 Total Volatile Fatty Acids

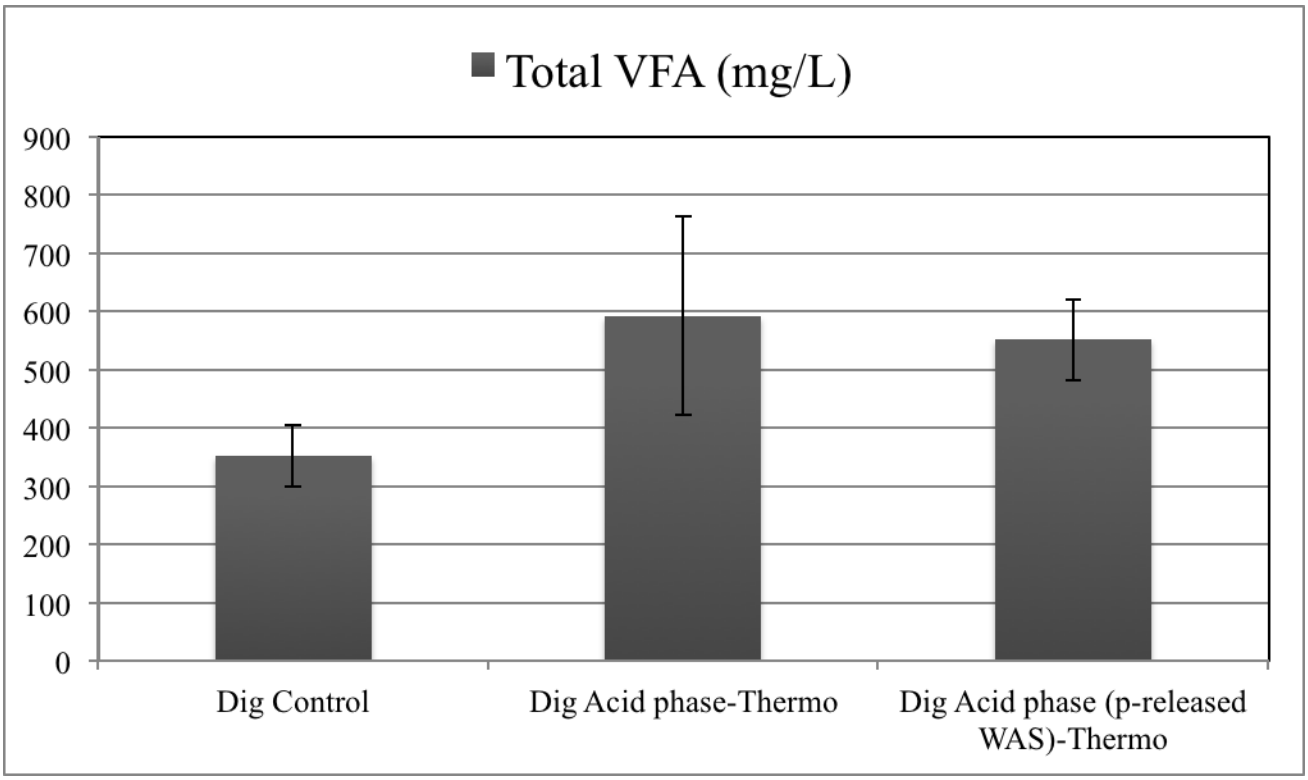


Figure 70: Phase 4 Total Volatile Fatty Acid

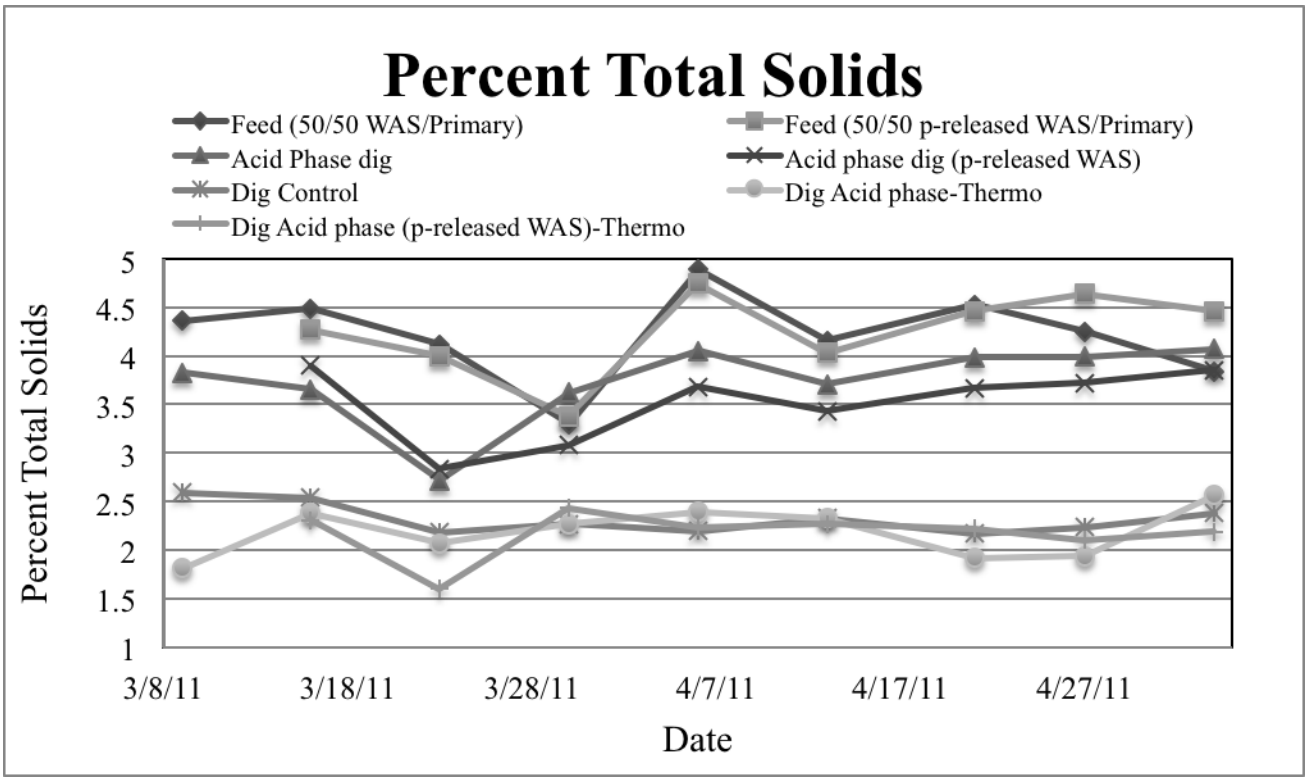


Figure 71: Phase 4 Percent Total Solids

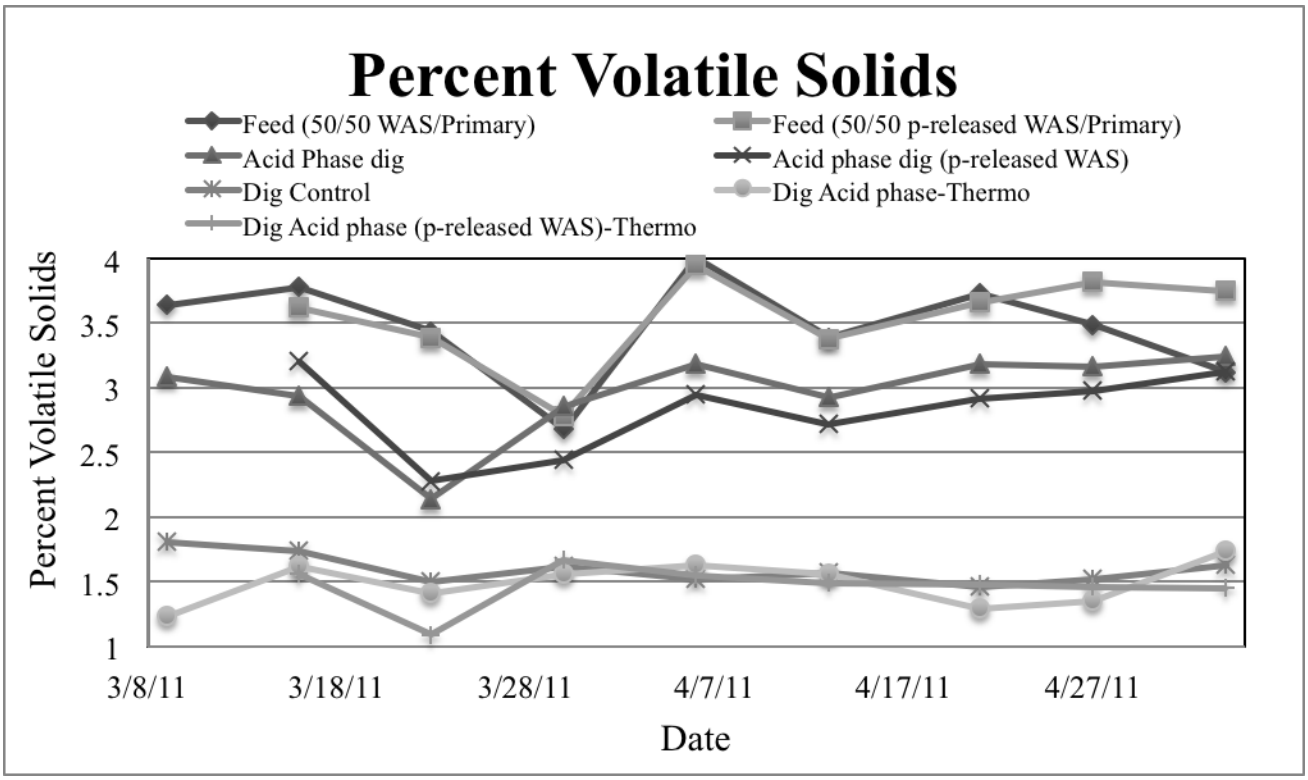


Figure 72: Phase 4 Percent Volatile Solids

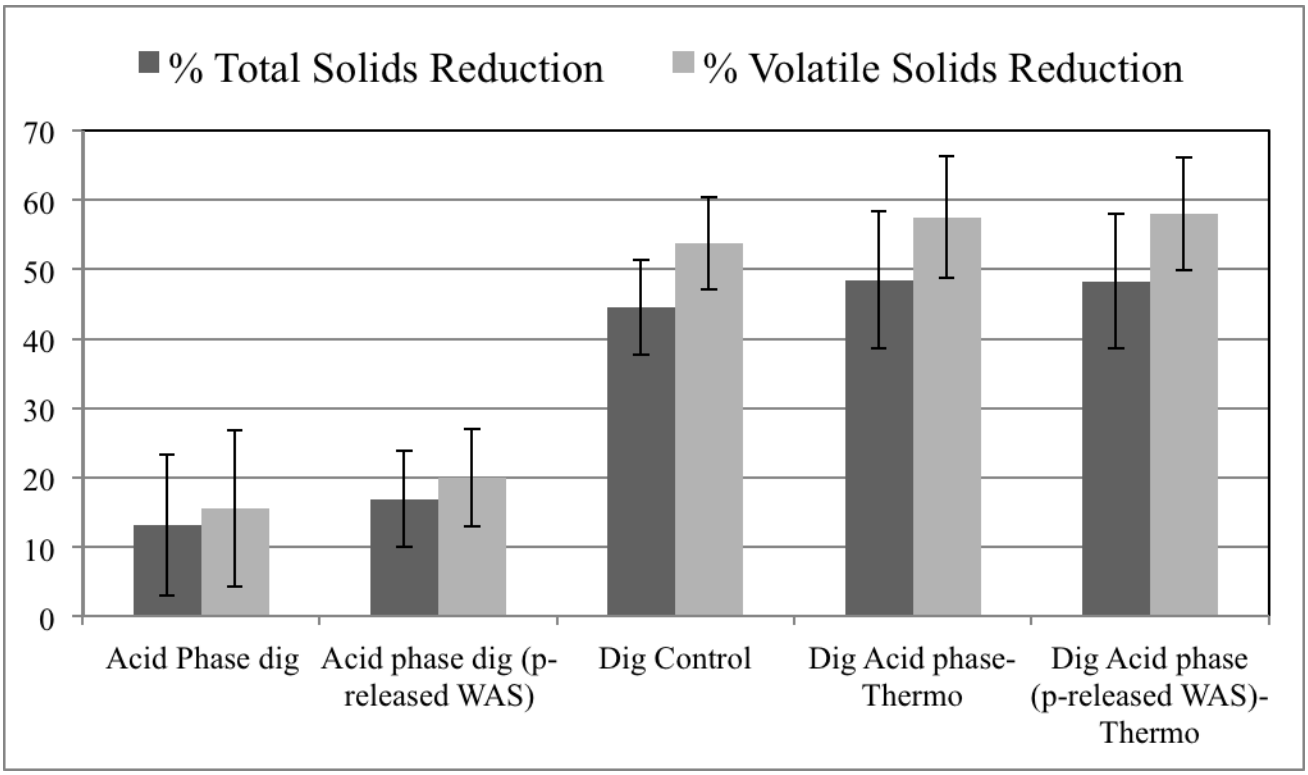


Figure 73: Phase 4 % Total Solids Reduction and % Volatile Solids Reduction

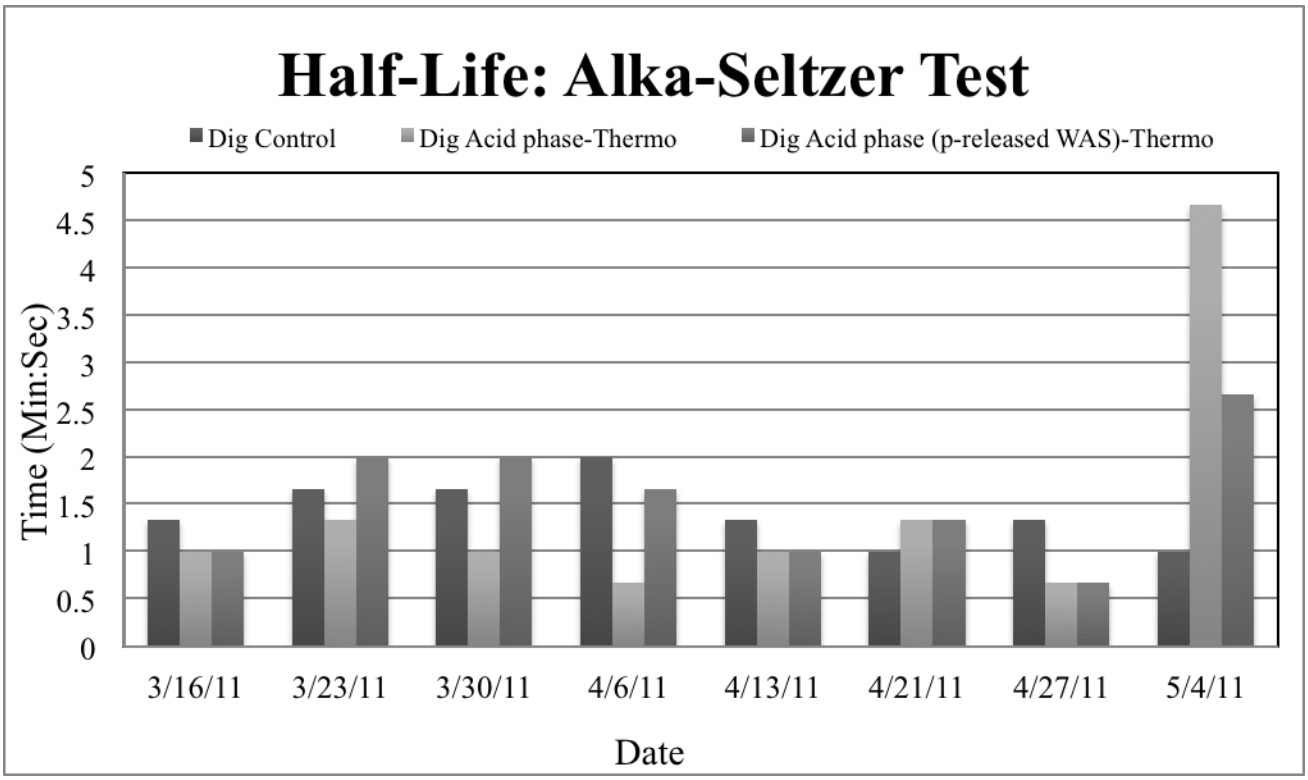


Figure 74: Phase 4 Half-Life: Alka-Seltzer Foaming Potential Test

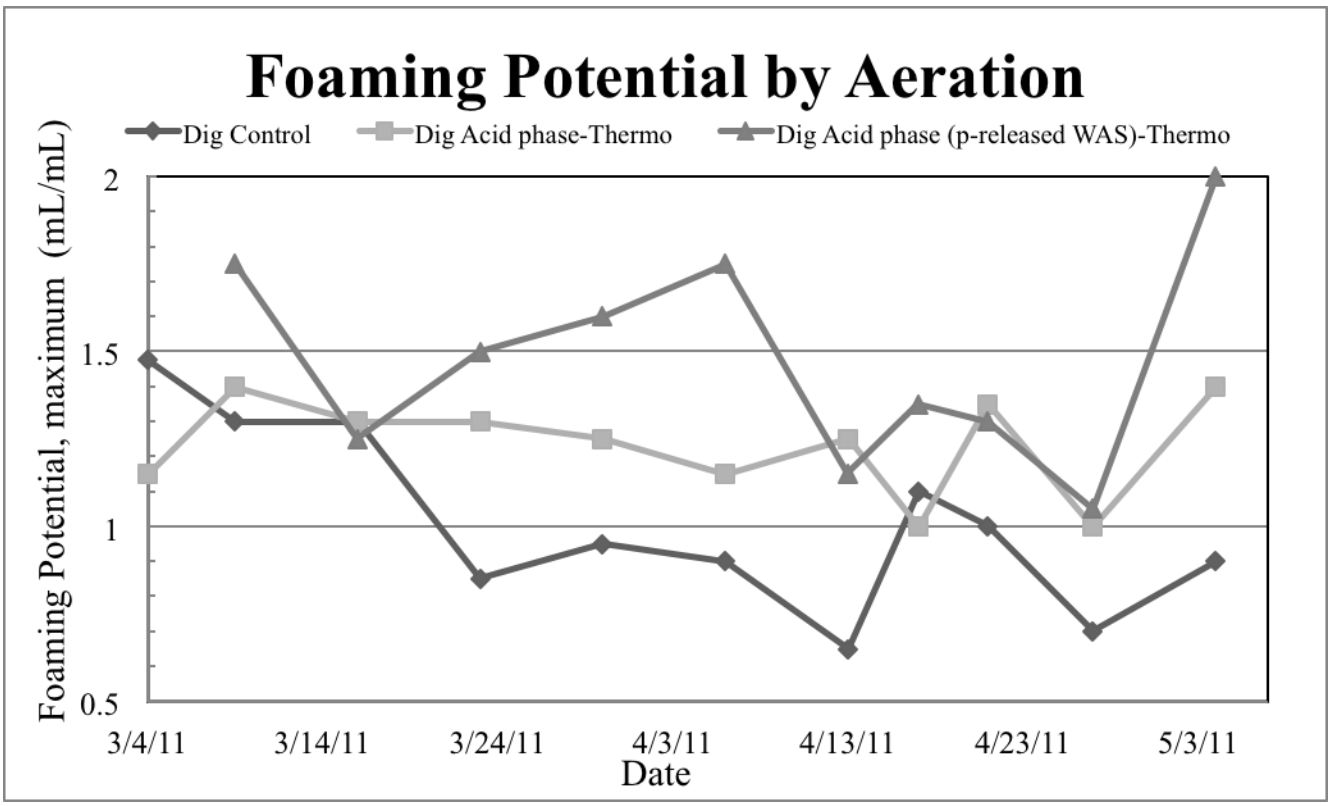


Figure 75: Phase 4 Foaming Potential by Aeration, Maximum Volume Observed

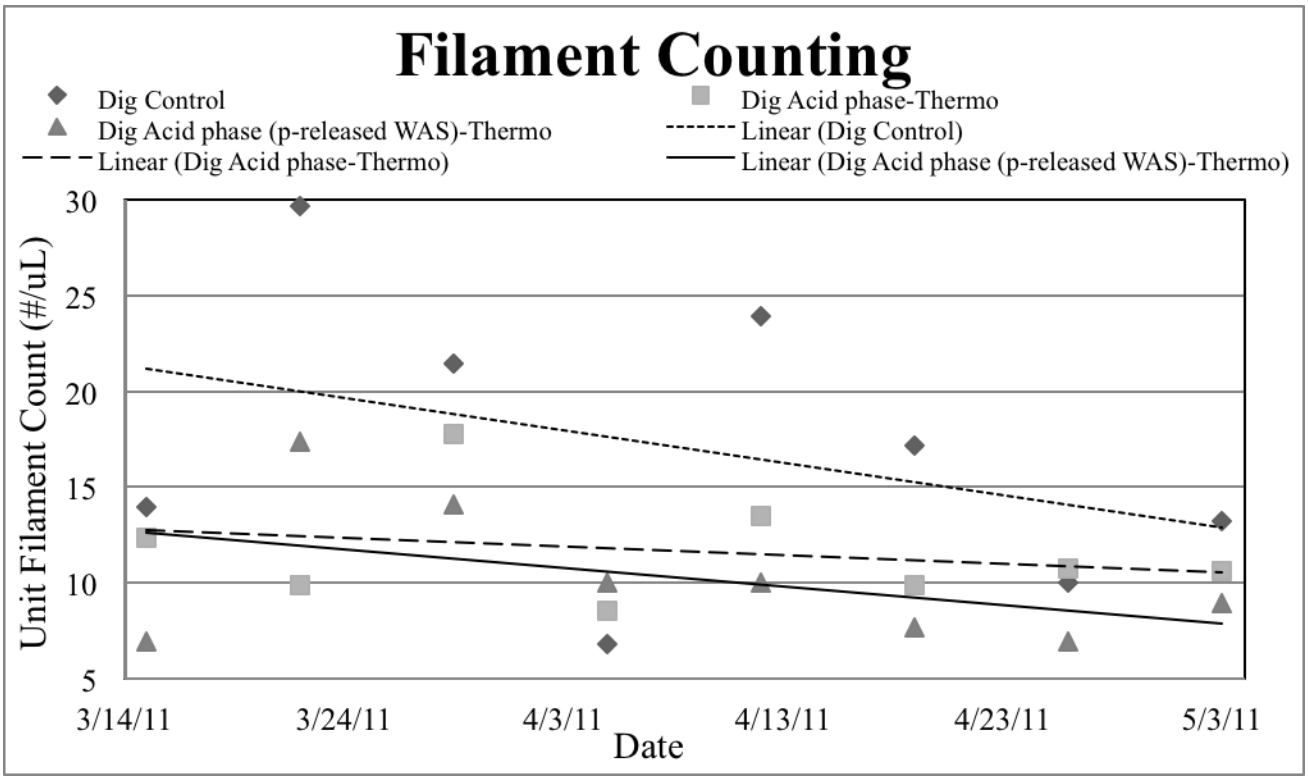


Figure 76: Phase 4 Filament Counting

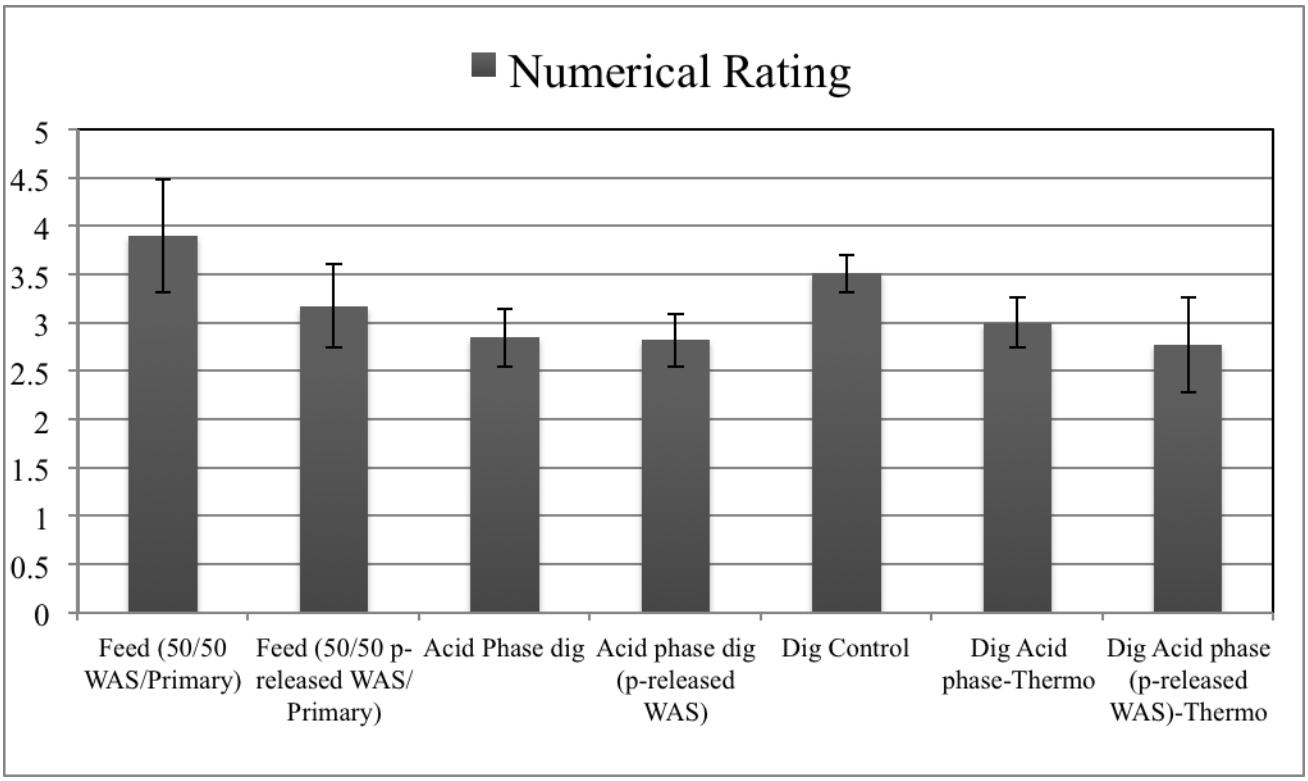


Figure 77: Phase 4 Filament Numerical Rating

Table 22: Phase 4 Summary of Correlation Coefficents

		Alkalinity	pH	TKN	TP	% Total Solids	VA-Acetic	VA-Iso-Butyric	VA-Iso-Valeric	VA-N Butyric	VA-N Valeric	VA-Propionic	VA-Sec Valeric	Total VFA	% Volatile Solids	Alka Seltzer	Half-life	VFA/Alkalinity	FP (max)	FP (5 min)	Numerical Rating	Filament Count
Alkalinity (ppm)		1																				
	p-value																					
pH	r^2*-value*	0.56	1																			
	p-value	<0.01																				
TKN	r^2*-value*	0.28	0.00	1																		
	p-value	<0.01	0.91																			
TP (ppm)	r^2*-value*	0.61	0.18	0.52	1																	
	p-value	<0.01	0.02	<0.01																		
% Total Solids	r^2*-value*	0.79	0.64	0.22	0.61	1																
	p-value	<0.01	<0.01	<0.01	<0.01																	
VA-Acetic (mg/L)	r^2*-value*	0.02	0.41	0.22	0.00	0.12	1															
	p-value	0.40	<0.01	<0.01	0.76	0.06																
VA-Iso-Butyric (mg/L)	r^2*-value*	0.02	0.49	0.23	0.00	0.12	0.76	1														
	p-value	0.44	<0.01	<0.01	0.80	0.05	<0.01															
VA-Iso-Valeric (mg/L)	r^2*-value*	0.02	0.47	0.22	0.00	0.12	0.78	0.98	1													
	p-value	0.50	<0.01	<0.01	0.81	0.05	<0.01	<0.01														
VA-N Butyric (mg/L)	r^2*-value*	0.02	0.47	0.23	0.00	0.12	0.86	0.97	0.97	1												
	p-value	0.49	<0.01	<0.01	0.82	0.05	<0.01	<0.01	<0.01													
VA-N-Valeric (mg/L)	r^2*-value*	0.01	0.46	0.24	0.00	0.11	0.81	0.98	0.99	0.99	1											
	p-value	0.55	<0.01	<0.01	0.76	0.06	<0.01	<0.01	<0.01	<0.01												
VA-Propionic (mg/L)	r^2*-value*	0.04	0.50	0.19	0.01	0.16	0.94	0.89	0.91	0.95	0.92	1										
	p-value	0.29	<0.01	0.01	0.69	0.02	<0.01	<0.01	<0.01	<0.01	<0.01											
VA-Sec Valeric (mg/L)	r^2*-value*	0.01	0.43	0.22	0.00	0.11	0.70	0.96	0.98	0.93	0.97	0.86	1									
	p-value	0.56	<0.01	<0.01	0.77	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01										
Total VFA (mg/L)	r^2*-value*	0.02	0.48	0.22	0.00	0.13	0.95	0.91	0.93	0.96	0.94	0.99	0.86	1								
	p-value	0.39	<0.01	<0.01	0.90	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01									
% Volatile Solids	r^2*-value*	0.83	0.66	0.22	0.61	0.99	0.12	0.12	0.11	0.12	0.12	0.11	0.16	0.10	0.13							
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	0.05	0.06	0.06	0.07	0.02	0.08	0.04								
Foaming potential alka seltzer		0.73	0.39	0.33	0.64	0.68	0.04	0.04	0.04	0.03	0.03	0.06	0.03	0.02	0.70	1						
	p-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.31	0.29	0.29	0.31	0.34	0.17	0.32	0.49	<0.01							
Half-life	r^2*-value*	0.05	0.31	0.08	0.00	0.11	0.38	0.32	0.30	0.37	0.30	0.36	0.21	0.35	0.12	0.05	1					
	p-value	0.07	<0.01	0.12	0.93	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.08						
VFA/Alkalinity	r^2*-value*	0.11	0.60	0.12	0.03	0.24	0.92	0.82	0.82	0.87	0.83	0.95	0.74	0.94	0.24	0.11	0.36	1				
	p-value	0.07	<0.01	0.06	0.32	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	<0.01					
Foaming Potential Aeration (max)		0.38	0.34	0.04	0.04	0.01	0.26	0.16	0.17	0.12	0.11	0.05	0.08	0.00	0.01	0.26	0.16	0.13	1			
	p-value	<0.01	<0.01	0.53	0.54	0.80	0.09	0.19	0.18	0.27	0.29	0.47	0.38	0.08	0.64	0.01	0.07	0.24				
Foaming Potential Aeration (5 min)		0.42	0.38	0.01	0.07	0.00	0.29	0.15	0.19	0.14	0.13	0.13	0.00	0.41	0.01	0.25	0.15	0.24	0.83	1		
	p-value	<0.01	<0.01	0.75	0.42	0.98	0.07	0.21	0.16	0.24	0.25	0.26	0.35	0.03	0.70	0.01	0.06	0.11	<0.01			
Numerical Rating	r^2*-value*	0.12	0.00	0.22	0.38	0.03	0.03	0.09	0.08	0.07	0.08	0.03	0.08	0.08	0.01	0.16	0.02	0.00	0.37	0.32	1	
	p-value	<0.01	0.70	0.01	<0.01	0.22	0.37	0.12	0.14	0.17	0.15	0.38	0.15	0.16	0.19	<0.01	0.31	0.66	0.04	0.06		
Filament Count	r^2*-value*	0.14	0.16	0.00	0.00	0.00	0.00	0.07	0.02	0.07	0.05	0.00	0.00	0.10	0.00	0.31	0.00	0.06	0.12	0.15	0.18	1
	p-value	0.07	0.05	0.82	0.91	0.95	0.95	0.39	0.62	0.42	0.47	0.91	0.81	0.31	0.89	<0.01	0.93	0.45	0.10	0.06	0.17	

Yellow shade: <0.01

Red Shade: <0.05

Table 23: Phase 4 Summary of ANOVA Results
Geometric Mean (Standard Deviation)

Parameter	Weak Foam	Fast Collapse Foam	Weak Foam but Stable	p-value
Alkalinity (mg/L)	4271 (985)	4306 (863)	4406 (590)	<0.01
pH	7.59 (0.22)	7.31 (0.33)	7.40 (0.31)	<0.01
TKN (ppm)	1095 (263)	1249 (265)	1154 (215)	0.08
TP (ppm)	279 (45)	277 (38)	281 (43)	0.269
%TS	1.90 (0.43)	1.67 (0.76)	1.70 (0.64)	<0.01
%VS	1.43 (1.08)	1.44 (2.69)	1.08 (0.42)	<0.01
Alka-Seltzer (mL)	337 (88)	395 (80)	364 (100)	<0.01
Aeration (mL)	100 (50)	125 (45)	100 (30)	<0.01
Total VFA (mg/L)	187 (240)	574 (1631)	189 (955)	<0.01
Filament Count	26.2 (9.4)	22.6 (8.1)	17.5 (12.9)	<0.01
Numerical Rating	3.63 (0.6)	3.40 (0.5)	3.62 (0.7)	0.163

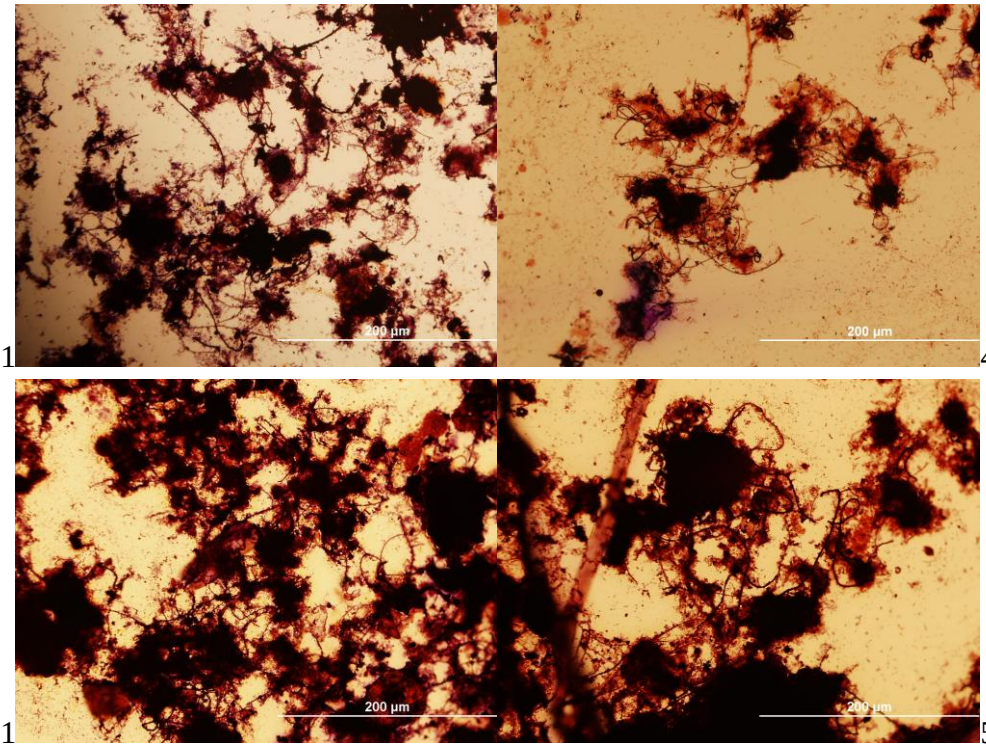


Figure 78: Phase 4 Gram Stain Analysis of 50/50 WAS/Primary Feed

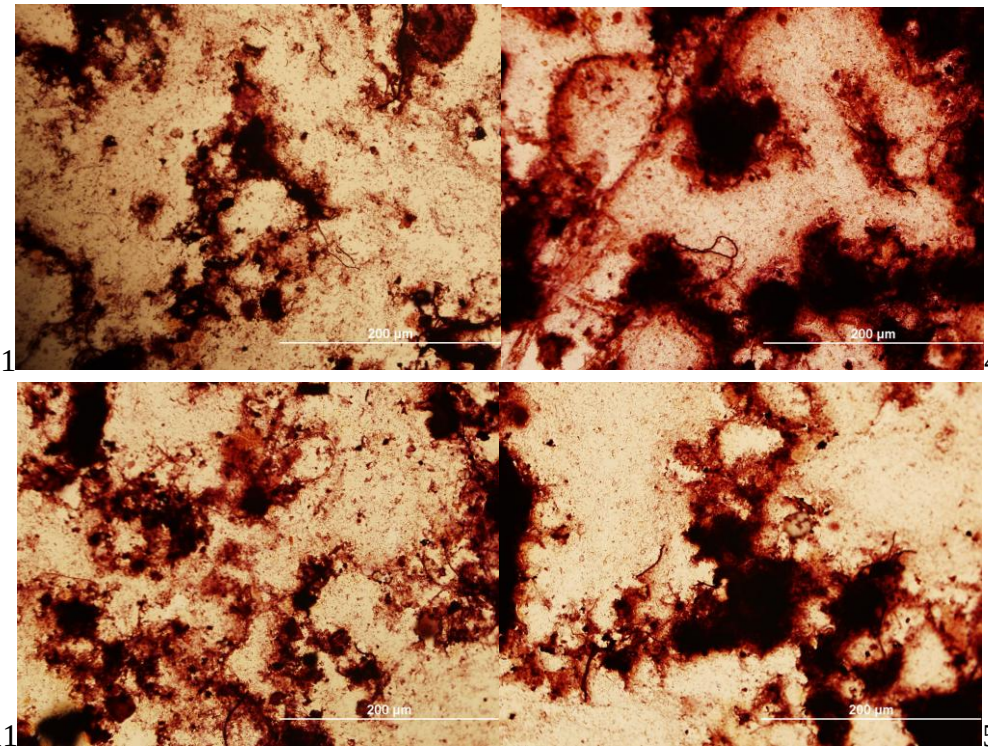
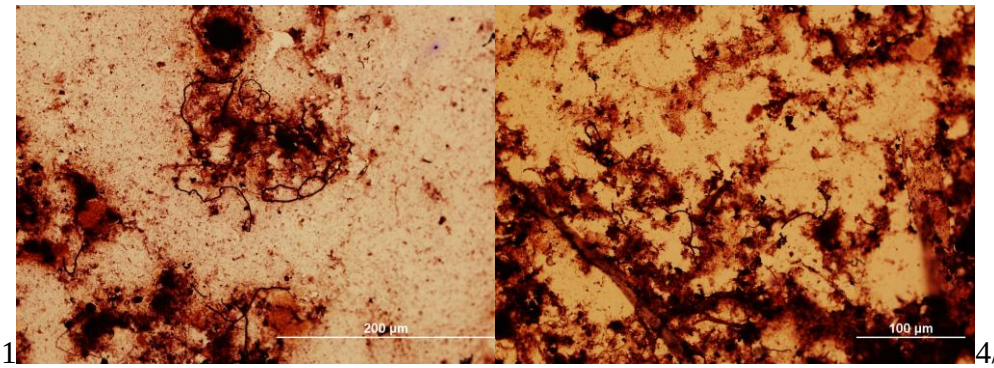
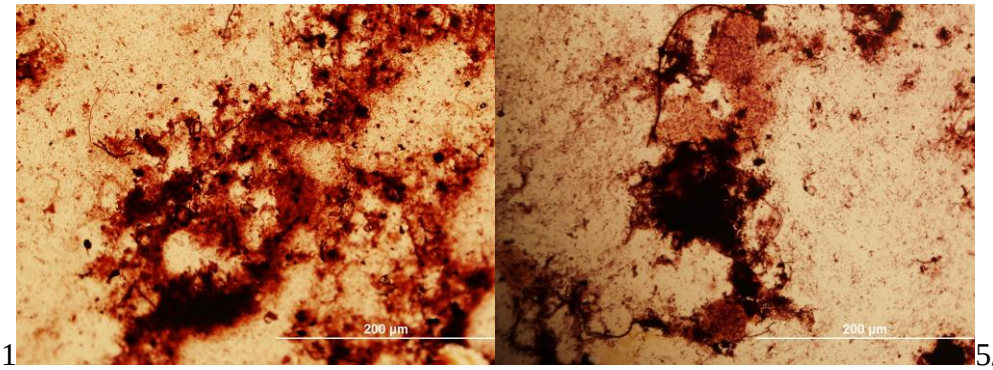


Figure 79: Phase 4 Gram Stain Analysis of Acid Phase Digester at 37ºC

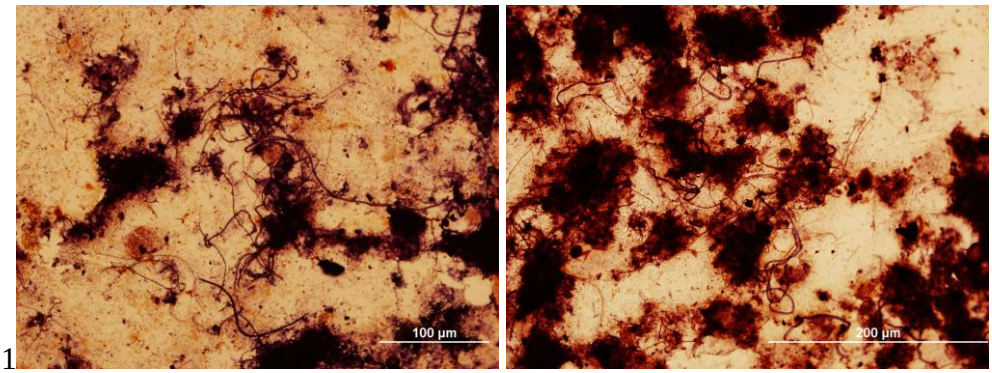
3/16/2011 

4/5/2011

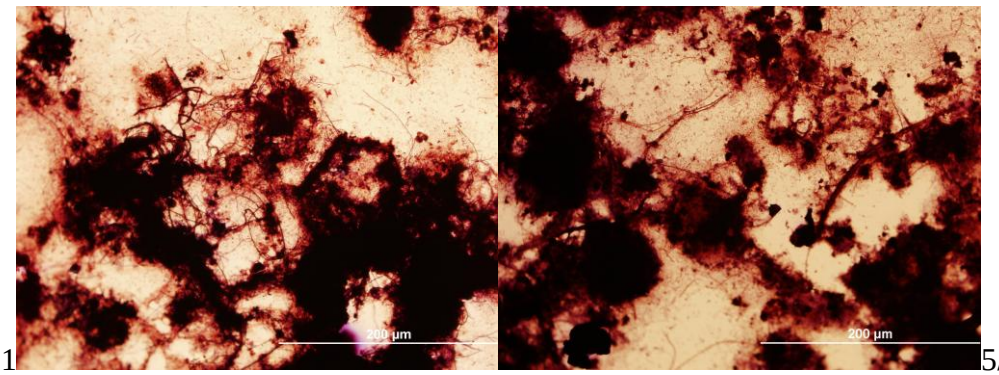


4/27/2011 5/3/2011

Figure 80: Phase 4 Gram Stain Analysis of Acid Phase, p-release Digester at 37ºC

3/16/2011 

4/5/2011



4/27/2011 5/3/2011

Figure 81: Phase 4 Gram Stain Analysis of Control Digester

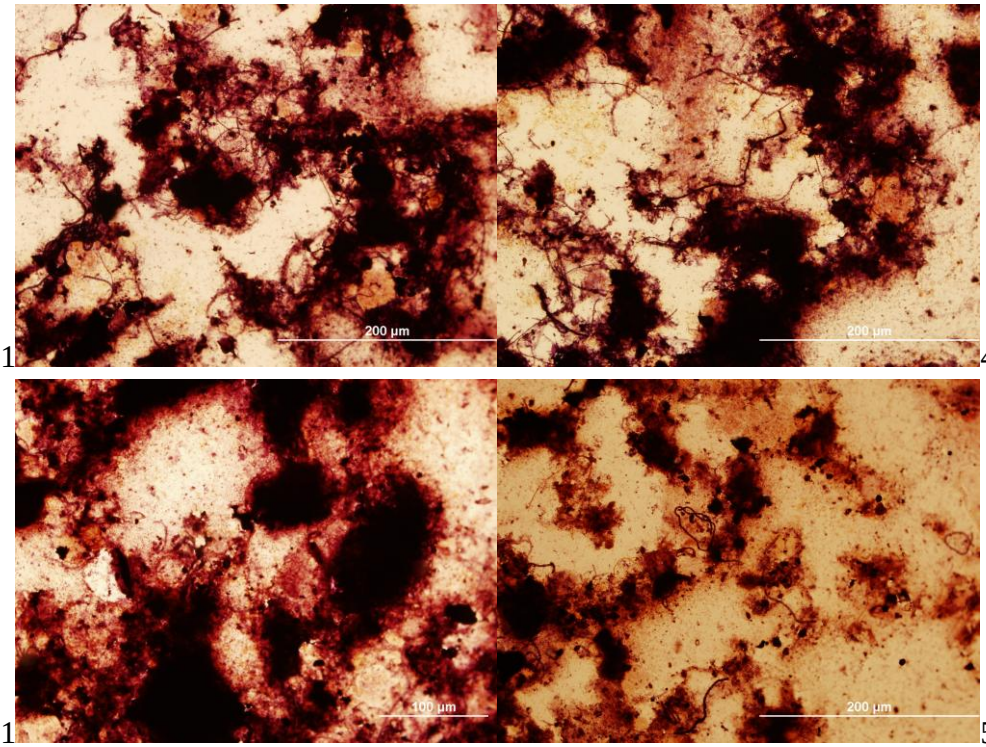


Figure 82: Phase 4 Gram Stain Analysis of Mesophilic Acid Phase-Thermophilic Digester

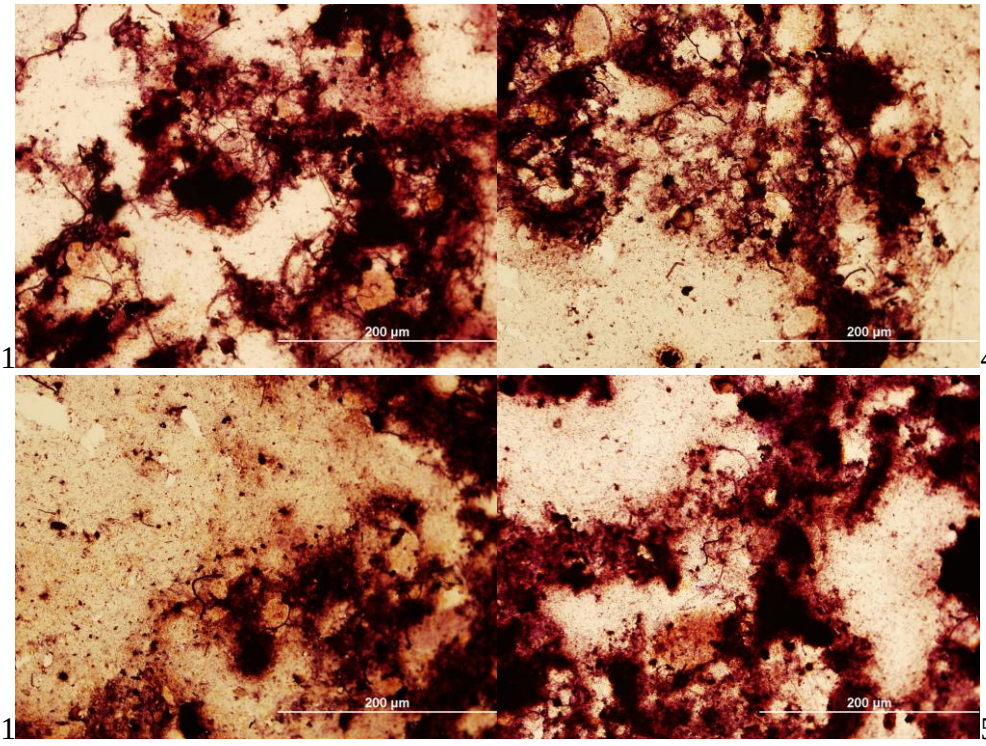


Figure 83: Phase 4 Gram Stain Analysis of Mesophilic Acid Phase (P-release)-Thermophilic Digester