The Effect of Steady-State Digestion Temperature on the Performance, Stability, and Biosolids Odor Production associated with Thermophilic Anaerobic Digestion

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Abstract

The performance and stability of a thermophilic anaerobic digestion system are inherently dependent on the engineered environment within each reactor. While the selection of operational parameters such as mixing, solids retention time, and digestion temperature are often selected on the basis of certain desirable outcomes such as the deactivation of human pathogens, these parameters have been shown to have a broad impact on the overall sludge digestion process. Since the current time-temperature requirements for biosolids pathogen reduction are most easily met at elevated digestion temperatures within the thermophilic range, it is certainly worth examining the effect of specific digestion temperatures on ancillary factors such as operational stability and the aesthetic quality of biosolids.

A series of experiments were carried out in which wastewater sludge was digested at a range of temperatures (35°C, 49°C, 51°C, 53°C, 55°C, 57.5 °C). Each reactor was operated for a period at steady state in order to make observations of microbial activity, digestion performance, and biosolids aesthetics as affected solely by digestion temperature. Results of this study show that poor operational stability arises in reactors operated at 57.5°C. Elevated concentrations of hydrogen and short-chain fatty acids in the 57.5°C digesters are evidence that the observed temperature-induced digester failures are related to the temperature sensitivity of hydrogenotrophic (CO_2-reducing) methanogens. Reactors operated at other temperatures performed equally well with respect to solids removal and operational stability.

In addition, peak volatile organic sulfur compound (VOSC) production from biosolids treated at 51°C and above was greatly reduced in comparison with mesophilic anaerobic digestion and a lower temperature (49°C) thermophilic system. Since the biosolids methanogenic community appeared to be equally capable of degrading VOSC over the range of thermophilic temperatures, the conclusion is that the activity of VOSC producing organisms in digested and dewatered biosolids is greatly reduced when operating temperature in excess of 51°C are used.

This study shows that small changes in an operationally defined parameter such as digestion temperature can have a large impact on the performance and stability of a digestion process. Single minded selection of digestion temperature in order to achieve effective pathogen reduction can result in poor digester performance and the production of an aesthetically unacceptable product. Careful selection, however, of an appropriate digestion temperature can not only ensure successful pathogen reduction in compliance with current regulations, but can also improve the performance, stability, and aesthetic quality of digestion systems employing thermophilic anaerobic digestion.

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Attribution

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Chapter 1

Introduction

Thermophilic digestion of wastewater sludge has been used with reasonable success for over a century; however, during that time the expectation for improved quality of digested material has steadily increased. Anaerobic digesters, which originally developed with the reliable reduction in hauled and disposed mass in mind, are now required to protect human health via pathogen reduction, provide a renewable source of energy in the form of CH_4 gas, and improve the aesthetics of wastewater treatment by producing a low-odor end product. These increased requirements of anaerobic digestion technology have spurred research into advanced treatment processes capable of meeting any combination of the treatment goals set forth by environmental laws and public opinion.

To address both legislative and public concerns associated with wastewater solids, and most notably to achieve substantial levels of pathogen reduction in biosolids, the development of advanced digestion processes has moved towards the implementation of thermophilic anaerobic digestion, operating within the typical temperature range of 50℃ and 60℃. Because biological activity at these temperatures requires specialized organisms equipped with thermostable cell components and enzymes, thermophilic anaerobic digestion has recently been recognized as a holistically unique biological process, rather than a close variation on mesophilic anaerobic digestion. As such, fundamental research into the capabilities and limitations of this technology, both on its own and as part of an advanced digestion process is needed before it can be reliably and successfully implemented at full-scale.

A review of current and past literature in fields having a direct impact on biosolids management is provided in Chapter 2. The aim of this review is to provide background information on past research and applications pertaining to thermophilic anaerobic digestion in order to provide a better understanding of these capabilities and limitations, and to determine the appropriate aim of current and future research such that thermophilic anaerobic digestion can be more beneficially applied. In addition, this review will address parameters such as methanogenesis, process stability, pathogen reduction, and odor reduction that have been previously shown to be affected by thermophilic anaerobic digestion.

Thermophilic anaerobic digestion microbiology

Based on the review of past literature pertaining to the microbiologic basis of thermophilic anaerobic digestion, an apparent contradiction exists in the recommended digestion temperature for these systems. The accumulation of VFA including propionic acid tends to set an upper limit on thermophilic anaerobic digestion temperature; however, the need for more complete deactivation of enteric pathogens serves as a driving force to investigate the temperature limitations of methanogenesis and anaerobic digestion. Previous literature comprehensively explains the role that acid fermentation and methanogenesis have on digester stability, and provides examples of VFA accumulation and digester instability at high temperatures. However, the specificity of the studies represented in the literature often lacks a thorough description of the mechanism of digester failure due to temperature limitations.

The research described in Chapter 3 aims to achieve a holistic picture of temperature induced digestion failure based on analysis of the significant intermediates and products of the electron transport from raw sludge to CH_4 gas. The goal of this research is to point at a particular microbial community (i.e. hydrogenotrophic methanogens, aceticlastic methanogens, and butyrate or propionate oxidizers) as playing a critical role in temperature sensitivity of the thermophilic digestion process. Future research will be conducted to determine the prevalence of these microbial communities at various phases of temperature-induced digester failure and to determine appropriate analyses that may be performed on high-temperature systems to provide an early warning indication of digester upset and/or failure.

Biosolids odor management

The available literature concerning the production and degradation of sulfur-based odor compounds is deficient in its description of the effect that operating parameters of the anaerobic digestion process have on biosolids odor production. The simultaneous need for pathogen reduction and odor minimization in biosolids supports the use of thermophilic anaerobic digestion as an integral part of biosolids management. While the literature and regulations support the idea that increased digestion temperature has a positive effect on pathogenic deactivation, the same has not been well established for the producers of VSC-based biosolids odors. Works in the fields of dental hygiene, freshwater sediment biochemistry, and biosolids odor management have provided a great deal of background information as to how exactly VSC are produced and regulated by the environment. This information can now be applied to engineered systems to enhance our control over these compounds.

The research described in Chapter 4 aims at evaluating the potential for biosolids VOSC reduction using a range of thermophilic digestion temperature, and also to determine the potential consequences at operating at digestion temperatures above or below the theoretical definition of thermophilic digestion, 55℃. This research ties together the ideas brought forth through previous research, and looks to better characterize the effect that operational parameters of anaerobic digestion may have on the homeostatic relationship between VOSC producers and degraders in dewatered biosolids.

Chapter 2

Literature Review

Thermophilic Anaerobic Digestion

Advanced digestion technologies using thermophilic anaerobic digestion

Thermophilic anaerobic digestion has been referred to as "an answer in search of a problem," (Iranpour and Cox, 2006) – suggesting that the use of thermophilic anaerobic digestion, though a technologically sound alternative, is not merited in many applications. In this statement, Iranpour and Cox were making reference to the state of residuals management from the inception of anaerobic digestion in the 1930's until as recently as the 1990's. Since then, regulatory requirements for pathogen reduction in residual biosolids have spurred research into and full-scale applications of thermophilic anaerobic digestion that have greatly increased our understanding of the capabilities of this process. In domestic wastewater treatment, thermophilic anaerobic digestion is often implemented in one of two manners; as a stand alone process using thermophilic anaerobic digestion as the sole solids stabilization and pathogen reduction technology or in conjunction with mesophilic anaerobic digestion as part of a process termed *temperature-phased anaerobic digestion* (TPAD).

Stand alone thermophilic anaerobic digestion processes have been operated with varying success. Hyperion Treatment Plant (HTP), operated by the City of Los Angeles Bureau of Sanitation may be considered a model process conducting thermophilic anaerobic digestion. HTP has exhibited the potential for residual biosolids termed as *Exceptional Quality* under United States, CFR 40 Part 503 (USEPA, 2003) in terms of residual concentrations of pathogenic indicators and inorganic constituents (Iranpour et al., 2005b). Additionally, the two-phased thermophilic process at HTP produces continually high quality effluent in terms of volatile fatty acids (VFA,

intermediates of anaerobic digestion) and carbonaceous oxygen demand (COD, an aggregate measurement of organic material). The biochemical and microbiological processes leading to the occurrence of VFA in digester effluent will be discussed in detail later, but for now high effluent VFA concentrations should be considered an indicator of relative digester instability and poor performance.

Inconsistencies in effluent quality associated with thermophilic anaerobic digestion have been reported and studied in the literature. Various researchers have shown markedly higher concentrations of effluent VFA in thermophilic anaerobic digesters in comparison to mesophilic anaerobic digesters. Data by Gray et al. show that operating digesters at steady-state temperatures at or greater than 53°C results in a sharp increase in effluent VFA concentrations versus mesophilic and low-temperature thermophilic digesters (Gray et al., 2006). In a different study conducted by Kim et al., thermophilic anaerobic digestion of synthetic domestic sludge (i.e. dog food) performed significantly more poorly than mesophilic digestion in terms of VFA production (Kim et al., 2002). During the study, the accumulation of VFA in the thermophilic reactor had to be relieved via operational changes and inorganic nutrient supplementation provided in order to prevent overall reactor failure from occurring.

Such issues with effluent quality associated with stand-alone thermophilic anaerobic digestion processes are often mitigated through the application of a downstream process for effluent polishing. TPAD is a popular system that includes a mesophilic effluent polishing reactor downstream of a thermophilic anaerobic digestion process. The net result of this process is potential additional pathogen reduction for certification of Class A biosolids as defined by United States CFR 40 Part 503, while maintaining the overall process effluent quality of a mesophilic digestion system. Pathogen indicator analyses conducted by Vandenburgh and Ellis over a range of loading conditions showed that TPAD could consistently produce Class A biosolids (Vandenburgh and Ellis, 2002). Previous research by Inman et al. compared the performance of single-stage thermophilic (SST) anaerobic digesters to TPAD systems and found the effluent VFA concentration of the TPAD systems (range = 192 mg/L-1031 mg/L as acetate) was always lower than that of the SST reactors (range = 1067 mg/L to 2367 mg/L as acetate) (Inman, 2004). Better effluent quality while maintaining the potential for Class A biosolids

production makes TPAD an improvement on conventional thermophilic anaerobic digestion systems.

Shifts in microbial community structure have been observed when increasing digestion temperature from mesophilic to thermophilic temperatures, or when varying digestion temperature within the thermophilic range (Bouskova et al., 2005; Zinder et al., 1984a). Information about the dominant microbial communities that exist at various temperatures may provide insight into the differences in reactor performance observed performance over various temperature ranges.

The microbiology of anaerobic digestion

The anaerobic degradation of wastewater sludge has been discussed at length in the literature (McCarty, 1964; McCarty and Smith, 1986; Parkin and Owen, 1986; Speece, 1983). Raw sludge represents a complex organic substrate that is available to a wide variety of anaerobic bacteria; however, methanogens exhibit specificity for very few substrates. Available substrates for methanogens fall into three distinct categories (Madigan et al., 2000):

1. CO_2-type substrates (including CO_2 with reducing equivalents typically supplied by H_2)
2. Acid-type substrates (including acetate and pyruvate)
3. Methylated substrates (including methanol, and methylated nitrogen and sulfur species)

Converting organic sludge particles to methanogenic substrates requires the syntrophic involvement of fermenting organisms that degrade complex organic material to methanogenic substrates. Recent research points to the relationship between the fermenting organisms that produce methanogenic substrates and the methanogens themselves as a key factor in maintaining the conversion of organic sludge particles into CH_4 (Kim et al., 2002; McCarty, 1964; McCarty and Smith, 1986; Parkin and Owen, 1986; Schink, 2002; Thiele et al., 1988). A schematic representation of the anaerobic digestion process is shown below (Figure 2.1). Because of the importance of carbon and electron shuttling between fermentative and methanogenic organisms, special consideration will be given for the production and fate of the compounds in the shaded regions of Figure 2.1 in this review.

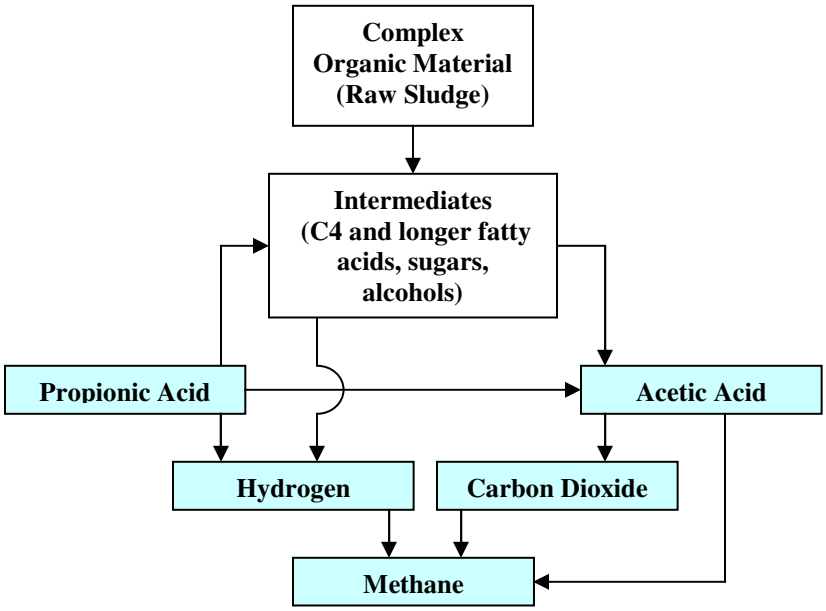
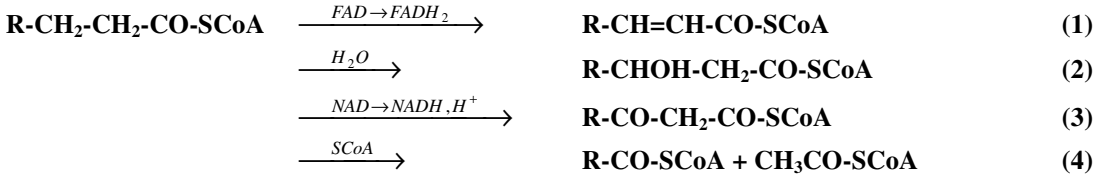


Figure 2.1: Carbon flow diagram for CH_4 formation during anaerobic digestion.

Acetate production in an anaerobic digester is the result of the degradation of organic macromolecules by fermentative bacteria. In general, β-oxidation of alcohols (Sharyshev et al., 1996), carbohydrates and long-chain fatty acids (Rittmann and McCarty, 2001), and particular degradation products of aromatic molecules (Harwood et al., 1998) result in the production of acetate or propionate, plus gaseous H_2 and CO_2. Each β-oxidation reaction is actually a four-stage process as described in Figure 2.2.

As mentioned above, either acetate or propionate can result from the β-oxidation of a longer organic fatty acid. Due to the nature of the two-carbon reaction with each β-oxidation cycle, the end product of successive reactions is dependent on the number of carbons in the parent fatty acid chain. β-oxidation of fatty acids with an even number of carbon atoms will result in the formation of acetate alone; however, oxidation of odd numbered fatty acids such as valeric (C5) or heptanoic (C7) acids will result in the production of the C3 VFA, propionate. Further β-oxidation of a fatty acid residue requires at least four carbon atoms for the formation of (at a minimum) two acetyl-CoA molecules. As such, propionic acid is neither a viable candidate for further β-oxidation, nor a methanogenic substrate. The implications of this situation and degradation pathways for propionate will be discussed later in this review.



(1) Oxidation (dehydrogenation) of the β-carbon atom by FAD (flavin-adenine dinucleotide) eventually resulting the production of H_2 as FAD is reoxidized.

(2) Hydrolysis of the bond between the C-2 and C-3, consuming water.

(3) Oxidation (dehydrogenation) of C-3 hydroxyl group to a keto group by NAD (nicotinamide-adenine dinucleotide).

(4) Thiolysis and activation of the keto group resulting in acteyl-SCoA (acetate) and an acyl-CoA two carbon atoms shorter than the original.

Figure 2.2: Single iteration of β-oxidation pathway degrading an activated fatty acid (acyl-CoA group). Process results in the production of acetyl-CoA, H_2, CO_2, and potentially, a residual fatty acid chain to undergo further β-oxidation (adapted from Rittman and McCarty, 2001).

The production of acetate, which serves as the carbon substrate for aceticlastic methanogenesis, is dependent on the steady β-oxidation of intermediate organic compounds. Under standard conditions, though, the oxidation of butyrate to acetate is thermodynamically unfavorable, meaning that the reaction requires an external driving force in the form of an input of energy or altered environmental conditions in order to occur. The thermodynamic feasibility of a biochemical reaction is related to Gibb's free energy as described by the following modified form of the Nernst Equation (Equation 2.1):

$$\Delta G = \Delta G^0 + RT \cdot \ln Q \qquad \text{Equation 2.1}$$

where ΔG and ΔG^0 are the actual and standard free energy associated with a specified reaction, R is the universal gas constant, T is the reaction temperature in Kelvin, and Q is the reaction quotient. The involvement of the reaction quotient in the expression of Gibb's free energy means that we can manipulate the thermodynamic condition of a biological reaction by affecting the relative concentrations of either reaction products or reactants. The chemical reaction that describes the oxidation of butyrate to propionate is shown below.

$$CH_3CH_3CH_2COO^-_{(aq)} + 2H_2O_{(l)} \rightarrow 2CH_3COO^-_{(aq)} + 2H_{2(g)} + 2H^+_{(aq)} \qquad \Delta G^{o'} = 48.30 \text{ kJ}$$

Considering this reaction as it applies to Equation 2.1, the likelihood of the β-oxidation of butyric acid to acetate taking place naturally can be improved by increasing the environmental concentration of butyrate, or decreasing the environmental concentrations of acetate and/or H_2. The thermodynamics of butyrate and propionate oxidation within the scope of anaerobic digestion have been previously discussed (McCarty and Smith, 1986; Scholten and Conrad, 2000). McCarty and Smith (1986) suggest that acetate and butyrate often exist at significant concentrations in an anaerobic digester such that their manipulation is simply not a feasible method for favorably affecting the reaction quotient, which is expressed below (Equation 2). Typical ranges of acetate concentration in anaerobic digestion are reported as 10^{-4} M to 10^{-1} M, whereas the concentration dissolved H_2 typically ranges between 10^{-8} and 10^{-5} M.

$$Q = \frac{\{H_2\}^2\{H^+\}^2\{CH_3COO^-\}^2}{\{CH_3CH_3CH_2COO^-\}} \qquad \text{Equation 2.2}$$

Since the concentration of dissolved H_2 is reported at anywhere from one to seven orders of magnitude less than that of acetate, stoichiometrically less H_2 must be removed from reactor environment to have an appreciable effect on Gibb's free energy.

Long chain fatty acids are degraded primarily through conversion to acetate. As described previously, β-oxidation of fatty acids results in the production of either butyric or propionic acid as an intermediate. Butyric acid can be directly converted to acetate via an additional β-oxidation step; however, propionate must be degraded via other means. Various methods for propionate degradation have been discussed in the literature, some of which do not rely on acetogenesis. For example, Güven et al. have suggested that propionate was both oxidized to CO_2 and assimilated into cell biomass by anaerobic ammonia oxidizing (anammox) bacteria (Guven et al., 2005). Such pathways however, are not well suited for an anaerobic digester environment. The more prevalent propionate degradation pathways during anaerobic digestion are those tied to acetogenesis.

Two pathways have been established for the oxidation of propionate to acetate. The first is via the methylmalonyl-CoA pathway (Figure 2.3) relies on the decarboxilation of pyruvate that is

formed as an intermediate (Kosaka et al., 2006). The pathway results in the production of CO_2 and acetate, and no H_2 is released.

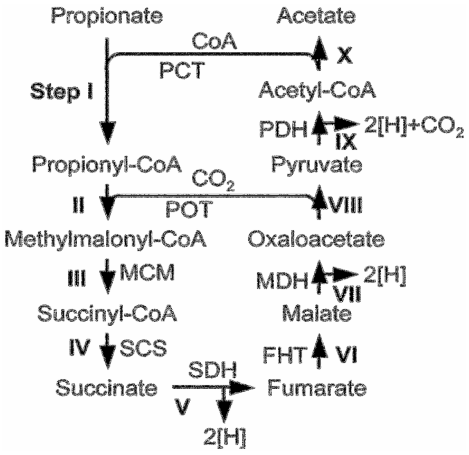


Figure 2.3: Methylmalonyl-CoA pathway for the oxidation of propionate to acetate under anoxic conditions. (Taken with permission from Kosaka et al. 2006)

The second acetogenic propionate oxidation pathway was first determined in cocultures of *Smithella propionica* and *Methanospirillum hungateii* that were observed to produce small quantities of butyric acid (Liu et al., 1999). The pathway for propionate oxidation by this same coculture was later established (de Bok et al., 2001). It was determined that two propionate molecules could be condensed by interaction of one C2 location with the carboxyl-end of the other. Linearization and rearrangement of the molecule would then result in the formation of 3-ketohexanoate (C6 VFA). Sequential β-oxidation of 3-ketohexanoate would result in the formation of three acetate molecules as previously described (Figure 2.4).

Because the oxidation of propionic acid to acetate via 3-ketohexanoate and butyrate requires two rounds of H_2-producing β-oxidation, this pathway is also heavily dependent on the removal of H_2 from the environment to ensure a favorable thermodynamic condition. Investigations of the feedback inhibition of H_2 on propionate oxidation have been carried out. Data by Scholten and Conrad (2000) support the importance of low H_2 concentration for maintaining an exergonic biochemical pathway for the conversion of propionate to acetate. Under standard reactor conditions, the buildup of H_2 in the reactor headspace limited the stoichiometric energy yield to approximately $^1/_{11}$ mol ATP per mol CH_4 produced, whereas flushing the headspace of the reactor to remove excess H_2 resulted in the equivalent production of $^1/_2$ mol ATP per mol CH_4.

Reactor headspace served as a pool of H_2 that caused an unfavorable thermodynamic condition for microbial growth on propionate. As previously discussed with butyric acid, the removal of H_2 from the reactor environment is essential to maintaining the effective shuttling of carbon from complex organics and long-chain fatty acids to acetate.

$$\mathbf{CH_3CH_2COO^- + CH_3CH_2COO^- \longrightarrow CH_3\text{-}CH_2\text{-}(C{=}O)\text{-}C(CH_3)H\text{-}COO^-} \quad \mathbf{(1)}$$

$$\mathbf{\longrightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}(C{=}O)\text{-}CH_2\text{-}COO^-} \quad \mathbf{(2)}$$

$$\mathbf{\longrightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}COO^- + CH_3COO^-} \quad \mathbf{(3)}$$

$$\mathbf{\longrightarrow CH_3COO^- + CH_3COO^- + CH_3COO^-} \quad \mathbf{(4)}$$

(1) C2-carboxyl end condensation of two propionic acid molecules.

(2) Linearization and rearrangement of molecule to form 3-ketohexanoate.

(3) β-oxidation of 3-ketohexanoate yielding butyrate and acetate.

(4) β-oxidation of butyrate yielding two additional acetates (3 CH_3COO^- total).

Figure 2.4: Propionate oxidation via butyrate intermediate. Process was isolated from coculture with *Smithella propionica* and *Methanospirillum hungateii.* (Adapted from de Bok et al. 2001)

The fermentative bacteria that are responsible for the production of acetate through propionic and butyric acids have no capacity for H_2 consumption. This means that a second group of organisms, the methanogens, must be responsible for regulating H_2 such that acetogenesis can proceed. Researchers have found that the butyrate-dependant pathway for propionate degradation does not occur in the absence of a methanogenic coculture, meaning that the metabolic activity of hydrogenotrophic methanogen is responsible for relieving the feedback inhibition by H_2.

Two predominant classes of methanogens exist in anaerobic digestion. One of these, the aceticlastic methanogens, is responsible for cleaving acetate produced through the β-oxidation of long-chain fatty acids and other complex organic compounds in wastewater sludge. This process, termed aceticlastis, results in the direct production of stoichiometric amounts of 1 mol CH_4 and 1 mol CO_2 per mol acetate (McCarty, 1964). The second class of methanogens, CO_2-reducing methanogens (also referred to as hydrogenotrophic methanogens) use CO_2 and reducing equivalents (electrons) typically supplied by H_2 to reduce C^{+4}-CO_2 to C^{-4}-CH_4. Previous

research provides varied reports of the fractionation of CH_4 that is formed by these two processes; however, a general consensus of approximately 65 to 80% is reported for CH_4 production via acetate. The smaller fraction of methanogenesis attributed to CO_2-reduction is the likely result of limited substrate availability.

As mentioned previously, the concentration of H_2 in an anaerobic digester ranges from one to seven orders of magnitude smaller than that of acetate, which limits the proportional mass of CH_4 that can be produced via CO_2 and H_2. However, community analysis of a thermophilic anaerobic digester has showed that over 99% of methanogenic organisms are hydrogenotrophic methanogens (Zinder et al., 1984c). The prevalence of CO_2-reducing metabolisms among methanogens suggests the importance of H_2 as an anaerobic digestion intermediate. For the conversion of carbon (+IV) to carbon (-IV), a total of eight electrons are required. If the reducing equivalents for this reaction are supplied by H_2 according the following reaction: $H_2 \rightarrow 2H^+ + 2e^-$, then four moles of H_2 are consumed for each mole of carbon converted to CH_4 (Jones et al., 1985; Madigan et al., 2000).

Microbial community assessment of archaeal populations in mesophilic and thermophilic anaerobic digesters using PCR-DGGE analysis have shown a highly simplified methanogenic community associated with thermophilic anaerobic digestion (Chen et al., 2005). Some genera of methanogenic organisms that have been identified from thermophilic environments are included in Table 2.1.

The simplified archaeal community observed in thermophilic anaerobic digesters may play a role in the potential upset of these systems. The inhibition of a dominant methanogenic population by the introduction of a toxicant or change in environmental conditions would result in the imbalance in the electron transport chain from complex organics, to intermediate organic acids, and eventually to CH_4.

Table 2.1: Methanogenic archaea identified from thermophilic anaerobic digestion.

	Archaeal Genera	Reference
Aceticlastic Methanogens	*Methanothrix sp.*	(Nozhevnikova and Yagodina, 1982)
		(Zinder et al., 1984c)
	Methanosarcina sp.	(Zinder and Mah, 1979)
		(Zinder et al., 1985)
		(Ahring et al., 2001)
	Methanosaeta sp.	(Kamagata and Mikami, 1991)
Hydrogenotrophic (CO_2-reducing) Methanogens	*Methanobacterium sp.*	(Marty and Bianchi, 1981)
		(Zinder et al., 1984c)
		(Laurinavichyus et al., 1988)
		(Stams et al., 1992)

Since the conversion of propionate and butyrate to acetate is strongly dependant on the H_2-oxidizing activity of hydrogenotrophic methanogens, a great deal of research has been focused on maintaining the effective flow of H_2 (and by association, electrons) between fermentative bacteria and hydrogen-consuming methanogens within an anaerobic digester. Because the environmental concentration of H_2 is important to both of these populations, the rate of H_2 transport across the intercellular space between methanogens and fermenters becomes an important design criterion. The rate of H_2 mass flow between cells can be described by Fick's first law of diffusion, shown as Equation 2.3 below, where J_{MT} is the mass flow rate (flux) of H_2 between the H_2 producing and consuming organisms, D is the diffusivity of H_2 in the intercellular fluid, A is the cross-sectional area across which H_2 flux occurs, C_2 and C_1 are the concentrations of H_2 at the surface of the H_2 consuming and producing organisms, respectively, and Δz is the intercellular gap between organisms (de Bok et al., 2004; McCarty and Smith, 1986; Thiele et al., 1988)

$$J_{MT} = -DA\frac{(C_2 - C_1)}{\Delta z} \qquad \text{Equation 2.3}$$

From the mass flux equation, the mass flow rate of H_2 from acetogens to methanogens can be increased by either *a)* increasing the magnitude of the H_2 concentration gradient between cells or by *b)* decreasing the intercellular gap distance. As previously described, research by Scholten and Conrad (2000) and McCarty and Smith (1986) provides a strong case for the need for a low environmental H_2 concentration for the oxidation of fatty acids to occur readily. This renders the manipulation of H_2 concentration gradient between organisms relatively useless in this case, and leaves the manipulation of the intercellular gap distance as a valuable parameter. Methanogenic cocultures (see Figure 2.5) tend to exist naturally in tightly bound clusters with fermenting organisms that are evidence of adaptation to their syntrophic relationship (de Bok et al., 2004; McCarty and Smith, 1986; Thiele et al., 1988).

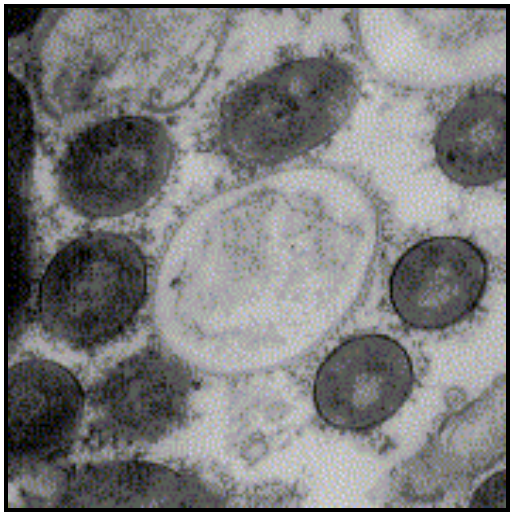


Figure 2.5: TEM image of syntrophic fermentative bacteria surrounded by hydrogenotrophic methanogens exhibiting the principle of close microbial consortia proximity. (Photo taken with permission from de Bok et al. 2004)

Apart from biological adaptations to syntrophic growth, other methods of for reducing the intercellular gap through a principle termed *close microbial consortia proximity* (Speece et al., 2006) are discussed in the literature. The first of these is an effort to keep biomass from passing from an anaerobic digester via the effluent, much like a secondary clarifier works to increase the biomass concentration of a conventional activated sludge system. Increased biomass in an anaerobic digester (assuming fixed reactor volume) would inherently decrease the intercellular gap between organisms, effectively raising the mass transfer rate of H_2 from fermenting bacteria to hydrogenotrophic methanogens. While allusions to membrane technology have been made in

the literature, it is generally conceded that such a technology would not be effective for sludge digestion (Speece et al., 2006). However, granularization of methanogenic cocultures has been used in the past in order to immobilize biomass within an anaerobic reactor (de Bok et al., 2004; Kim et al., 2004).

An alternate method for microbial compaction that has been investigated is the simple manipulation of mixing conditions within a CSTR. Kim et al. (2002) conducted a comparative study of mesophilic and thermophilic chemostat reactors in which mixing conditions were varied. Data from this research show that within both temperature ranges, the absence of regular mixing greatly reduced the concentration of VFA in the reactor effluent, presumably due to more effective shuttling of H_2 to the hydrogenotrophic methanogens and more favorable thermodynamics for fatty acid oxidation. Whether non-mixed conditions and immobilized biomass are deemed feasible by treatment plant operators or not, the knowledge of the syntrophic relationship between organic acid fermenting bacteria and hydrogenotrophic methanogens gained by through past research is crucial to the understanding of thermophilic anaerobic digestion.

Biosolids Odors

Odor Production and Degradation

Nuisance odors associated with anaerobically digested biosolids can be traced to the presence of certain groups of volatile compounds. The primary categories of odor-causing compounds associated with biosolids are as follows:

1. Volatile Sulfur Compounds (VSC) – hydrogen sulfide (H_2S), methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), carbonyl sulfide (COS), carbon disulfide (CS_2).
2. Volatile Nitrogen Compounds (VNC) – trimethyl amine (TMA), indole, skatole.
3. Volatile Fatty Acids (VFA) – acetate, propionate, butyrate, etc.

Of particular interest to wastewater treatment are the volatile sulfur compounds. Previous studies have reported a direct correlation between the concentration of these compounds and human olfactory assessments of digested biosolids (Higgins et al., 2006; Witherspoon et al., 2004). As such, much of the research in the field of biosolids odor production is focused on VSC.

The study of odor-producing processes associated with wastewater treatment is still relatively nascent; however, a great deal of research into the production and control of VSC within freshwater sediments and the human oral cavity has led to a fair understanding of the physical, chemical, and biological reactions that cause VSC odor in biosolids. Past dental research has focused on the production of VSC due to their prime involvement in clinically defined halitosis. Such research has concluded that the level of VSC produced in the oral cavity of patients that had been diagnosed with halitosis based on olfactory observation can be up to twenty-five times higher than in control patients (Oho et al., 2001). Efforts to mitigate malodors in patients have led to the identification of important microbiological communities associated with VSC production in the absence (Persson et al., 1989; Persson et al., 1990; Tanaka et al., 2004) and presence of periodontal disease (Figueiredo et al., 2002). Evidence of VSC production in the absence of corroborating conditions, such as acute periodontal disease, helps researchers

interested in VSC levels in biosolids to effectively focus their efforts on biologically mediated production routes.

Dental research, in conjunction with investigations of nuisance odors from freshwater sediments, has provided a great deal of understanding of the mechanisms of biological VSC production. Based on previous investigations of VSC production in both environments, the direct biological production of H_2S and MT can be directly attributed to the degradation of the sulfur containing amino acids, L-cysteine and L-methionine. Researchers have observed H_2S production from free cell extracts of *E. coli* (Awano et al., 2005; Ohigashi et al., 1951) and *Desulfovibrio sp.* (Forsberg, 1980) when L-cysteine was available as a carbon substrate. Similar experiments with L-methionine supplementation of free cell extracts of *Clostridium sp.* (Wiesendanger and Nisman, 1953), *E. coli* (Ohigashi et al. 1951) and *Pseudomonas sp.* (Kallio and Larson, 1955; Miwatani et al., 1954) show MT production. Further research by Segal et al. (1969) shows that a mixed culture of soil microbes was capable of degrading L-methionine by successive oxidative deamination and demethiolation, resulting in the production of α-ketobutyric acid, ammonia, and MT. Cysteine is degraded by a similar process of deamination and hydrolysis to produce pyruvate, ammonia, and H_2S (Awano et al., 2005; Forsberg, 1980). Apart from amino acid degradation, additional production of H_2S and MT occurs in natural systems as a result of sulfur reducing bacteria and methylation of H_2S, respectively (Bak et al., 1992; Higgins et al., 2004).

In wastewater systems, malodors are often attributed to volatile organic sulfur (or organo-sulfur) compounds (VOSC), a specialized classification of VSC that signifies the inclusion of one or more methyl groups in the chemical structure. Along with MT, the production of which was described above, DMS, DMDS, and DMTS are characterized as VOSC. As mentioned above, the methylation of H_2S has been credited with the production of additional MT. In a similar process, the methylation of MT results in the production of DMS. In a previous experiment (Segal and Starkey, 1969), when MT was provided as the sole carbon substrate and sulfur source, certain bacteria isolated from soil cultures converted up to 90% of the initial MT-Sulfur to DMS-Sulfur over a period of thirty minutes. This reaction was shown to be irreversible under these conditions, as cultures initially supplemented with DMS showed no capacity for MT production.

These previously described mechanisms show how H_2S, MT, and DMS can be produced biologically from the parent compounds, cysteine and methionine. DMDS and DMTS however, are likely produced through abiotic pathways involving metal catalyzed oligomers of H_2S, MT, and DMDS. Previous research (Chin and Lindsay, 1994) has concluded that the oxidation of MT with either oxidized iron (or copper) as an electron acceptor could result in the formation of thiyl radicals ($CH_3S\bullet$) and reduced iron (Follow Table 2.2). The combination of reduced iron and an oxidant, such as hydrogen peroxide, would then result in the production of hydroxyl radicals ($\bullet OH$) which could react with thiyl radicals to form sulfenic acid (CH_3SOH). DMTS is formed ether by reaction of sulfenic acid with H_2S, or by dehydration of sulfenic acid to from methyl methanethiosulfinate ($CH_3(S=O)SCH_3$) and subsequent reaction with H_2S.

Table 2.2: Biological reactions involved in the oxidation of MT to DMTS. (Adapted from Chin and Lindsay, 1993)

			Eq.
CH_3SH (MT) + Fe(III)	→	$CH_3S\bullet$ + Fe(II) + H^+	1
Fe(II) + H_2O_2	→	Fe(III) + $\bullet OH$ + OH^-	2
$CH_3S\bullet$ + $\bullet OH$	→	CH_3SOH	3
2 CH_3SOH + H_2S	→	CH_3SSSCH_3 (DMTS) + 2 H_2O	4
2 CH_3SOH	→	$CH_3(S=O)SCH_3$ + H_2O	5
2 $CH_3(S=O)SCH_3$ + H_2S	→	CH_3SSSCH_3 (DMTS) + 2 CH_3SOH	6

Additional data (Korycka-Dahl and Richardson, 1978) suggest that a similar mechanism exists for the production of DMDS. When sodium benzoate was added to reaction vessels in order to scavenge hydroxyl radicals, the production of both DMDS and DMTS was greatly reduced. These results support the abiotic metal-catalyzed pathway for DMDS and DMTS proposed by Chin and Lindsay (1993).

Though the VSC production and degradation pathways in the literature are based on data collected from freshwater sediment, oral hygiene, and food chemistry research, the environmental conditions under which past research has been conducted reveal underlying similarities with biosolids odor management. Whether biotic or abiotic, the parent compounds for VSC-based odors are the sulfur containing amino acids, cysteine and methionine. Waste activated sludge, one of the primary components of anaerobic digester feed, contains extracellular polymeric substances (EPS) that are necessary when considering biological floc structure and settling characteristics (Dignac et al., 1998; Higgins and Novak, 1997). Higgins

and Novak (1997) have proposed a biological floc model that explains the interaction of mono- and divalent cations, polysaccharide chains, and lectin-like protein within EPS. Extraction and characterization of EPS protein in full-scale and laboratory activated sludge reactors revealed that both cysteine and methionine were present at a rate of approximately 100-200 μmol per gram of total protein (approximately 1.5-3.0 percent of total protein, by mass). The presence of the cysteine and methionine in extracted EPS from activated sludge is further supported in the literature (Dignac et al., 1998; Morgan et al., 1991). Higgins et al. (2006) later hypothesized that the breakdown of this EPS bound cysteine and methionine would result in the VSC perceived as nuisance biosolids odor as described by the biotic and abiotic pathways for VSC production described herein.

As Lomans et al. (2002) describe, various mechanisms leading to VSC production in marine and freshwater environments have been identified, yet atmospheric VSC concentrations have not been observed to fluctuate over time. This leads to the conclusion that mechanisms that serve as VSC sinks exist in relative balance with VSC production. The biological conversion of methylated compounds (e.g. methanol) to CH_4 has been well established through previous research. Various studies have identified a direct route for methylotrophic methanogenesis from methanol, using methyl-CoM as an intermediate (Muller et al., 1986; Smith and Mah, 1978; Zandvoort et al., 2002a; Zandvoort et al., 2002b). During methanogenesis from methanol, the simultaneous oxidation of methyl-CoM provides the necessary reducing equivalents for reduction to CH_4; a process which is also hypothesized as a viable mechanism for metylotrophic methanogenesis from MT, DMS, DMDS, and DMTS (Lomans et al., 2002). The recent isolation of a freshwater methanogen, *Methanomethylovorans hollandica* (optimal growth at 0.04 M NaCl), capable of performing methanogenesis for growth with MT and DMS as sole carbon substrates (Lomans et al., 1999) lends support for the hypothesized VSC degradation mechanism.

Researchers have noted that, when incubated under closed anaerobic conditions, biosolids odors are observed to initially increase due to VSC production, and then decrease to below the level of olfactory detection (Figure 2.6) presumably through the breakdown of these same chemicals (Higgins et al., 2006). Due to previous findings of VSC degradation within freshwater sediments,

the involvement of methanogenic activity on the reduction of VSC odors associated with stabilized biosolids has recently been investigated. Although *M. hollandica* has not yet been isolated from an anaerobic digester community, previous digestion studies have identified *methanosarcina sp.*, a methanogen capable of methylotrophic methanogenesis using methanol, as an ecologically significant microbial population (estimated 10^8 CFU per ml or reactor) in thermophilic anaerobic digestion (Zinder et al., 1984a). Applying the link between methanol degradation and VOSC degradation by methanogens proposed by Lomans et al. (2002), VOSC degradation via methylotrophic methanogenesis is a likely cause of the decrease in biosolids odor observed over long incubation times.

In order to better determine the role that methanogens play in the degradation of VOSC in biosolids headspace, experiments have been conducted in which methanogenesis was inhibited through chemical addition in order to effectively uncouple VSC production and VOSC degradation (Chen et al., 2005). The specific methanogenic inhibitor used in this study was 2-bromoethanesulfonic acid (BESA). Various researchers (Chen et al., 2005; Zehnder and Brock, 1980; Zinder et al., 1984b) have shown that a single addition of BESA to a methanogenic culture at a concentration of 10^{-5} to 10^{-4} M was sufficient to cause complete inhibition of methanogenesis. Various problems with the use of BESA for the inhibition of methanogenesis have been proposed, such as the development of strains of methanogens resistant to BESA and the potential degradation of BESA (Bouwer and McCarty, 1983). These difficulties with BESA addition would not likely be applicable to anaerobically digested biosolids because of the lack of previous methanogenic exposure to chlorinated aliphatic compounds leading to the development of resistance or a functional metabolic pathway for BESA degradation. As such, the use of BESA for the purposes of studying biosolids odor is likely appropriate.

As mentioned before, digested and dewatered biosolids typically exhibit an initial increase in odor followed by eventual dissipation. When Chen et al. (2005) added BESA to anaerobically digested biosolids, quantitative measurements of headspace VSC increased to an approximate peak value, but did not exhibit the typical decrease observed in unammended samples. Because measurements of peak VSC in the presence of BESA provides information about a system's capacity for producing odor compounds in the absence of degraders, such data are often referred

to as “biosolids odor potentials” in the literature. The lack of VSC dissipation in the presence of the methanogenic inhibitor BESA provides strong evidence of the role that methylotrophic methanogenesis plays in biosolids odor reduction. In addition, Chen et al. found a direct correlation between integrated optical density (IOD) of archaeal PCR-DGGE fingerprints of various digesters and peak biosolids headspace MT (Figure 2.7). IOD is a measure of cumulative light intensity, in this case, contained in a DGGE image; and as such, IOD can be used as a basis of qualitative comparison between the abundance of methanogenic organisms in various anaerobic digesters.

Research in the area of odor management pertaining to oral health, freshwater and marine sediments, and domestically produced biosolids has helped to develop a better understanding of the mechanisms that are responsible for the production and degradation of VSC. Figure 2.8 provides a summary of the biotic and abiotic reactions that are involved in VSC cycling in these environments (revised from Higgins et al. 2006).

The bell shape of a typical biosolids growth and consumption curve means that there is an imbalance between the rate of VSC production and degradation throughout the sampling interval. The lag in methanogenic activity results in the accumulation of VSC, and is responsible for what olfactory observations perceive as odor. Researchers have suggested various explanations for the lag in methanogenic activity after biosolids dewatering including mechanical shear, aeration, and cell lysis (Higgins et al. 2006). In any event, the inhibition of methanogenesis in dewatered biosolids disrupts the natural homeostasis that exists between VSC production and degradation. Studies aimed at the mitigation of biosolids odor have arrived at similar conclusions; that an effective method for controlling biosolids odor is to maintain an active methanogenic community in digested and dewatered biosolids, and thus enhance VSC degradation as it is produced (Chen et al., 2005; Higgins et al., 2006).

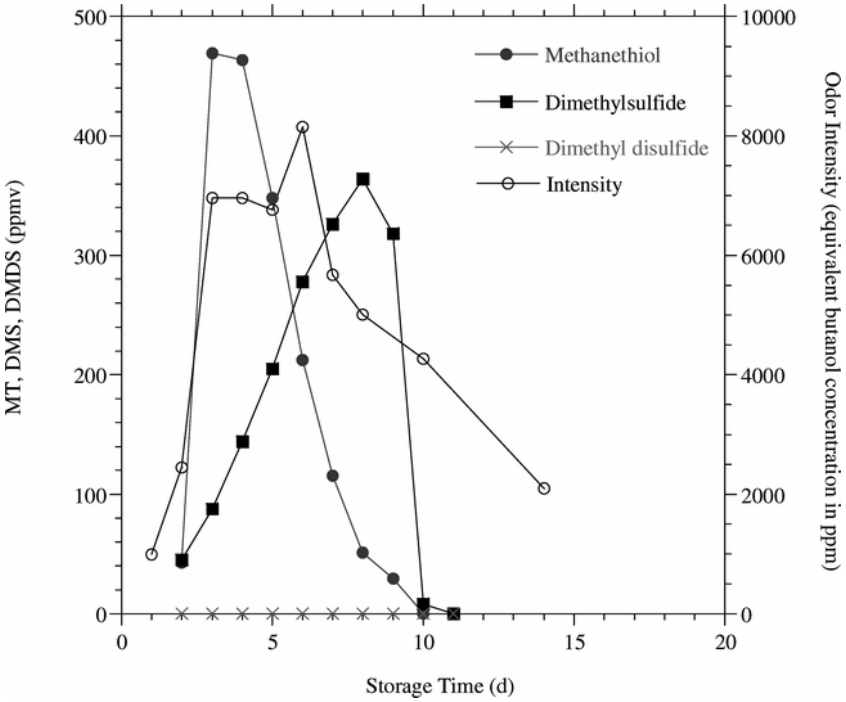


Figure 2.6: Growth and consumption curves for MT, DMS, DMDS, and human-perceived odor intensity throughout biosolids incubation and storage. (taken with permission from Higgins et al. 2006)

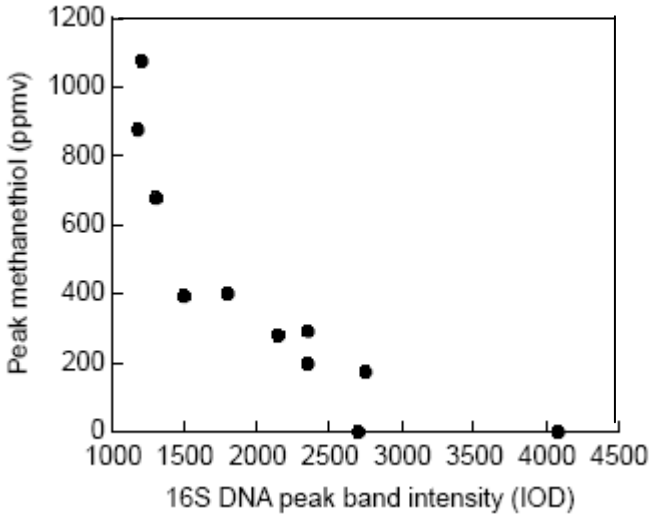


Figure 2.7: Correlation between peak MT and IOD (band intensity) attained from archaeal 16S rDNA PCR-DGGE analysis. (taken with permission from Chen et al. 2005)

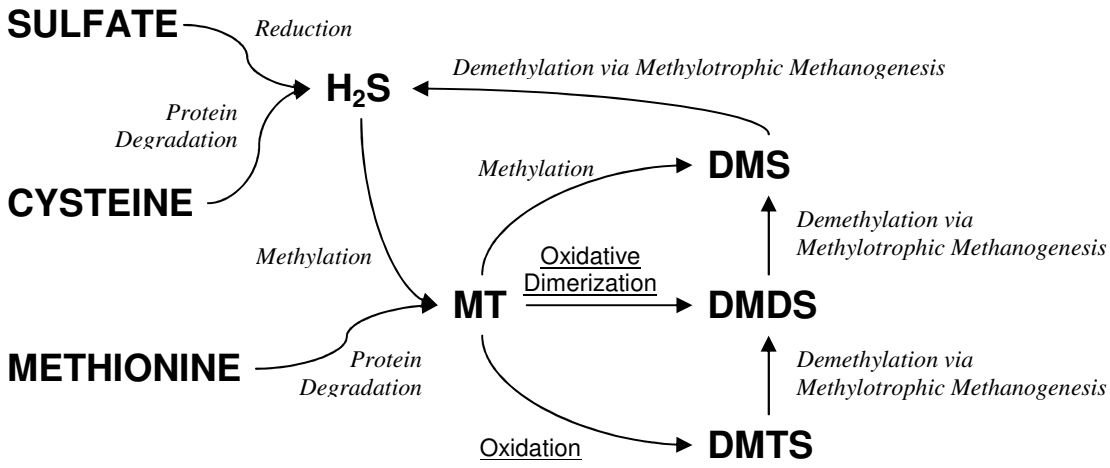


Figure 2.8: Conversions involved in VSC cycling. Biochemical conversions are shown in *italics*, while abiotic chemical reactions are underlined. Nuisance odor production is a result of the imbalance between VSC production and degradation within a biological system. (Revised from Higgins et al, 2006)

Effect of dewatering practices on biosolids odor

The operating factors that effect the production of VSC are not only important to full scale efforts to mitigate biosolids odor, but are also important to the development of laboratory techniques that simulate field conditions such that odor production from biosolids is more easily observed. The generation of VSC-based biosolids odor is dependent on the degradation of cysteine and methionine within sludge floc-associated EPS. Thus, factors affecting the bioavailability of proteinaceous EPS during processing and dewatering will also affect the potential for biosolids odor production.

Major factors that influence biosolids during post digestion processing (dewatering, storage, and disposal) include mechanical shear and polymer addition. Both of these factors are necessary, at least in some regard, when developing an effective and economical biosolids management plan. Muller et al. (2004) have proposed a mechanism by which mechanical shear in the presence of cationic polymer renders EPS-associated protein bioavailable in post-dewatered biosolids. As the researchers describe, floc-bound protein is released into solution when digested sludge is exposed to significant shear forces. The quantitative shear force applied by dewatering equipment can be measured by the unitless parameter *Gt*, which is the product of the instantaneous velocity gradient between solids particles within the shear device and the time over which this gradient is applied. Estimations of *Gt* for widely employed dewatering equipment as stated in the literature are as follows: Belt filter press, 10,000; Low-Solids Centrifuge, 10,000; Mid-Solids Centrifuge, 25,000; High-Solids Centrifuge, 75,000 (WERF, 2006). The magnitude of *Gt*, along with various other parameters, has a significant impact on the degree of dewatering, and thus, the final biosolids concentration. Muller et al. (2004) have determined that there is a positive correlation between cake solid concentration and normalized peak headspace VOSC (Figure 2.9) – however, cake solids concentration appears to be dependent on the applied shear force during dewatering as evident by the designations given to low-, mid-, and high-solids centrifuges. The relationship between solids concentration, shear force, and VOSC production suggests that increased applied shear force during dewatering may indeed have a direct impact on biosolids odors as excess bioavailable EPS-bound protein is solubilized (Muller et al., 2004).

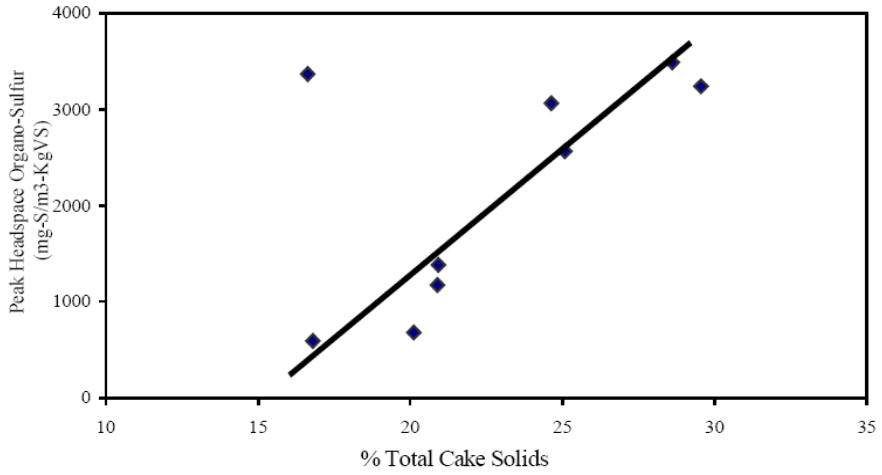


Figure 2.9: Correlation between VOSC production and final cake solids concentration after dewatering. Final cake concentration is a function of, among other parameters, shear input during dewatering. The data suggest that dewatering equipment may play a role in the production of nuisance odors from biosolids. (taken with permission from Muller et al. 2004)

In the same study, when shear was applied in the absence of polymer addition or with post polymer addition, no appreciable VSC production was reported. These results suggest that concurrent polymer addition with applied shear is necessary for biosolids odor production. As shown in Figure 2.10, a necessary step in the conversion of EPS-bound protein to VSC is the denaturation of protein (Higgins et al., 2006). The conditions that cause denaturation are a direct result of the mechanical shear input during dewatering. The removal of these conditions in the absence of a binding agent for the polypeptide chains could result in the refolding of a large fraction of the protein bound as EPS. The researchers hypothesize that protein material that undergoes hydrolysis and denaturation due to shear input are bound in a polymer-protein complex by cationic polymer and disallowed from rejoining the biological floc. The protein bound in this complex is available for degradation and conversion into VSC resulting in nuisance odor production.

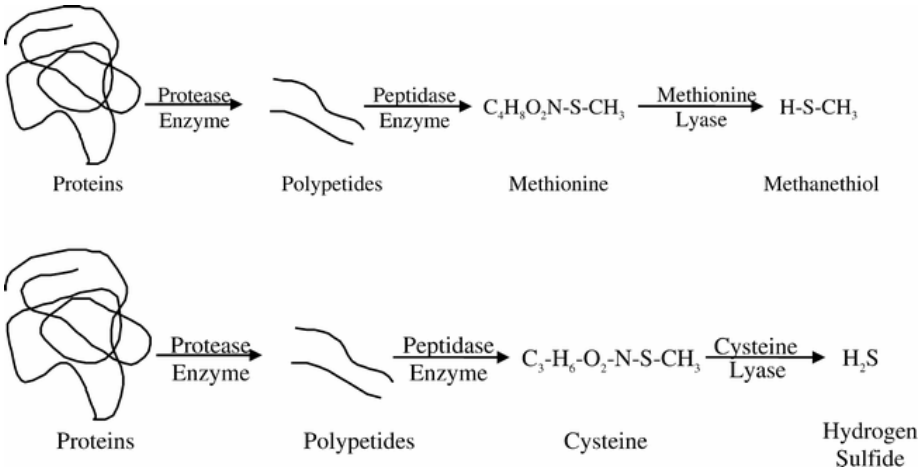


Figure 2.10: Proposed steps in the bioconversion of floc associated protein to VSC. Cationic polymer has proved to play an important role in this pathway by binding polypeptides and preventing their reincorporation into the biological floc. (taken with permission from Higgins et al., 2006)

Effect of digestion temperature on biosolids odor

Research concerning the effect of digestion temperature on the production of VOSC from anaerobically digested biosolids is scarce. The production of VSC from anaerobically digested biosolids has been well correlated to the biological activity of organisms that are involved of the production or degradation of organic sulfur compounds. As such, the manipulation of these microbial communities through the use of an easily controlled operational parameter could have a large impact on biosolids odor production.

The manipulation of a specific microbial community through easy to control operational parameters is not new to anaerobic digestion. The potential deactivation of various human pathogens has recently been recognized as an advantage of various high temperature biological sludge stabilization technologies. The United States Environmental Protection Agency (EPA) currently lists thermophilic aerobic digestion (Athermophilic Anaerobic Digestion™), in-vessel anaerobic composting (En-vessel™, IPS Process™) and various multi-phased anaerobic digestion processes (CBI ATP™ Process, Two-Phased Thermo-Meso Feed Sequencing Anaerobic Digestion) as Processes to Further Reduce Pathogens (USEPA, 2003). In this case, the specific microbial community of interest is a group of enteric human pathogens accustomed to life at standard body temperature (37°C), or mesophilic conditions. Previous research has shown that the viability of pathogenic organisms can be significantly decreased by high temperature incubation and/or treatment (Berg and Berman, 1980; Ghosh, 1998; Iranpour, 2006;

Watanabe et al., 1997). The aforementioned processes each use increased temperatures between 50°C-65°C to deactivate non-thermostable components of the pathogenic cells and viruses. Protein degrading organisms in biosolids are similarly accustomed to mesophilic temperatures; therefore, the application of thermophilic anaerobic digestion may similarly affect VSC production.

Few comparisons evaluating odor potentials of biosolids digested at various temperatures have been made. Investigations at between two full scale thermophilic digestion facilities have been conducted to determine the effect of intermittent temperature increases on the production of digester headspace VSC (Iranpour et al., 2005a). The researchers found that in both conditions, the concentration of H_2S, MT, and DMS increased in response to these transient temperature fluxes. At Terminal Island Treatment Plant (TITP), San Pedros, California, VSC monitoring during a transient temperature increase from 57.5℃ to 65.5℃ showed enhanced production of H_2S and MT in the digester headspace. No change in DMS concentration was observed during this trial. At Hyperion Treatment Plant (HTP), Playa del Ray, California, an increase in digester temperature from 54.4℃ to 58℃ caused sharp increased in MT and DMS headspace concentrations.

Previous research concerning methanogenic population dynamics with changes in thermophilic digestion temperature suggests that higher digestion temperatures tend to select for non-methylotrophic methanogenic species. Zinder et al. (1984) observed a shift from *methanosarcina sp.* to *methanothrix sp.* as the dominant aceticlastic methanogenic population when the digestion temperature of a laboratory digester was increased to 58℃. Organisms of the genus *methanosarcina* have been identified as potential methylotrophic methanogens (Muller et al., 1986; Smith and Mah, 1978). The supplantation of this community by a community of methanogens incapable of methylotrophic methanogenesis would result in increased organo-sulfur compounds in the digester headspace.

The relative instability of the methanogenic population in thermophilic anaerobic digesters has been supported by simplified PCR-DGGE archaeal fingerprints of thermophilic digester communities when compared to their mesophilic counterparts (Chen et al., 2005). Considering

this instability, and the supplantation of *methanosarcina sp.* observed by Zinder et al., the fluxes in headspace VSC production measured at HTP and TITP were likely due to stress imposed on the methanogenic community due to transient digestion temperature, and not due to changes in VSC production stemming from degradation of proteinaceous material. Though the accumulation of VSC in the headspace of an anaerobic digester and the accumulation of VSC associated with dewatered biosolids are both the likely result of the pathways described in this review, very different mechanisms are responsible for these two cases. Dewatered biosolids odor is greatly affected by shear input and polymer addition during dewatering (Chen et al., 2005; Higgins et al., 2006; Muller et al., 2004) while digester headspace odors are dependent digester operation (Iranpour et al., 2005a). Though previous research on digester headspace odor provides useful information about the relationship between methanogens and VSC producers, these distinctions between digester headspace and dewatered biosolids VSC production make comparisons from previous literature problematic.

Previous research has shown that the peak concentration of headspace VSC in sealed bottles containing dewatered biosolids digested thermophilically was reduced by approximately 50% when compared samples digested at 35°C (Kumar et al., 2006). This comparison supports the hypothesis that biosolids digested at higher temperatures support less VSC production. However, changes in reactor operation throughout the study including variations in feed solids concentration, thermophilic temperature fluctuation, and investigations of various solids retention times tend to limit the value of this research when examining the effect of steady-state digestion temperature on the biosolids VSC odor potential. In order to better determine the effect that digestion temperature may have on the activity of VSC producers in digested and dewatered biosolids, investigations of steady state digestion temperature across a range of thermophilic temperature should be conducted under operationally defined standard conditions (i.e. constant SRT, solids concentration, and temperature control).

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Chapter 3

Manuscript 1

Title:

The Effect of Temperature on CO_2-Reducing Methanogens in a Thermophilic Anaerobic Sewage Sludge Digester

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The Effect of Temperature on CO_2-Reducing Methanogens in a Thermophilic Anaerobic Sewage Sludge Digester

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Abstract Sustainable operation of an anaerobic sewage sludge digester requires the effective shuttling of organic carbon from dense solid material to CH_4 gas. The accumulation of intermediates and metabolic products such as short chain fatty acids and hydrogen gas not only reveal inefficiency within the digestion process, but can be detrimental to reactor operation at sufficiently high levels. In this study, the effect of steady state digestion temperature on the accumulation of fatty acids and hydrogen gas in thermophilic (49°C-57.5°C) anaerobic digesters treating sewage sludge was used to determine the mechanism of methanogenic inhibition at high temperatures. Methane production at 57.5°C was reduced by approximately 80% compared to the other digestion temperatures. In addition, data showed that the accumulation of volatile fatty acids (C2-C7 fatty acids, VFA) accounted for approximately 19% of volatile solids reduction (VSR) on a COD basis in the 57.5°C digester, while VFA accumulation at temperatures less than 55°C only accounted for 3.6% ± 1.2% of VSR.

Thermodynamic calculations considering the conversion of propionic acid to acetic acid under each experimental reactor condition support the observed accumulation of longer chain fatty acids at 57.5°C. Considering the steady state concentrations of acetate, propionate, CO_2, and H_2 at each observed digestion temperature, data suggest that the oxidation of propionate yields a beneficial release of energy only when it is coupled with effective CO_2-reducing (hydrogenotrophic) methanogenesis. Accumulation of headspace hydrogen in high temperature reactors (55°C, 57.5°C), resulting in feedback inhibition of fatty acid oxidation and subsequent VFA accumulation at 57.5°C, suggests that hydrogenotrophic CO_2-reducing methanogens are likely the most sensitive methanogenic population to increased digestion temperature. Inefficient CO_2-reduction at 57.5°C, effectively uncoupling syntrophic propionate oxidation from methanogenesis, is a likely explanation for the data observed in this study.

The observations in this study are supported by PCR-DGGE microbial community assessment at selected digestion temperatures. The result of this analysis shows greatly reduced archaeal community diversity in the 57.5°C digester. Decreased archaeal community diversity at high digestion temperatures suggests that one or more important methanogenic populations, such as CO_2-reducers, may be missing or severely impacted at digestion temperatures greater than 53°C.

Keywords Digestion temperature; hydrogen; methanogen; thermophilic anaerobic digestion

INTRODUCTION

Stringent pathogen regulations required for the land application of domestic wastewater biosolids have revitalized interest in thermophilic anaerobic digestion. Since biological activity at these temperatures requires specialized organisms equipped with thermostable cell components and enzymes, thermophilic anaerobic digestion has recently been recognized as holistically unique biological process, rather than a close variation on mesophilic anaerobic digestion. As such, much of the fundamental discussion regarding anaerobic digestion technology, keeping mesophilic anaerobic digestion in mind, is inadequate for the development of a thorough understanding of the design parameters and operational variables that greatly affect thermophilic performance.

Several recent studies have focused on the effect of operational parameters such as solids retention time (SRT), feeding pattern, and digestion temperature on the operation of thermophilic anaerobic digestion systems. Since the legal definition of the level of treatment required for adequate pathogen deactivation as provided by CFR 40 Part 503 is based directly on the length of treatment (essentially SRT) and the temperature at which treatment takes place (reactor temperature in the case of anaerobic digestion), the effect of these parameters on the operability of a digestion system are of particular interest.

Decisions concerning digestion process design with the goal of achieving a prescribed level of pathogen destruction often favor high temperature, short SRT systems due to the high cost of large volume systems. However, comparisons between reactors operated over a range of thermophilic temperatures provide support for the contrary. It has been observed that under a constant SRT of approximately 15 days (Gray et al., 2006), the accumulation of organic digestion intermediates is greatly increased by elevated steady-state digestion temperature (53°C, 62°C) when compared to lower temperature systems (35°C, 49°C). Specifically, the concentrations of acetic and propionic acid accumulated to greater than 500 mg/L and 800 mg/L, respectively, at digestion temperatures at or above 53°C. The concentration of these acids also varied with SRT as operation of thermophilic anaerobic digesters at retention times as low as 2 days resulted in an increase in total volatile fatty acid concentrations (VFA) by a factor of two to six. Organic acids, along with hydrogen gas (H_2) and carbon dioxide (CO_2) have been identified

as important intermediates during the anaerobic conversion of complex organic substrates such as domestic wastewater sludge to methane gas (CH_4). Investigations of metabolic intermediates within the anaerobic digestion process have yielded useful information as to the relationship between the relative activity of methanogenic and fermentative communities, and the stability of an anaerobic digestion process.

Similar results showing the accumulation of digestion intermediates during single-staged thermophilic digestion are found in the literature. A comparative study of mesophilic and thermophilic reactors (Kim et al., 2002) showed that both batch and continuous flow thermophilic anaerobic digestion systems operated at 55°C and an SRT of 20 days exhibited significant accumulation of VFA, specifically propionic acid. By comparison, mesophilic anaerobic digesters maintained an average steady-state propionic acid concentration of less than 60 mg/L, whereas propionic acid levels at 55°C approached 3000 mg/L. VFA accumulation within the thermophilic reactor was partially relieved by separating the 20 day SRT digester into primary and secondary thermophilic reactors having retention times of 2 and 18 days, respectively.

A full scale investigation of the impact of digestion temperature on digester stability concluded that a thermophilic anaerobic digester could be reliably operated with no perceived VFA accumulation (Iranpour et al., 2005). A relatively quick temperature increase from 54°C to 58°C within a single stage thermophilic anaerobic digester caused an increase in total VFA concentration from 450 mg/L to 1050 mg/L as acetate. A subsequent decrease in digestion temperature resulted in a decrease in VFA concentrations to below 600 mg/L as acetate. However, when the digestion temperature was gradually increased from 53.9°C to 57.2°C, random variation in total VFA concentrations were observed between 150 mg/L and 450 mg/L with no apparent correlation with digestion temperature. The ability of the digester in the long term temperature change experiment to acclimate to gradual temperature change suggests that digester instability may indeed be due to microbial population dynamics between different environmental conditions.

Bouškovᡠet al. (2005) similarly determined that microbial community composition is likely a determining factor in the potential buildup of VFA under thermophilic anaerobic digestion. Anaerobic digester cultures acclimated to 37°C were subjected to temperature increases, both stepwise and at once, to 55°C. It was determined that the step-wise increase resulted in comparatively higher steady-state total VFA concentrations during subsequent thermophilic digestion. The researchers proposed that this was due to a truly thermophilic community that developed during an abrupt shift to thermophilic temperatures. Conversely, a step-wise increase in digestion temperature allowed survival of mesophilic organisms that were not able to effectively metabolize VFA at high temperatures.

The results of previous research suggest that the stability of an anaerobic digestion process is effectively enhanced at lower digestion temperature and longer retention times, which is unfavorable in terms of relatively large reactor volumes needed for efficient pathogen reduction. Secondly, the mechanisms that allow full-scale thermophilic systems to be operated with relative success in terms of process stability and digestion performance are not well understood. A better understanding of such mechanisms could promote the reliable application of high-temperature digestion systems for enhanced pathogen destruction without sacrificing process stability. This study aims to determine the effect that specific digestion temperatures have on anaerobic digestion performance and to elucidate the mechanistic effect that elevated digestion temperature plays on reduced process stability, leading to digester upset and failure.

EXPERIMENTAL METHODS AND MATERIALS

Anaerobic digester setup and operation

High-density polyethylene batch fermentation reactors supplied by Hobby Beverage Equipment Company (Temecula, California) were chosen for this study. The conical bottom of these vessels was thought to be advantageous in terms of mixing and suspension of grit, similar to the full-scale application of egg-shaped anaerobic digesters. The nominal volume of each vessel was 25 liters (L) and was operated with an active volume of 22.5 L. The reactor vessels were modified to accept a threaded stainless steel thermometer, also supplied by Hobby Beverage. Digesters were operated at 35°C, 49°C, 51°C, 53°C, 55°C, and 57.5°C. Replicate digesters at

53°C and 57.5°C were constructed and operated in order to investigate the reproducibility of the results of this study. For the purpose of this paper, the reactors are named according to their operating digestion temperature. Replicate reactors of the same temperature are designated by appropriate subscripts. The digesters were heated using an external circulating water bath (Haake DC10-W19) attached to 13 mm i.d. vinyl tube water jacket around each digester. This heating method controlled the internal temperature of the digesters within ± 0.2°C of the nominal temperature.

A conical stainless steel batch fermentation reactor produced by Blichmann Engineering, LLC was used in the operation of reactor 49°C. The reactor had similar dimensions and the same nominal volume as the HDPE reactors produced by Hobby Beverage and was therefore determined to be an equivalent reaction vessel for anaerobic digestion. Unfortunately, the reactor lid consisted of a stainless steel to stainless steel joint and leaking of digester gas from the reactor was unpreventable. Compositional analysis of the digester headspace did not yield significant quantities of ambient air (N_2 plus O_2), so the digester was simply maintained in the absence of volumetric digester gas production data.

At startup, digesters were seeded with approximately 15 L of mesophilic anaerobically digested sludge from Pepper's Ferry Regional Wastewater Treatment Facility (PFRWTF; Radford, Virginia). Previous research has shown that relatively quick acclimation of an anaerobic digester microbial community can be achieved by immediately exposing mesophilic sludge to a new thermophilic temperature (Bouskova et al., 2005). Following initial seeding, digester gas production ceased within approximately five days and then gradually increased to a steady rate for each digester. This suggests that the mesophilic microbial community had been deactivated due to the increased temperature, and a lag phase was required for the thermophilic microbial community to grow within each reactor. Rebel™ wet-tip gas flow meters (Nashville, Tennessee) were used to monitor daily gas production rates by the anaerobic digesters. This startup regime helped to ensure that the microbial community within each anaerobic digester was unique, and solely dependant on the applied digestion temperature. After approximately 15 days of acclimation to the desired digestion temperature, daily feeding in the absence of wastage was used to bring the digester contents up to its final operating volume. Each reactor was monitored

in terms of pH (variation of less than 0.1 over one retention time) and gas production (daily production not deviating by more than 25% from average over one retention time) in order to determine that a steady state microbial community had been reached prior to sampling for the experimental analyses described in the following sections. In the case of the 49°C digester from which quantification of biogas production was not possible, monitoring of pH was used solely as an indication of steady-state performance.

The digester feed consisted of a 1:1 ratio of primary and secondary solids from DCWASA Blue Plains Advanced Wastewater Treatment Facility (BPAWWTF) measured on a mass basis and diluted to approximately 3% total solids. In order to achieve the desired SRT, the digesters were daily batch fed 1.5 L of blended sludge. Ice-packed primary and secondary solids were received weekly from BPAWWTF via overnight shipment. Solids were then blended at the appropriate ratio and homogenized using a 200-watt Hamilton Beach kitchen stick blender. Pre-screening of the feed sludge to remove large particles was not required.

Suspension and mixing of the reactor contents was achieved by digester gas recirculation. Variable speed (6-600 rpm control) peristaltic pumps manufactured by Cole Parmer (Vernon Hills, Illinois) and Cole Parmer Masterflex Tygon LFL-17 pump tubing were used for gas pumping. General gas recirculation throughout reactor operation was controlled at approximately 0.8 liters per min. (50% max.). Homogenization of the reactor contents prior to sampling was accomplished by increasing the recirculation rate to approximately 1.5 L per min. (100% max.) A fixed solids mass balance across the digesters was performed and revealed greater than 99% recovery on average using this mixing regime. The increased recirculation rate was also applied for about 10 min. after feeding to disperse the raw sludge throughout the digester.

Gibbs free energy change

The Gibbs free energy for the reactor under non-standard conditions ($\Delta G'$) was calculated on the basis of previously published (McCarty and Smith, 1986; Rossini et al., 1952) standard Gibbs free energies for the individual reactions involved in syntrophic propionate oxidation. The calculation of $\Delta G'$ included temperature corrections to standard Gibbs free energy ($\Delta G^{o\prime}$) for the

various reactor conditions by applying the Gibbs-Helmholtz equation (Scholten and Conrad, 2000) as well as measured concentrations of appropriate reactants and reaction products.

Headspace analyses

Headspace hydrogen (H_2) concentrations were analyzed using a reduction gas detector (Trace Analytical RGA5). Nitrogen was used as the carrier and actuator gasses at approximately 275 kPa and 414 kPa respectively.

Headspace methane (CH_4) and carbon dioxide (CO_2) were analyzed on a Shimadzu Gas Chromatograph (Model GC-14A) with a thermal conductivity detector (TCD). The column was constructed from a 4 meter length of copper tubing with a 6.35 mm inner diameter. The column was coiled to fit in the GC-14A oven and packed with Haysep Q media (Supelco, Bellefonte, PA). Helium was used as the carrier gas at a flow rate of approximately 17 ml/min.

Calculation of $\Delta G'$ of specific chemical reactions required the conversion of headspace H_2, CH_4, and CO_2 concentrations to associated dissolved gas concentrations. The calculation of dissolved species concentration was based on temperature corrected Henry's law constants to achieve molar concentrations of dissolved gasses within each reactor's liquid volume.

Volatile fatty acid concentrations

Volatile fatty acids (VFA) were measured weekly on the solution phase of each digester. Samples for VFA analysis were passed through a 0.45 μm nitrocellulose membrane filter and frozen prior to analysis. VFA were measured using a Shimadzu gas chromatograph (Model GC-14A) with flame ionization detector (FID). Species separation was performed using a Nukol™ fused silica 15 m x 0.53 mm capillary column with 0.5 μm film thickness. A Shimadzu computer integrator (Model CR501 Chromatopak) was used for data analysis. Helium was used as the carrier gas at a flow rate of 17 ml/min. Additional gasses are as follows: Hydrogen, 45 ml/min; Air, 450 ml/min; Nitrogen, 13 ml/min.

Volatile fatty acids are expressed as mg/L of individual species (C2-C7 fatty acids). Individual acid concentrations are converted to acetate on a theoretical oxygen demand basis and summed to report Total VFA as mg/L as acetate.

PCR-DGGE community analysis

Samples were collected aseptically from the digesters and feed source, and shipped on dry ice to Bucknell University (Lewisburg, PA) for community analysis. Prior to analysis samples were stored at -50°C to prevent changes in community structure. DNA extraction and amplification (Ovreas et al., 1997) and denaturing gradient gel electrophoresis (DGGE) (LaPara et al., 2000) were performed as previously described. DGGE was carried out for bacterial and archaeal analyses at run times of 5.5 hours and 3.5 hours, respectively, and with an applied voltage of 200V as previously described (Nakatsu et al., 2000). Primers PRBA338f and PRUN518r were used for community analysis of organisms in the bacterial domain, and primers PARCH340f and PARCH519r were used for community analysis of the archaeal community (Chen et al., 2005).

Other analyses

Total and volatile solids concentrations (Method 2540-G) as well as total alkalinity (Method 2320-G) were analyzed as specified in Standard Methods for the Examination of Water and Wastewater (APHA, 1995).

RESULTS AND DISCUSSION

Statistical analysis of reactor performance

Reactor performance is determined by the activity of fermentative and methanogenic organisms across a range of thermophilic temperatures. While dissolved hydrogen and organic acid concentrations have been supported as early warning indicators of digester upset and failure in past studies (CordRuwisch et al., 1997), fundamental discussions of anaerobic digestion technology also point out reactor pH and methane output as effective measures of overall digester health (Parkin and Owen, 1986). Since steady-state reactor pH and daily methane

production were well characterized throughout the course of this study, statistical analysis of reactor operation is based on these analyses.

Statistical analyses were performed using NCSS statistical software package (Kaysville, Utah). All statistical analyses were carried out at the 95% confidence level ($\alpha = 0.05$). Reactor pH data ($n \geq 25$) were found to be normally distributed over the period of steady-state data collection based; random variation in methane production rates caused these data ($n \geq 21$) to be non-normal. Since it was desired that the same statistical analysis be used for pH and methane data, a nonparametric test was required for this condition. The Kruskal-Wallis Z-test was used in this case since it did not require the assumption of data normality.

Kruskal-Wallis one-way analyses of variations (ANOVA) determined that significant differences existed in both reactor pH and methane production data between at least two reactors. The following remarks resulted from the Kruskal-Wallis multiple comparison z-value tests conducted on these data.

Replicate reactors 57.5°C_1 and 57.5°C_2 had statistically similar steady state pH. Methane production by the same reactors was statistically different; however comparison of the closeness of the z-statistic (2.1886) and z-critical (1.9600) for this analysis shows that relative similarities exist between these reactors that were slightly beyond the confidence level of this statistical analysis. With respect to reactor pH, reactors 49°C, 53°C_2, and 55°C were statistically similar; and, the same is true for reactors 51°C and 53°C_1. Similarity of replicate reactors operated at 53°C were slightly outside the confidence interval set for this analysis (z-statistic = 2.1490). The appearance of replicate reactors operated at 53°C in two statistically dissimilar groups suggests the inherent variability of the operation of mixed culture reactors for the degradation of a complex substrate such as raw wastewater sludge.

In all cases, methane production by reactors operated at 55°C and below was statistically dissimilar from that of reactors operated at 57.5°C. Statistical similarity was observed in methane production rates between reactors 53°C_1, 53°C_2, and 55°C. Analysis of methane output of reactor 51°C was affected by three low methane gas production readings ranging from 2.4 to

6.3 liters of methane per day and was statistically dissimilar in terms of methane production to all other reactors in this study. Theses methane production values are were likely erroneous since low methane output by this digester is not supported by additional data (VSR, VFA, pH) collected during this study. Intermittent leaking of gas from the reactor or failure of the gas flow meter for this reactor are likely explanations.

In general, the statistical analyses that were conducted on steady-state reactor pH and methane production support the separation of digesters in this study into two groups: those reactors operated at 57.5°C and those operated at temperatures between 49°C and 55°C. Replicate reactors were also observed to show considerable agreement, supporting the repeatability of the results achieved during this study. The consideration of these two groups is a useful starting point for further discussion of the results of this study in terms of digestion performance and stability.

Digestion performance

Laboratory-scaled anaerobic digesters were operated in order to study the effect of specific thermophilic digestion temperatures on methanogenic and reactor stability. As discussed previously, reactor seeding, start-up, and steady-state operation was carried out such that a distinct thermophilic microbial community would develop that could be adequately compared on the basis of digestion temperature. For each reactor in this study, the daily biogas production rate stabilized after approximately two retention times of routine feeding and wasting (data not shown).

Average steady state daily production rates of CH_4, CO_2, and total digester gas are listed in Table 3.1 below. From the data, it appears that optimal CH_4 production occurred in the range of 53°C to 55°C, with a maximum specific CH_4 production rate of 0.40 L CH_4/g VS_{Feed} at 53°C (Feed = 3.0% TS, VS/TS= 0.78). Operation of anaerobic digesters at 57.5°C showed a reduction in specific CH_4 production by up to 78% (57.5°C_2 vs. 53°C_2). In addition, the presence of CO_2 in the digester headspace as compared to CH_4 is greatly increased at 57.5°C.

Table 3.1. Average steady-state digester gas production and composition (n≥20).

	Total Digester Gas	Methane (CH_4)		Carbon Dioxide (CO_2)	
	L/day	L/day	% of total	L/day	% of total
35°C	11.18 ± 0.7	7.0 ± 0.3	62.9 ± 2.5	3.8 ± 0.1	33.8 ± 1.0
51°C	20.6 ± 5.7	10.8 ± 3.0	54.5 ± 3.7	6.1 ± 1.7	30.4 ± 4.2
53°C_1	20.2 ± 5.1	12.9 ± 1.2	63.9 ± 1.3	6.8 ± 0.8	33.4 ± 0.8
53°C_2	23.1 ± 1.9	14.1 ± 1.1	61.2 ± 0.2	7.6 ± 0.7	33.0 ± 2.0
55°C	22.2 ± 2.0	13.0 ±1.3	58.6 ± 1.4	6.3 ± 2.4	31.7 ± 3.5
57.5°C_1	7.0 ± 1.7	3.2 ± 0.5	45.9 ± 6.7	2.5 ± 0.4	38.5 ± 1.5
57.5°C_2	10.0 ± 1.1	4.8± 0.5	47.8 ± 1.6	3.9 ± 0.5	38.7± 1.4

Note: Leaking from reactor 49°C made quantification of digester gas production unreliable. As such, this reactor is not included in analyses considering volumetric gas production throughout this study.

Generally, the ratio of headspace CO_2:CH_4 for digesters operated at or below 55°C ranged between 0.48 and 0.56, whereas the same ratio at 57.5°C was 0.78 to 0.81. The increased ratio of CO_2:CH_4 at 57.5°C suggests an imbalance in the natural homeostasis of between CO_2-producing and CO_2-utilizing organisms at this temperature.

As described previously (McCarty, 1964; McCarty and Smith, 1986; Parkin and Owen, 1986), the production of CH_4 and CO_2 from an anaerobic digester is the result of the biodegradation of organic material in the raw sludge feed via hydrolysis, fermentation, and methanogenesis. The rate of CH_4 gas production then, is directly related the rate of removal of solids from the digester, measured as volatile solids reduction (VSR). Assuming similar feed to each reactor, a plot of daily CH_4 production versus VSR should result in a close linear correlation; however, as shown in Figure 3.1, the correlation between measured (raw) VSR and CH_4 production only yields R^2 = 0.66, suggesting that there exists an alternate fate for particulate organics separate from CH_4 production.

As mentioned above, the full conversion of complex particulate organic material to CH_4 and CO_2 requires the involvement of reactions including hydrolysis and acid fermentation. VSR is more directly a measurement of these preliminary reactions, rather than methanogenesis. Previous studies have identified C2-C7 fatty acids, or volatile fatty acids (VFA), as important

intermediates immediately preceding CH_4 fermentation during anaerobic digestion. The accumulation of such intermediates could signify the diversion of some organic carbon away from CH_4 formation either by inefficiency or inhibition of methanogenesis. Table 3.2 shows that the concentrations of VFA in digesters across the temperature range are widely varied. The differences in the concentration of soluble digestion intermediates represent organic material that is reflected under the analysis of VSR but not CH_4 production.

The use of volatile solids analysis as an indication of the net degradation of organic material is insufficient in the case of widely varied VFA concentrations. During anaerobic digestion, high molecular weight polysaccharides, proteins, fats, and carbohydrates are converted to relatively low molecular weight VFA. VFA are operationally defined as those fatty acids that can be removed from solution via simple distillation (Rittmann and McCarty, 2001) which occurs during total solids analysis (at 104°C). The net loss of volatile organic material during total solids analysis tends to cause the misrepresentation of organic material during subsequent volatile solids analysis. In order account for the volatilization of VFA during total solids analysis in the correlation between CH_4 production and VSR as previously described in Figure 1, the total VFA concentrations listed in Table 3.2 were converted on the basis of carbonaceous oxygen demand (COD) to represent volatile solids. The characterization of biological volatile solids fed to the laboratory digesters is assumed to be approximated by the previously published empirical formula for heterotrophic biomass, $C_{10}H_{19}O_3N$ (Rittmann and McCarty, 2001). Alternate empirical formulas are available to characterize various sludges (e.g. nitrifying activated sludge, BioP sludge); however, no data were available during this study that supported their use. Measurements of organic nitrogen in the digester feed throughout this study reveal an average concentration of 2130 mg/L ± 240 mg/L as nitrogen (measured on 4% TS blended sludge), or 6.8% of volatile solids by mass. These data support the use of the empirical formula for wastewater sludge, $C_{10}H_{19}O_3N$, which exhibits reasonable agreement with our organic nitrogen measurements at approximately 7.0% nitrogen, by mass.

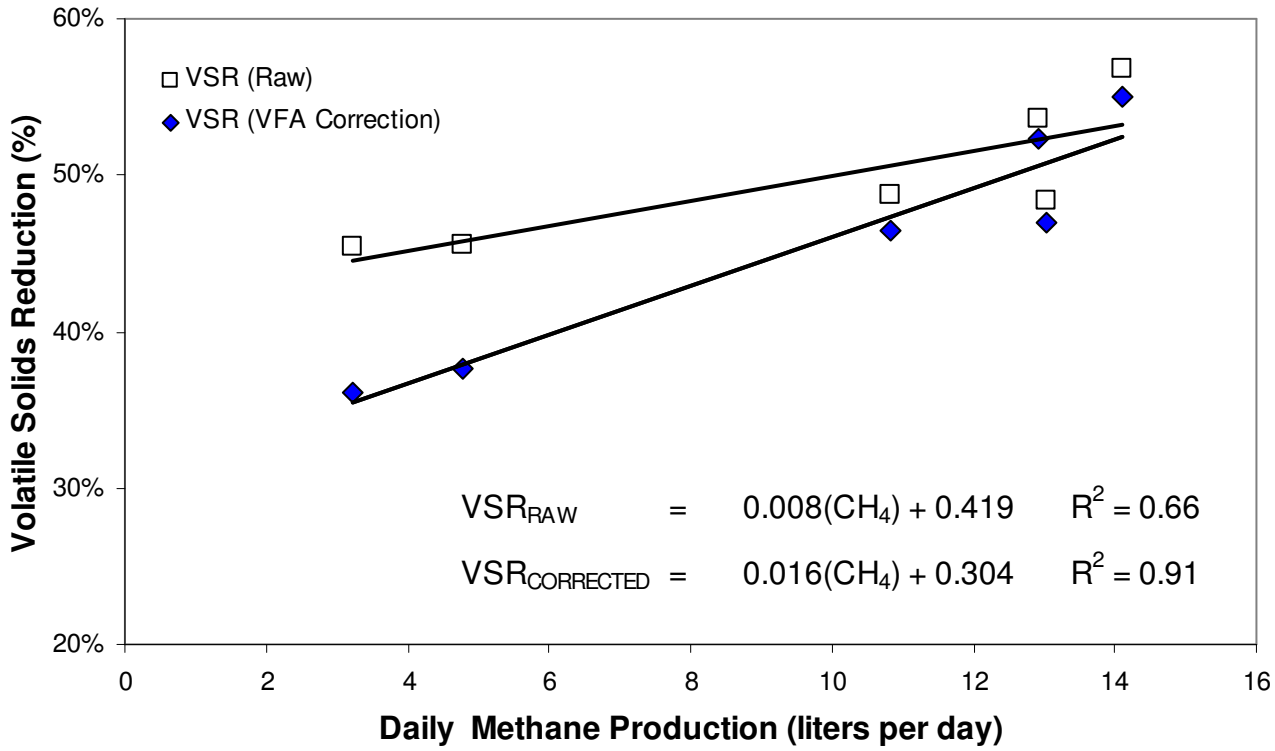


Figure 3.1: Correlation of average steady-state CH_4 production with raw and VFA-corrected VSR. Reactor 35°C (mesophilic digester) was not included in this analysis due to evidence of a largely different fermentative and methanogenic microbial community from the thermophilic digesters, as discussed later.

Using the concentration of VFA in each digester, converted to an equivalent concentration of biological volatile solids, the corrected VSR for each digester as shown in Table 3.3 could then be calculated. When the corrected values for VSR are compared to daily CH_4 production in Table 3.1, the predicted linear correlation ($R^2 = 0.91$) between the two analyses can be seen. The remaining variation between corrected VSR and CH_4 is likely the result of various solubilized digestion products including protein, polysaccharides, and reduced alcohols. The difference between raw and corrected VSR data suggests that a significant portion of organic material that is solubilized at 57.5°C does not undergo complete fermentation and persists as unstabilized COD within the digester in the form of VFA.

Table 3.2: Average steady-state VFA concentrations in laboratory digesters (n≥5).

	Digester Temperature							
	35°C	**49°C**	**51°C**	**53°C$_1$**[a]	**53°C$_2$**	**55°C**	**57.5°C$_1$**	**57.5°C$_2$**
Acetic Acid[b]	23.6	259	219	75	324	70	334	642
Propionic Acid	BDL[d]	352	364	294	328	332	971	1047
Isobutyric Acid	4.9	66	30	27	36	18	362	191
Butyric Acid	BDL	35	35	BDL	99	BDL	308	78
Isovaleric Acid	BDL	68	70	BDL	BDL	6	487	362
n-Valeric Acid	BDL	BDL	13	BDL	BDL	BDL	84	53
Isocaproic Acid	0.8	BDL	5	BDL	BDL	BDL	BDL	17
Hexanoic Acid	BDL	BDL	12	BDL	BDL	BDL	84	BDL
Heptanoic Acid	2.5	76	BDL	BDL	BDL	BDL	BDL	BDL
VFA_{TOTAL}[c]	**38.9 ± 3.5**	**1226 ± 564**	**1039 ±151**	**537 ± 88**	**814 ± 367**	**590 ± 235**	**4052 ± 255**	**3411 ± 523**

[a] Subscripts designate replicate reactors operated at same digestion temperature.
[b] Concentrations given in ppm of individual acid species, unless otherwise noted.
[c] Total VFA expressed as ppm ± STDV as Acetic Acid, converted on a COD basis.
[d] BDL = Below detectable limit.

While the presence of VFA within an anaerobic digester is representative of inefficient conversion of complex organic substrates to CH_4, fatty acids can also be particularly damaging to the digester environment in themselves. The accumulation of VFA serves as a pool of weak acid, of which, dissociation would cause the release of free hydrogen ions into solution, thus lowering the reactor pH. Since the pKa values of acetate, propionate, and butyrate are all less than 5.0, these important VFA species tend to exist in a predominantly deprotonated form at near neutral pH (Rittmann and McCarty, 2001). The effect of VFA in this context is that the steady-state operating pH of digesters operated at 57.5°C was significantly lower than that of digesters operated at 55°C or below, contributing to the relative instability of the higher temperature thermophilic digesters (Table 3.3).

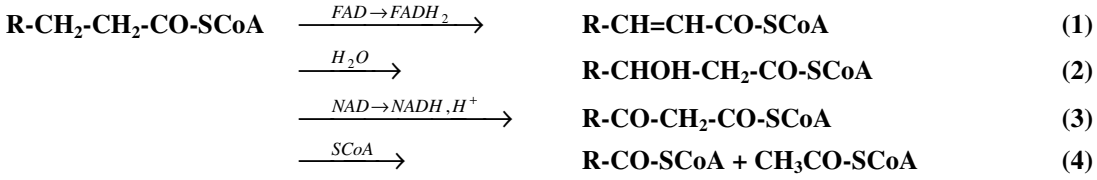
Table 3.3: Average steady-state VSR (n≥5) in laboratory digesters reflecting raw and VFA-corrected data. Steady state pH values are provided as a basis for comparison of the effect that VFA accumulation has on digester stability.

	pH	Volatile Solids Reduction (%)	
	± STDV	Raw	VFA Correction
49°C	7.45 ± 0.04	57.9	55.0
51°C[b]	7.38 ± 0.04	48.8	46.4
53°C$_1$	7.40 ± 0.06	53.6	52.4
53°C$_2$	7.46 ± 0.05	56.8	55.0
55°C	7.46 ± 0.03	48.4	47.0
57.5°C$_1$	7.13 ± 0.07	45.4	36.1
57.5°C$_2$	7.13 ± 0.04	45.5	37.7

Mechanism of temperature-induced digester instability

Previous research has provided evidence of volatile fatty acid accumulation as a result of anaerobic digester upset (Inanc et al., 1996; Iranpour et al., 2005; Kim et al., 2002). As such, the use of short-chain fatty acid buildup as an early warning indicator of an imbalance in the various metabolisms that are involved in anaerobic digestion leading to digester failure has become a common practice. Determining the nature of this imbalance however, requires consideration for the method of VFA degradation under anaerobic conditions as well as degradation by-products.

Degradation of VFA within an anaerobic digester occurs by β-oxidation, a process which produces C2 and C3 fatty acids (acetic and propionic acid) as well as H_2 and CO_2. Each β-oxidation reaction is actually a four-stage process as described in Figure 3.2.



(5) Oxidation (dehydrogenation) of the β-carbon atom by FAD (flavin-adenine dinucleotide) eventually resulting the production of H_2 as FAD is reoxidized.

(6) Hydrolysis of the bond between the C-2 and C-3.

(7) Oxidation (dehydrogenation) of C-3 hydroxyl group to a keto group by NAD (nicotinamide-adenine dinucleotide).

(8) Thiolysis and activation of the keto group resulting in acteyl-SCoA (acetate) and an acyl-CoA two carbon atoms shorter than the original.

Figure 3.2: Single iteration of β-oxidation pathway degrading an activated fatty acid (acyl-CoA group). Process results in the production of acetyl-CoA, H_2, CO_2, and potentially, a residual fatty acid chain to undergo further β-oxidation (Rittmann and McCarty, 2001).

Due to the nature of the two-carbon reaction with each β-oxidation cycle, the end product of successive reactions is dependent on the number of carbons in the parent fatty acid chain. β-oxidation of fatty acids with an even number of carbon atoms will result in the formation of acetate alone; however, oxidation of odd numbered fatty acids such as valeric (C5) acid or heptanoic (C7) acid will result in the production of the C3 VFA, propionate. Propionate is not a viable substrate for further degradation by β-oxidation, as only three carbon atoms are available for the formation of (at a minimum) two acetyl-CoA molecules. The potential for propionate accumulation as a result of β-oxidation of fatty acids having an odd number of carbon atoms means that propionate is an important organic intermediate during anaerobic digestion.

The oxidation of propionate is a bioenergetically disadvantageous reaction. That is, under standard conditions, energy is consumed during the microbial conversion of propionate to acetate. The thermodynamic feasibility of a biochemical reaction is related to Gibb's free energy as described by the following modified form of the Nernst Equation:

$$\Delta G = \Delta G^0 + RT \cdot \ln Q \qquad \text{Equation 3.1}$$

where ΔG and ΔG^0 are the actual and standard free energy associated with a specified reaction, R is the universal gas constant, T is the reaction temperature in Kelvin, and Q is the reaction

quotient. The standard free energy for the direct oxidation of propionic acid to acetate has a standard free energy of $\Delta G^0 \approx 71.6$ kJ, and therefore is endergonic and will not naturally occur without an external driving force (de Bok et al., 2004; McCarty and Smith, 1986; Thiele et al., 1988). The involvement of the reaction quotient, *Q*, in Equation 3.1 allows us to apply this external driving force by creating an environment in which the concentrations of reaction products, acetate and H_2, are sufficiently reduced by synergistic biological reactions.

Various pathways for the oxidation of propionate to acetate under anaerobic conditions have been proposed; however most environmental instances of this reaction have been shown to rely on either syntrophic (de Bok et al., 2001; Liu et al., 1999) or cometabolic (Guven et al., 2005) metabolism. These studies provide evidence of the dependence of propionate oxidation on the environmental concentration of acetate and H_2. It has been previously asserted the control of H_2 rather than acetate is a feasible means of manipulating the equilibrium of propionate oxidation (McCarty and Smith, 1986). In this study, dissolved H_2 concentrations ranged between 10^{-8} M and 10^{-7} M, while acetate was observed between 10^{-5} M and 10^{-2} M. Since dissolved H_2 concentration is several orders of magnitude less than that of acetate, a large effect on the reaction quotient can be attained by a relatively small removal of H_2 from the digester.

In a methanogenic environment, the regulation of H_2 is carried out by CO_2-reducing methanogens (also referred to as hydrogenotrophic methanogens). As described by the chemical reaction for CO_2-reduction in Table 3.4 (Equation 3.3), four mol of H_2 are consumed in the reduction of a single mol of CO_2 by these organisms. Previous research has suggested that under healthy methanogenic conditions, hydrogenotrophic methanogenesis accounts for approximately one-third of total CH_4 production. The stoichiometric need for H_2 shows that coupling propionate oxidation with CO_2-reduction is an effective means for controlling dissolved H_2 such that the syntrophic oxidation of propionate to acetate remains thermodynamically favorable.

Table 3.4: Biochemical reactions involved in the net syntrophic oxidation of propionic acid to acetic acid.

Description	Chemical Reaction	
Propionate Oxidation	$CH_3CH_2COO^- + 2\ H_2O \rightarrow CH_3COO^- + 3\ H_2 + CO_2$	Eq. 2
Hydrogenotrophic Methanogenesis (CO_2-Reduction)	$3\ H_2 + ¾\ CO_2 \rightarrow ¾\ CH_4 + 1.5\ H_2O$	Eq. 3
Syntrophic Propionate Oxidation (Net Reaction)	$CH_3CH_2COO^- + ½\ H_2O \rightarrow CH_3COO^- + ¾\ CH_4 + ¼\ CO_2$	Eq. 4

The steady buildup of dissolved H_2 gas with increased temperature shown in Table 3.5 suggests that the microbial communities present in reactors 55°C and 57.5°C_2 are inefficient at scavenging H_2 to ensure uninhibited removal of propionic acid and other VFA. When considering the non-standard conditions (i.e. reactor temperature and concentration of products and reactants) of each reactor, the oxidation of propionic acid is thermodynamically unfavorable ($\Delta G' > 0$ kJ). It is only when oxidation of propionate is coupled with the reduction of CO_2 to CH_4 that a beneficial energy release is achieved ($\Delta G' < 0$ kJ). Assuming that hydrogenotrophic methanogens are the primary consumers of H_2 under methanogenic conditions, factors that tend to inhibit this process would likely promote increased H_2 measurements within the reactor. The likely result of methanogenic inhibition then, is feedback inhibition of H_2 producing metabolisms, including the β-oxidation of fatty acids and the oxidation of propionic acid to acetate. Sufficiently low activity of hydrogenotrophic methanogens could effectively result in the decoupling of VFA oxidation by fermentative bacteria and hydrogen consumption by methanogens. As such, the buildup of VFA in reactors 57.5°C_1 and 57.5°C_2 is likely the effect low CO_2-reducing methanogenic activity as affected by this high digestion temperature.

The concentrations of individual and total VFA listed above in Table 3.2 reveal the effect of the hypothesized feedback inhibition of β-oxidation by H_2. In digesters operated at or below 55°C, the concentration of propionic acid was maintained below the maximum steady state average of 364 mg/L at 51°C. By comparison, the average steady state concentration of propionic acid at 57.5°C_1 was 971 mg/L, an increase of approximately 270%. Similarly, reactor 57.5°C_2 exhibited an increase in propionic acid concentration of 290% over reactor 51°C. It is interesting to note that the concentrations of H_2 and propionic acid have a similar effect on C5 fatty acid oxidation as H_2 and acetic acid have on propionate oxidation. The increased presence of isovaleric and n-

valeric acid, both C5 fatty acids and direct predecessors to propionic acid during β-oxidation, provides support for the notion that VFA accumulation can be caused by inhibition of H_2-utilizing methanogens at high temperatures leading to propionic acid accumulation and further inhibition of the oxidation of longer chain fatty acids.

The low total VFA concentrations observed at 55°C in the presence of elevated headspace H_2 measurements suggests that a threshold for digestion temperature likely exists below which the effective oxidation of VFA takes place, and above which syntrophic fatty acid oxidation and hydrogenotrophic methanogenesis are effectively decoupled. The effect of increased H_2 on the thermodynamics of propionate oxidation can be seen in Table 3.5. The oxidation of propionic acid to acetate exerts an increasing requirement for external energy as digestion temperature is increased. This need for energy can be effectively satisfied by hydrogenotrophic methanogenesis, as is the apparent case in reactors operated at 55°C or less. The calculated free energy release under these reactor conditions is consistent with the previously reported range required for syntrophic propionate oxidation within methanogenic environments (Scholten and Conrad, 2000). Although the thermodynamic conditions within reactor 57.5°C_2 could apparently support microbial growth, an extenuating factor such as thermal inhibition of hydrogenotrophic methanogens is likely responsible for inefficient propionate oxidation.

Table 3.5: Calculation of Gibbs free energy release during syntrophic propionate oxidation under measured environmental and chemical reactor conditions.

	Conc. H_2 µM dissolved (ppm in headspace)		Free Energy Release (ΔG´)		
			Propionate Oxidation	Hydrogenotrophic Methanogenesis (CO_2-Reduction)	Net Syntrophic Propionate Oxidation
35°C	1.0 x 10^{-2}	(13.9)	NA[a]	-52.4 kJ	NA
49°C	1.8 x 10^{-2}	(26.5)	44.1 kJ	-61.4 kJ	-17.4 kJ
51°C[b]	4.2 x 10^{-2}	(62.2)	50.0 kJ	-71.6 kJ	-21.7 kJ
53°C_2[c]	2.6 x 10^{-2}	(39.2)	46.3 kJ	-66.1 kJ	-19.7 kJ
55°C	5.1 x 10^{-2}	(75.6)	45.6 kJ	-73.3 kJ	-27.7 kJ
57.5°C_2	7.4 x 10^{-2}	(112.3)	53.1 kJ	-81.4 kJ	-28.3 kJ

[a] Propionic acid was not detected at 35°C. Reaction quotient is undefined for this condition.
[b] 51°C digester used in this analysis was operated at 7.5 SRT.
[c] Headspace hydrogen measurements were not made for reactors 53°C_1 and 57.5°C_2.

Microbial community assessment of selected digester samples

As described in this study, the concurrent accumulation of H_2 and fatty acids such as that observed in reactors 57.5°C_1 and 57.5°C_2, are indicative of hydrogenotrophic methanogenic inhibition. In order to better elucidate the effect that steady state digestion temperature has on the methanogenic community within each anaerobic digester, PCR-DGGE analysis was performed on selected samples across the temperature range. In this way, a qualitative microbial fingerprint of each digester could be effectively observed. The bacterial community in the feed, mesophilic digester, and three thermophilic digesters represent three distinct microbial communities (Figure 3.3a.). The variation in bacterial community between the feed solids and both classes of anaerobic digesters reveals that the seeding protocol was indeed effective in producing a microbial community solely affected by digestion temperature as a variable. It is also interesting to note the similarities in the bacterial communities between reactors 49°C, 53°C_2, and 57.5°C_2. Possible explanations for these similarities are as follows: 1. Digester seed obtained from PFRWTF included a small dormant population of thermophilic organisms that were better expressed by the operating scheme of our laboratory digesters. 2. The primary and secondary sludge blend attained from BPAWWTF contained thermophilic organisms capable of carrying out anaerobic metabolisms. Evidence for this alternative has been previously provided by studies in which anaerobic thermophilic digesters were seeded solely with aerobic mixed liquor (Kim and Speece, 2002). The researchers concluded that thermophilic anaerobic fermentation of glucose and subsequent methanogenesis could be achieved with little or no lag phase using the aerobic seed. Both of these explanations seem to be a plausible explanation for the similarities observed between microbial communities in the thermophilic range. As such, the actual microbial communities that developed in the laboratory digesters are the likely result of the combined effect of the digester seed and regular blended sludge feed.

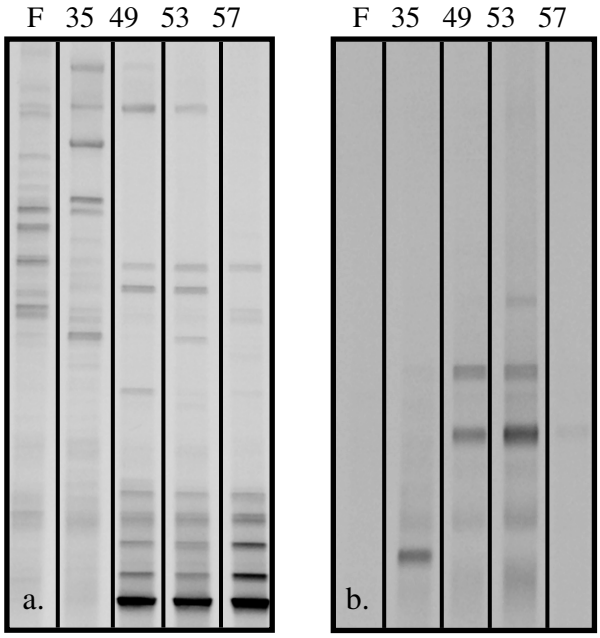


Figure 3.3: Bacterial (a.) and Archaeal (b.) community profiles of blended sludge feed (F), and reactors 35°C (35), 49°C (49), 53°C_2 (53), and 57.5°C_2 (57).

The archaeal community PCR-DGGE (Figure 3.3b.) analysis provided useful insight into methanogenic inhibition occurring at high digestion temperatures. In an anaerobic digester, analysis of total archaeal population is considered to be representative of the methanogenic population (Chen et al., 2005). Analysis of reactors 35°C, 49°C, and 53°C_2 yielded the presence of distinct bands, representing individual populations of methanogenic organisms. Interestingly, fewer bands were identified in the mesophilic reactor, signifying less microbial diversity among methanogens. Similar results in previous research have been interpreted as a community lacking the biological diversity to resist significant environmental stressors; however, data from this study suggest that the limited archaeal community observed at 35°C is indeed effective in regulating fatty acids and H_2 within the digester. Methanogenic communities observed in reactors 49°C and 53°C_2 were robust, having multiple well defined bands. Reactor 57.5°C_2, however, failed to produce any well defined bands representing methanogenic DNA. The minimal production of CH_4 by this digester suggests that the poorly defined shadows seen in Figure 2b at 57.5°C represent some quantity of methanogenic biomass. The results of the PCR-DGGE analysis for the three thermophilic digesters analyzed in this study support the hypothesis of methanogenic inhibition at high digestion temperature. This information in conjunction with the accumulation of H_2 in high temperature reactors (55°C, 57.5°C) resulting in feedback

inhibition of fatty acid oxidation and subsequent VFA accumulation at 57.5°C suggests that hydrogenotrophic CO_2-reducing methanogens are likely the most sensitive methanogenic population to increased digestion temperature.

CONCLUSION

The results of this study, in conjunction with previous research (vanLier, 1996), suggest that the performance and stability of anaerobic digestion may be limited by digestion temperatures exceeding 55°C. The consideration of anaerobic digestion intermediates and products including volatile fatty acids, gaseous hydrogen, carbon dioxide, and methane helps to describe why digester instability and VFA accumulation are often observed at high operating temperatures. The accumulation of certain digestion intermediates can likely be correlated to the temperature induced shift in microbial community at various steady-state digestion temperatures. While further analysis of microbial population and methanogenic pathway shifts at various temperatures would be useful in order to better ascertain the responsible mechanism(s) of the temperature-sensitivity observed during thermophilic anaerobic digestion, current data point to the thermal inhibition of hydrogenotrophic methanogens as a likely cause.

While much of the previous research in VFA accumulation during anaerobic digestion has been primarily focused on the thermodynamics of syntrophic fatty acid oxidation, the mere calculation of $\Delta G^{\circ\prime}$ for a given reactor conditions may not sufficiently predict stable operation. Extenuating factors such as the thermal inhibition of a key microbial population may tend to limit the application of high temperature thermophilic anaerobic digestion, even under thermodynamically favorable conditions.

The implication of this research is that the control strategies for domestic waste treatment plants wishing to perform thermophilic anaerobic digestion in order to achieve more complete and/or rapid destruction of enteric pathogens would need to consider the potential for decreased activity of hydrogenotrophic methanogens. The artificial removal of H_2 from the headspace of anaerobic reactors has been shown to be an effective method for increasing the effectiveness of propionate metabolism under unfavorable conditions (Scholten and Conrad, 2000). The application of such a practice may be effective for full-scale installations experiencing similar temperature sensitivity as are described by this study.

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Chapter 4

Manuscript 2

Title:

The Effect of Digester Temperature on the Production of Volatile Organic Sulfur Compounds associated with Thermophilic Anaerobic Biosolids

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THE EFFECT OF DIGESTER TEMPERATURE ON THE PRODUCTION OF VOLATILE ORGANIC SULFUR COMPOUNDS ASSOCIATED WITH THERMOPHILIC ANAEROBIC BIOSOLIDS

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ABSTRACT

Volatile organic sulfur compounds (VOSC) are recognized as major contributors to malodors associated with dewatered and land applied biosolids. Recent research in the area of VOSC production and control has revealed that microbial degradation of sulfur containing amino acids is the likely mechanism in the formation of hydrogen sulfide (H_2S) and methanethiol (MT). The objective of this research was to determine the effect of increased digestion temperatures within the thermophilic range (49°C-57.5°C) on microbial production of VOSC. In addition, observations of digester performance and stability were made in order to determine the feasibility of operating at these high temperatures. Results of this research suggest that high thermophilic temperatures are an effective means of biosolids odor abatement. Biosolids associated with digestion temperatures greater than 53°C yielded an 83% reduction in total headspace sulfur when compared with mesophilic biosolids and a 71% reduction in total headspace sulfur when compared to low-temperature thermophilic (49°C) biosolids. However, treatment at higher temperatures (55°C, 57.5°C) showed evidence of inhibition of methanogenic activity. These data suggest that simple operational parameters such as temperature can be utilized to manipulate the activity of various microbial communities of interest within an anaerobic digester, and that increased digestion temperature can indeed be effective in the control of biosolids odor.

KEYWORDS
Thermophilic anaerobic digestion, biosolids odors, odor control, digestion temperature

INTRODUCTION

The control of odors associated with dewatered biosolids is of practical concern to wastewater utilities looking to utilize biosolids land application. Encroachment of residential development into rural and agricultural areas requires that biosolids are not only safe, but also aesthetically pleasing to the general public. In response to these concerns, considerable research in recent years has been focused both on the mechanism of biosolids odor production and methods for odor abatement (Witherspoon et al., 2004).

Volatile organic sulfur compounds, including methanethiol (MT), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), have been shown to correlate well with the human perception of "biosolids odor," and thus, headspace VOSC concentrations are the focus of this research (Witherspoon et al., 2004). The production of these compounds has been linked to biological activity within post-dewatered biosolids. Specifically, the production of hydrogen sulfide (H_2S) and MT are attributable to the activity of protein degrading bacteria and the anaerobic biodegradation of the sulfur containing amino acids cysteine and methionine, respectively. The production of DMS and DMDS are the result of methylation and oxidative dimerization of MT, respectively (Higgins et al., 2006; Oho et al., 2000; Segal and Starkey, 1969).

The degradation of methylated sulfur compounds by methylotrophic methanogens has also been shown to be an important mechanism in the assessment of VOSC associated biosolids odor. Previous work in the area of biosolids odor has shown that when dewatered biosolids are stored in anaerobic serum bottles, the headspace VOSC concentration increases over time to a particular peak concentration, and then decays to below detectable levels (Muller et al., 2004). In contrast, when a methanogenic inhibitor such as 2-bromoethanesulfonic acid (BESA) is added to dewatered biosolids, the decay of VOSC does not occur or is significantly slowed (Chen et al., 2005; Higgins et al., 2006). Though the methanogenic species responsible for VOSC degradation have not yet been identified within an anaerobic digester, supporting evidence for the mechanism of VOSC degradation has been previously provided (Lomans et al., 1999a; Lomans et al., 1999b). This indicates that the activity of methanogenic organisms capable of degrading methylated sulfur compounds is a key mechanism in the observed decay of biosolids

VOSC. For this reason, methanogenic activity must be considered when developing control strategies for biosolids odor.

Cysteine and methionine, the parent compounds for the biological production of H_2S and MT have previously shown to be present in both anaerobic digester feed and anaerobically digested sludges (Dignac et al., 1998; Higgins et al., 2004; Higgins and Novak, 1997). Though the specific microbial populations within anaerobic sludge digesters that are responsible for VOSC production are not well established, previous studies have shown that sulfur-containing amino acids can be broken down within an anaerobic digester environment resulting in the formation of H_2S and MT (Segal and Starkey, 1969). This suggests that for feeds containing cysteine and methionine (e.g. waste activated sludge), VOSC production is likely tied to a community of protein metabolizing organisms within the anaerobic digester. This manipulation of this population within the anaerobic digester could result in an advantageous odor control method for treatment plant operators.

The manipulation of a specific microbial community through easy to control operational parameters is not new to anaerobic digestion. The potential deactivation of various human pathogens has recently been recognized as an advantage of various biological sludge stabilization technologies. The United States Environmental Protection Agency (EPA) currently lists thermophilic aerobic digestion (ATAD™), in-vessel anaerobic composting (En-vessel™, IPS Process™) and various multi-phased anaerobic digestion processes (CBI ATP™ Process, Two-Phased Thermo-Meso Feed Sequencing Anaerobic Digestion) as Processes to Further Reduce Pathogens (EPA, 2003). In this case, the specific microbial community of interest is a group of enteric human pathogens accustomed to life at standard body temperature (37°C), or mesophilic conditions. Previous research has shown that the viability of pathogenic organisms can be significantly affected by high temperature incubation and/or treatment (Berg and Berman, 1980; Ghosh, 1998; Iranpour et al., 2006; Watanabe et al., 1997). The aforementioned processes each use increased temperatures between 50°C-65°C to deactivate non-thermostable components of the pathogenic cells and viruses.

Few comparisons evaluating odor potentials of biosolids digested at various temperatures have been made. Investigations at two full scale thermophilic digestion facilities have been conducted to determine the effect of intermittent temperature increases on the production of digester headspace VSC (Iranpour et al., 2005). The researchers found that in both cases, the concentration of H_2S, MT, and DMS increased in response to these transient temperature fluxes. At the Terminal Island Treatment Plant (TITP), San Pedros, California, VSC monitoring during a transient temperature increase from 57.5°C to 65.5°C caused enhanced production of H_2S and MT in the digester headspace. No change in DMS concentration was observed during this trial. At Hyperion Treatment Plant (HTP), Playa del Ray, California, an increase in digester temperature from 54.4°C to 58°C caused sharp increased in MT and DMS headspace concentrations.

Previous research concerning methanogenic population dynamics with changes in thermophilic digestion temperature suggests that higher digestion temperatures tend to select for non-methylotrophic methanogenic species. Zinder et al. (1984) observed a shift from *methanosarcina sp.* to *methanothrix sp.* as the dominant aceticlastic methanogenic population when the digestion temperature of a laboratory digester was increased to 58°C. Organisms of the genus *methanosarcina* have been identified as potential methylotrophic methanogens (Muller et al., 1986; Smith and Mah, 1978). The supplantation of this community by a community of methanogens incapable of methylotrophic methanogenesis would result in increased organo-sulfur compounds in the digester headspace.

The relative instability of the methanogenic population in thermophilic anaerobic digesters has been supported by simplified PCR-DGGE archaeal fingerprints of thermophilic digester communities when compared to their mesophilic counterparts (Chen et al., 2005). Considering this instability, and the supplantation of *methanosarcina sp.* observed by Zinder et al. (2004), the fluxes in headspace VSC production measured at HTP and TITP were likely due to stress imposed on the methanogenic community due to transient digestion temperature, and not due to changes in VSC production stemming from degradation of proteinaceous material. Though the accumulation of VSC in the headspace of an anaerobic digester and the accumulation of VSC associated with dewatered biosolids are both the likely result of the pathways described in this

review, very different mechanisms are responsible for these two cases. Dewatered biosolids odor has been previously shown to be greatly affected by shear input and polymer addition during dewatering (Chen et al., 2005; Higgins et al., 2006; Muller et al., 2004), while digester headspace odors are dependent on digester operation (Iranpour et al., 2005). Though previous research on digester headspace odor provides useful information about the relationship between methanogens and VSC producers, these distinctions between digester headspace and dewatered biosolids VSC production make comparisons from previous literature problematic.

OBJECTIVES

This study was conducted in order to determine whether increased digestion temperature during thermophilic anaerobic digestion can reduce the activity of VOSC producing organisms in dewatered biosolids and to determine how sufficiently high temperatures impact digester performance and stability. The information collected from this study could be applied to full-scale digestion systems in order to manipulate the various microbial communities of interest, including methanogens, VOSC producing bacteria, and human pathogens.

The experiments included in this study were conducted such that the following research questions could be adequately assessed:

1. Can increased digestion temperature be used to affect the production of VOSC compounds, and thus, decrease the potential for odor generation associated with dewatered and land applied biosolids?

2. Does increased digestion temperature represent a viable odor control method for wastewater treatment operators without sacrificing process stability?

3. Do differences in the odor production trends at different digestion temperature provide useful information for treatment plant operators when developing a biosolids storage and land application regime?

EXPERIMENTAL METHODS AND MATERIALS

Anaerobic digester setup and operation:

High-density polyethylene batch fermentation reactors supplied by Hobby Beverage Equipment Company (Temecula, California) were chosen for this study. The conical bottom of these vessels was thought to be advantageous in terms of mixing and suspension of grit, similar to the full-scale application of egg-shaped anaerobic digesters. The nominal volume of each vessel was 25 liters (L) and was operated with an active volume of 22.5 L. The reactor vessels were modified to accept a threaded stainless steel thermometer, also supplied by Hobby Beverage. For the purpose of this paper, the reactors are named according to their operating digestion temperature. Replicate reactors of the same temperature are designated by appropriate subscripts.

At startup, digesters were seeded with approximately 15 L of mesophilic anaerobic digested sludge from Pepper's Ferry Regional Wastewater Treatment Facility (Radford, Virginia). Previous research has shown that relatively quick acclimation of an anaerobic digester microbial community can be achieved by immediately exposing mesophilic sludge to a new thermophilic temperature (Bouskova et al., 2005). Following initial seeding, observed digester gas production ceased within approximately 5 days and then gradually increased to a steady rate for each digester. This suggests that the mesophilic microbial community had been deactivated due to the increased temperature, and a lag phase was required for the thermophilic microbial community to grow within each reactor. Rebel^{TM} wet-tip gas flow meters (Nashville, Tennessee) were used to monitor daily gas production rates by the anaerobic digesters. This startup regime helped to ensure that the microbial community within each anaerobic digester was unique, and solely dependant on the applied digestion temperature. After approximately 15 days of acclimation to the desired digestion temperature, daily feeding in the absence of wastage was used to bring the digester contents up to its final operating volume. Each reactor was monitored in terms of pH, gas production, and volatile solids destruction (data not shown) in order to determine that a steady state microbial community had been reached prior to sampling for the experimental analyses described in the following sections.

The digester feed consisted of a 1:1 ratio of primary and secondary solids from DCWASA Blue Plains Advanced Wastewater Treatment Facility (BPAWWTF) measured on a mass basis and diluted to approximately 3% total solids. In order to achieve the desired SRT, the digesters were daily batch fed 1.5 L of blended sludge. Ice-packed primary and secondary solids were received weekly from BPAWWTF via overnight shipment. Solids were then blended at the appropriate ratio and homogenized using a 200-watt Hamilton Beach kitchen stick blender. Pre-screening of the feed sludge to remove large particles was not required.

Suspension and mixing of the reactor contents was achieved by digester gas recirculation. Variable speed (6-600 rpm control) peristaltic pumps manufactured by Cole Parmer (Vernon Hills, Illinois) and Cole Parmer Masterflex Tygon LFL-17 pump tubing were used for gas pumping. General gas recirculation throughout reactor operation was controlled at approximately 0.8 L per min. (50% max.). Homogenization of the reactor contents prior to sampling was accomplished by increasing the recirculation rate to approximately 1.5 L per min. (100% max.) A fixed solids mass balance across the digesters was performed and revealed greater than 99% recovery on average using this mixing regime. The increased recirculation rate was also applied for about 10 min. after feeding to disperse the raw sludge throughout the digester.

Biosolids simulation:

A simulation method was used that has been shown to adequately mimic the full-scale production of dewatered biosolids through the use of high solids centrifugation (Muller et al., 2004). The dewatering method includes three distinct phases as listed below:

1. *Polymer conditioning and shearing*: Minimum capillary suction time was used to determine the optimal cationic polymer dose for sludge dewatering. Triton CST apparatus Types P304M and 165 with Whatman 17-CHR chromatography paper were used to determine optimal polymer dosage (Clarifloc 3275, 1% $^{w}/_{w}$). Polymer was incorporated by shearing for 30 seconds using a $^{1}/_{5}$ hp Waring Blender at approximately 12,000 rpm.

2. *Centrifugation/Dewatering*: 400 ml aliquots of conditioned sludge were centrifuged at approximately 17,700 x G on a Beckman-Coulter Avanti-JE centrifuge for 15 minutes. The centrate was decanted and the solids pellet was further dewatered using a pneumatic piston press assembly over Whatman 41 filter paper and a 40 μm porous metal filter (Mott Corporation; Farmington, Connecticut). The solids were subjected to a pressure of 38 psi for 10 minutes in order to achieve the increased cake solids concentration typical of full-scale high solids centrifuges.

3. *Headspace bottle preparation/Incubation*: Sealed incubation bottles were used to limit the exposure of the dewatered biosolids to atmospheric air, thus replicating the environment within a biosolids pile. 250 ml glass bottles with screw caps and Teflon™-lined septa were used as incubation containers in this study. Dewatered solids were cut into pieces approximately 5 mm^3 in size in order to facilitate gas transfer between the biosolids and headspace. Each headspace bottle was loaded with approximately 25 grams of dewatered solids and was incubated at ambient room temperature (~22°C) during the period of headspace sulfur assessment. Samples to be amended with BESA as a methanogenic inhibitor were treated after loading into the headspace bottles. Data from BESA amended samples are termed "headspace sulfur potential" as it reflects the conversion of bioavailable sulfur into VOSC without the effect of VOSC degradation by methylotrophic methanogenic organisms.

Headspace sulfur assessment:

The measurement of specific VOSC species associated with dewatered biosolids was done using a cryotrapping gas chromatograph (GC, HP 5890) – mass selective detector (MS, HP 5970). The GC column was a Supelco Equity-5, 30 m x 0.25 mm capillary column with 1.0 μm film thickness. A liquid nitrogen cryotrap was used to achieve species separation and was controlled by AMA Systems (Germany) cryotrap heaters and control equipment. Compounds of interest including MT, DMS, DMDS and DMTS were individually assessed using HP Chemstation integration software and then summed on the basis of mass sulfur in order to report "total

headspace sulfur" concentrations. The reported concentrations are normalized with respect to mass of volatile solids loaded to each headspace bottle in order to remove the effect of heterogeneity in sample dryness and mass.

H_2S is also of interest as it is a product of the degradation of the cysteine and tied to biosolids associated odor. While H_2S production through protein degradation (i.e. the mechanism of interest for VOSC production) has been well documented (Segal and Starkey, 1969), production of H_2S as a metabolic product of MT and DMS degradation by methylotrophic methanogenic organisms can be expected to greatly affect H_2S concentrations as well (Lomans et al., 1999b). Due to the combination of H_2S production via VOSC degradation and the potential for the speciation of reduced sulfur with iron present in the feed sludge, H_2S assessment throughout this study was difficult and unreliable. Additionally, H_2S was never identified during this study at concentrations that significantly contributed to the total sulfur concentration of a given sample.

Headspace sulfur measurements were made periodically in order to produce a time-scaled sulfur profile for each biosolids headspace bottle. A representative headspace sulfur profile is shown in Figure 4.1. The parameters discussed in this paper are the peak headspace sulfur, peak headspace sulfur potential, and time to peak for each profile. For clarification, the peak headspace sulfur and sulfur potential in Figure 4.1 are annotated by the arrows. Headspace samples were spaced such that the shape of each sulfur profile could be well established and the highest point on each curve was representative of either peak headspace sulfur or sulfur potential.

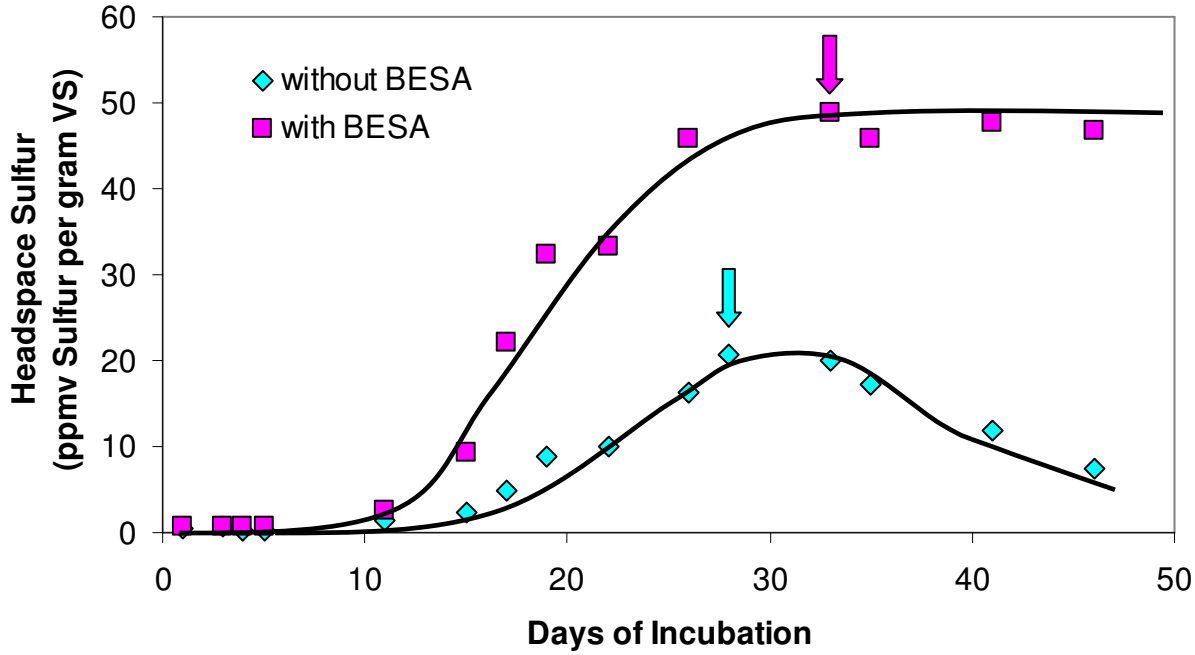

Figure 4.1: Representative odor growth and decay curve. Data shown were attained from dewatered biosolids digested at 55°C.

Volatile fatty acid analysis:

Volatile fatty acids (VFA) were measured weekly on the solution phase each digester. Effluent sludge samples were passed through a 0.45 µm filter (Fisher Scientific; Pittsburgh, Pennsylvania) and frozen until analysis was performed. VFA were measured using a gas chromatograph with flame ionization detector (GC-FID, Shimadzu GC-14A). Species separation was achieved using a Nukol™ fused silica 15 m x 0.53 mm capillary column with 0.5 µm film thickness. A computer integrator (Shimadzu CR501 Chromatopak) was used for data analysis. Helium was used as the carrier gas at a pressure of 49.04 kPa. Additional gasses are as follows: Hydrogen (H_2) = 58.84 kPa, Air = 58.84 kPa, Nitrogen (N_2) = 49.04 kPa.

Hydrogen gas analysis:

Headspace hydrogen concentrations were analyzed using a reduction gas detector (Trace Analytical RGA5). Nitrogen was used as the carrier and actuator gasses at approximately 275 kPa and 414 kPa, respectively.

Additional analyses:

Total and volatile solids concentrations, as well as total alkalinity were analyzed as specified in Standard Methods for the Examination of Water and Wastewater (APHA, 1995).

RESULTS AND DISCUSSION

Impact of increased digestion temperature on the production of VOSC

The production of VOSC associated with dewatered biosolids is related to the biological conversion of sulfur containing amino acids (cysteine and methionine) to H_2S and MT. Therefore, an increase in digestion temperature above that at which cysteine- and methionine-degrading organisms are thermostable should result in a decrease in the production these specific odor compounds. VOSC-producer inhibition by increased digestion temperature is evident from a comparison between peak headspace sulfur concentrations of biosolids produced from wastewater sludge digested at a range of temperatures (Figure 4.2). It can be seen that digestion temperatures greater than 51°C produce biosolids that support significantly less VOSC production than is observed from mesophilic and low temperature thermophilic biosolids.

As has been previously described (Chen et al., 2005), the production and decay of biosolids odor has shown to be a function of the rate at which VOSC are produced and the rate at which VOSC is consumed by methylotrophic methanogens. In practice, biosolids dewatering mechanisms are strongly inhibitory to methanogenic organisms and a perceived lag phase exists before methanogenic metabolisms are restored within the biosolids cake (Muller et al., 2004). During the lag phase, the rate of VOSC production is greater than that of VOSC degradation, causing MT, DMS, DMDS and DMTS to accumulate. Chen et al. (2005) have shown that the end of this lag phase is marked by the reactivation of methanogenic organisms, as evident by the increased presence methane in the headspace and decreased rate of net VOSC production. The variations in peak headspace sulfur in this study can then be explained by differences in the production rate of VOSC prior to the reactivation and/or regrowth of methanogens.

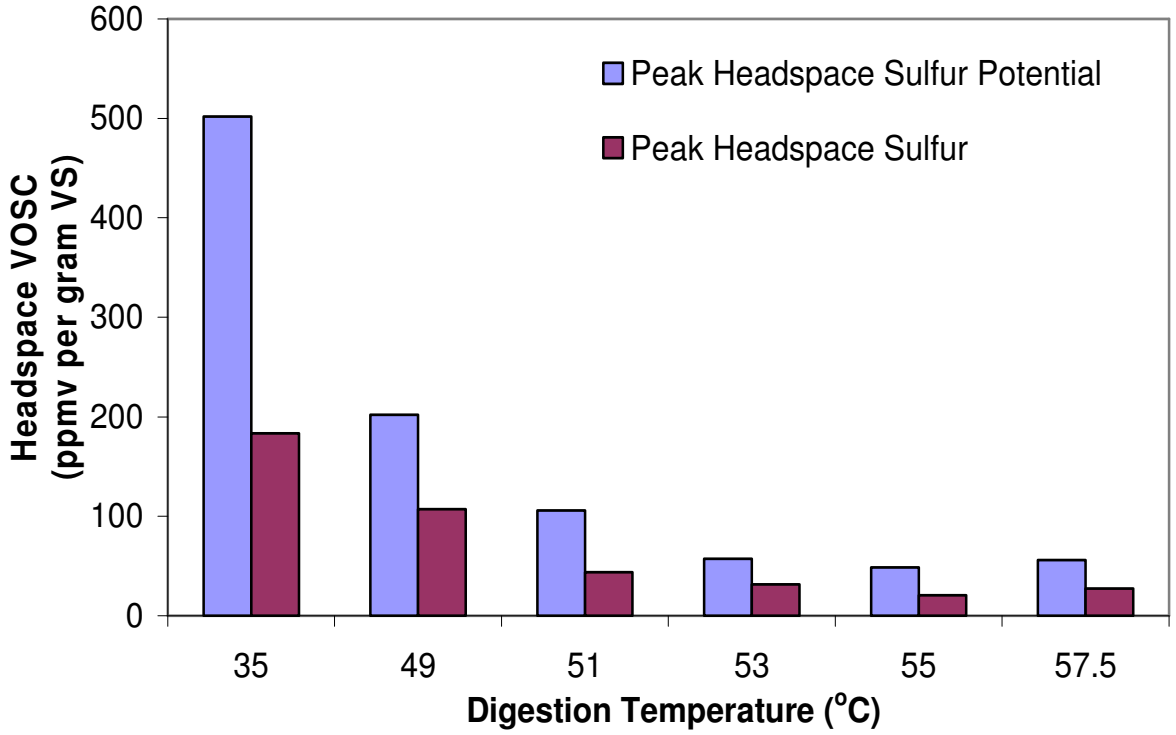

Figure 4.2: Overall trend of peak sulfur concentration with increasing temperature. Data suggest that increased digestion temperature may be a viable operational odor control method.

Interestingly, a similar trend between peak headspace sulfur and peak headspace sulfur potential exists. Whereas peak headspace sulfur concentrations suggest a decreased rate of VOSC production, peak headspace sulfur potential concentrations can be used to assess the overall ability of the microbial community within dewatered solids to convert bioavailable sulfur (cysteine and methionine) to VOSC in the absence of VOSC degradation. The similar trend between amended and non-amended samples suggests that not only the activity of VOSC producers is affected by increased digestion temperature but also that the overall presence of these organisms is decreased such that their metabolic products are not allowed to accumulate. This is an important outcome for odor control at land application sites. Further assessment of bioavailable organic sulfur in digested and dewatered sludge at these temperatures would be useful in verifying the role of VOSC producer deactivation as a likely mechanism of odor control.

Over the same range of temperatures, experimental data show that volatile solids reduction (VSR) within each digester was not significantly different from 50% (data not shown). The consistent VSR data throughout this study suggests that the release of bioavailable protein necessary for the production of H_2S and MT occurs to a similar extent at each temperature. Assuming that the required substrates for VOSC production are equally present in dewatered biosolids digested at a range of temperatures, differences in VOSC production can then be attributed to thermal inhibition of the microbial community that is responsible for cysteine and methionine degradation. Therefore, the observed effect of increased digestion temperature on VOSC production can be directly related to biological activity within the dewatered biosolids.

In each case, MT and DMS are the dominant VOSC species identified in the headspace bottles. On average, 90% of peak headspace sulfur can be accounted for by MT and DMS, with much of the remaining sulfur attributable to DMDS. Since the production of these compounds is directly related to separate amino acid metabolisms, their production can be expected to be independent; therefore, looking at the production of the dominant headspace sulfur compounds can provide additional information about the overall production of biosolids sulfur odor.

The individual concentrations of peak headspace MT and peak headspace DMS across the temperature range (Figure 4.3) suggest that significant differences in sulfur-protein metabolism exist between mesophilic and thermophilic digestion systems. In all cases, the accumulation of MT in thermophilic samples exceeds that of DMS, with DMS being the dominant organic sulfur species in the mesophilic sample. In addition, the approximate minimum production of DMS occurs at a lower temperature (51°C) than MT (55°C). These observations suggest that the metabolism that produces DMS (i.e. methionine degradation to MT and subsequent biological methylation) is less thermostable than the production of MT via direct methionine degradation or methylation of H_2S. Additional identification of the proteins that are degraded throughout the period of VOSC production would be useful in further understanding the differences between thermophilic and mesophilic biosolids associated odors.

While the concentration of DMS reaches a relative sustained minimum at 51°C, the minimum MT concentration at 55°C is followed by a relatively large increase between 55° and 57.5°C

(approximately 105%). Over this same range, the peak headspace MT potential (data not shown) increases only slightly (approximately 13%). Such an increase in peak MT without a similar increase in peak MT potential suggests that the activity of methylotrophic VOSC degrading methanogens within 57.5°C-digested solids is significantly less than at 55°C. Perhaps when the digestion temperature is raised above the tolerance threshold for certain methylotrophic methanogens, the balance between production and decay of VOSC degraders favors MT production. These data suggest that higher temperatures than those used in this study may result in poor removal of VOSC, and points to an upper limit of the utility of increasing digestion temperature in order to control the production of biosolids odor.

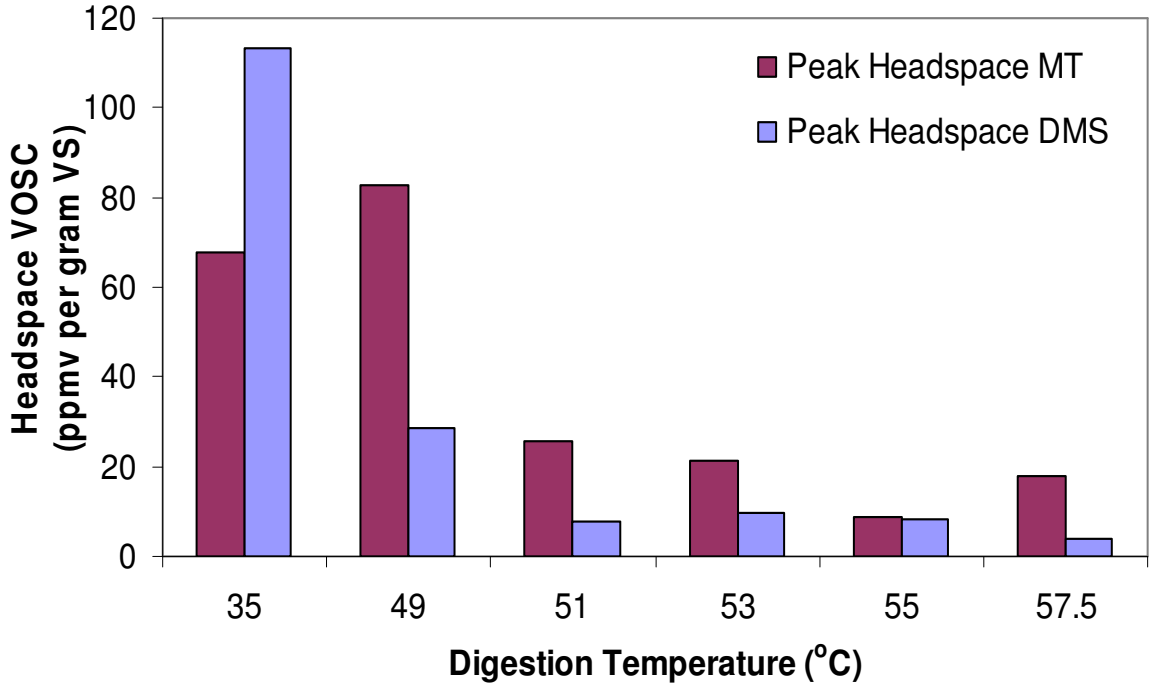

Figure 4.3: The production of MT and DMS across a range of digestion temperatures. MT and DMS represent approximately 90% of total headspace sulfur produced from dewatered biosolids in this study.

Implication for on-site biosolids storage and land application

As discussed above, the peak of a biosolids odor growth and decay curve indicates the time point at which the rate of methanogenic degradation of VOSC increases over the rate of VOSC production by protein degrading bacteria. VOSC accumulation immediately following biosolids dewatering suggest that the process of biosolids dewatering is particularly inhibitory to methanogenic activity. The lag phase between dewatering and methanogenic regrowth is

governed by the nature and severity of this initial inhibitory condition. Hypothesized mechanisms for methanogenic inhibition during dewatering include shear intensity stemming from high solids centrifugation, introduction of oxygen as a result of open-air dewatering systems, and thermal shock as the biosolids exit a temperature controlled reactor and are processed and stored at ambient temperature.

No effort was made throughout this study to simulate the effect of various types and intensities of shearing on odor production; however, the introduction of oxygen, and more importantly the issue of thermal shock were inherent as a result of the dewatering and incubation methods that were used. While aeration of the biosolids during dewatering and processing certainly added to the methanogenic lag-phase, no effort to measure the oxidation-reduction potential of various samples was made prior to incubation. As such, the role of oxygen is considered to be constant across all samples and is not considered in this discussion.

In terms of thermal shock, thermophilic digestion represents a larger change in temperature between ambient and digestion temperatures than mesophilic digestion. Therefore, a longer lag phase is expected for biosolids digested at higher temperatures. The results of this study show that increased digestion temperature can be positively correlated to the length of time before the peak headspace sulfur concentration is seen (Figure 4.4).

The reason for this relationship is likely two-fold. Primarily, as described above, methanogenic activity is strongly inhibited by the action of biosolids dewatering. A later peak at higher temperatures shows that methanogenic inhibition due to thermal shock may last upwards of four weeks. Secondly, though VOSC degraders have been shown to be less sensitive to the effects of biosolids dewatering, a short lag phase likely exists at high temperatures that allows protein degraders to acclimate to lower temperatures. The likely effect of this is that the maximum rate of VOSC production is pushed to later in the odor growth and decay curve.

This phenomenon is of definite concern when considering the on-site storage of dewatered biosolids prior to land application. Biosolids possessing a longer lag time are more manageable in terms of in-plant aesthetics. The maximum release of VOSC by the biosolids would likely not

occur until after spreading and incorporation into agricultural soil, making application of high temperature biosolids particularly advantageous.

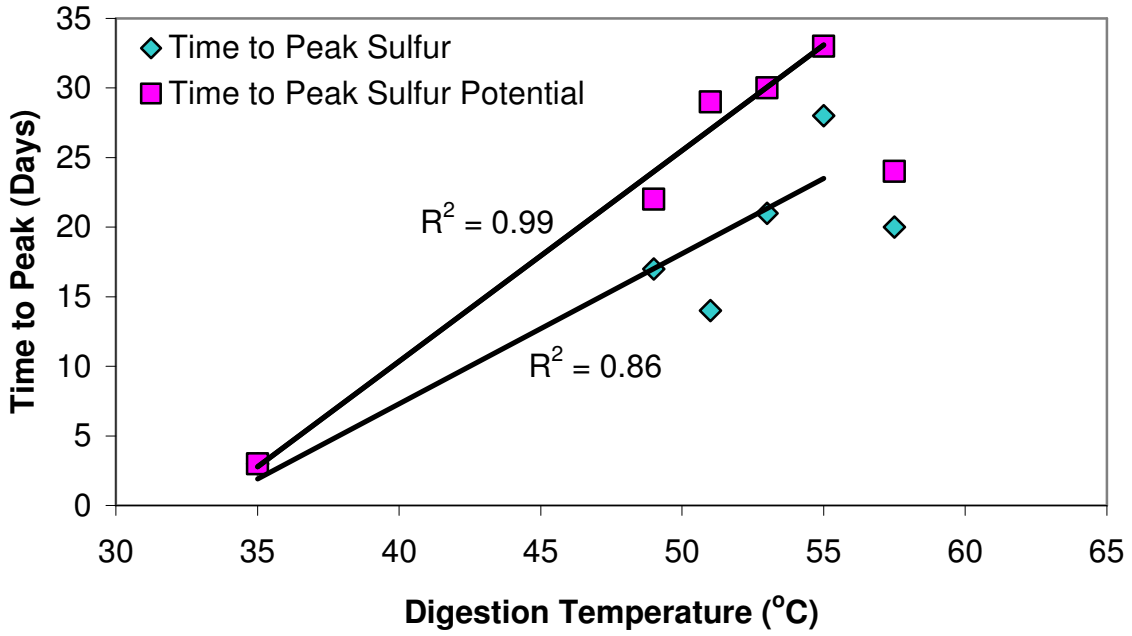

Figure 4.4: Correlation between increased digestion temperature and time to peak headspace sulfur concentrations. Data suggest that increased digestion temperatures result in an increased acclimation period for VOSC producing organisms after biosolids dewatering. Data from the 57.5°C digester were not included in the linear regression analysis due to evidence of a largely different microbiological community at that temperature.

Chen et al. have shown that a freeze thaw cycle following the dewatering of mesophilically digested biosolids results in a large increase in MT concentration after thawing and incubation at 25°C (Chen et al., 2005). This is attributable to a near total inhibition of methanogenesis during the freeze cycle and fast recovery of protein degrading bacteria after thawing. High temperature digestion has been shown in this study to not only slow VOSC production, but to limit the potential for odor release. As described by Kumar et al., it can be reasonably expected that biosolids digested at high temperatures would perform more desirably in terms of VOSC production than mesophilic digested solids following a freeze thaw cycle (Kumar et al., 2006).

The viability of high-temperature thermal treatment as a odor control option and its implication on digestion process stability

In order to use increased digestion temperature to control biosolids odor, the system must be able to perform adequately in terms of solids removal and process stability in order to be a truly viable and useful method for VOSC odor abatement. While several full-scale thermophilic digestion systems have been shown to operate well at temperature greater than 55°C (Zinder, 1984; Zinder et al., 1984), most laboratory research into higher temperatures (> 60°C) has been focused on fixed and granular growth anaerobic system designed to treat specific industrial or agricultural wastes (Bouallagui et al., 2004; Van Lier et al., 1996). A likely reason for this focus is the relatively poor performance of carbon-dioxide (CO_2) reducing methanogens at digestion temperatures approaching 60°C.

CO_2 reducing methanogens are critical in maintaining the process stability of an anaerobic digester because they consume large quantities of hydrogen gas (H_2) in the metabolic conversion of CO_2 to methane (CH_4) (Jones et al., 1985). The importance of H_2 is in the conversion of VFA to acetate via β-oxidation, and eventually to CH_4. Accumulation of hydrogen due to poor CO_2-reducer performance can cause feedback inhibition of β-oxidation, leading to an accumulation of VFA within the anaerobic digester, and potential problems with process stability. When it becomes desirable to operate an anaerobic digester at high temperatures, non-mixed and fixed growth reactors can be used to improve the efficiency of the methanogenic conversion of CO_2 and H_2 to CH_4. This method for operating reactors in order to achieve close microbial proximity between H_2 producers and H_2 consumers, termed interspecies hydrogen transfer, has been discussed in depth in the literature (Kim et al., 2002; McCarty and Smith, 1986).

The application for full-scale sewage sludge digestion, however, does not typically allow for fixed growth or non-mixed conditions in order to achieve efficient hydrogen removal at high temperatures. For this reason, a digestion temperature must be selected such that the performance of CO_2-reducing methanogens can be maintained, and that H_2 can be efficiently regulated under completely missed conditions. The results of this study show the effect that temperature has on the ability of CO_2-reducing methanogens to adequately regulate headspace H_2 (Figure 4.5). Over the range of temperatures shown, there is clear upward trend in headspace

H_2 concentrations, suggesting methanogenic organism inhibition at high temperatures. While each thermophilic digester shows an increased headspace H_2 concentration in comparison to that of the mesophilic digester, only the 57.5°C digester can be characterized as being in imminent danger of digester failure.

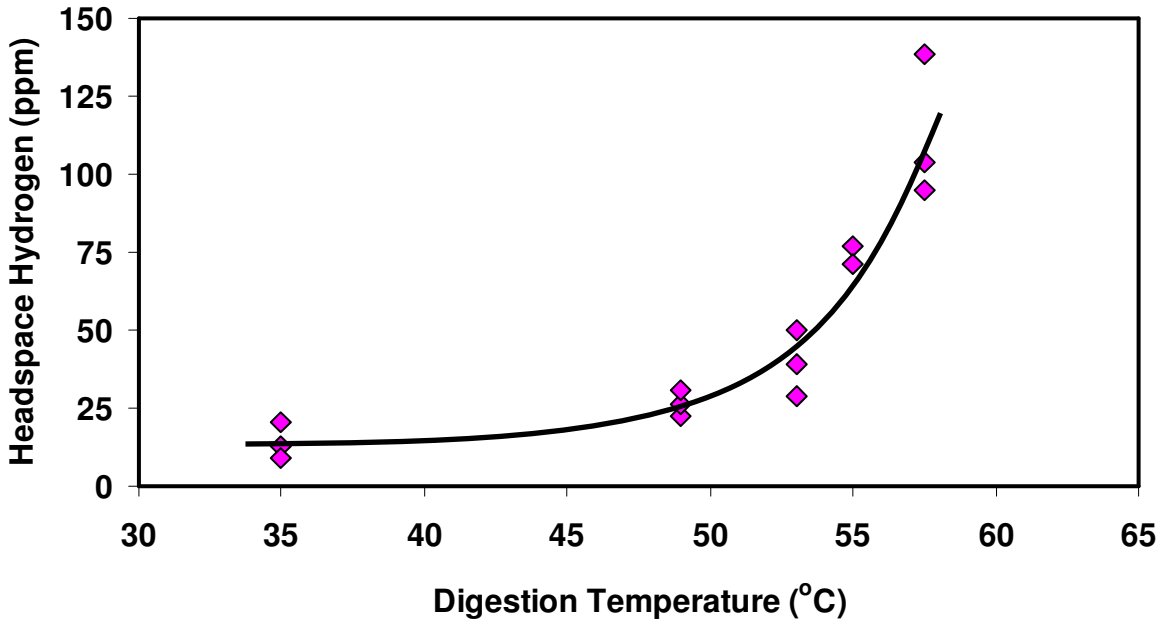

Figure 4.5: Headspace H_2 accumulation with increased digestion temperature. Trend reveals the effect of high temperature digestion of the activity of CO_2-reducing methanogens.

Based on the headspace hydrogen values shown in Figure 4.5, VFA accumulation should be evident in the 57.5°C digester. A comparison between total alkalinity and total VFA concentration can be useful in order to assess the buffering capacity that a digester may have in order to resist downward pressure on reactor pH. Table 4.1 shows the ratio of total VFA (expressed as acetate) to total alkalinity for each digester included in this study. This ratio (α) presents a useful method for quantifying reactor stability; a ratio approaching 1.0 suggests an absence of buffering capacity to resist changes in reactor pH in response to any type of digester upset that causes an increase in organic acid concentrations.

From the above relationships, it becomes apparent that reactor operation at or above 57.5°C is infeasible for this sludge. This temperature is shown to cause methanogenic inhibition, resulting

in the accumulation of headspace H_2 and organic acids. Thermophilic digesters below 55°C show a tendency for H_2 accumulation with increasing temperature suggesting slight inhibition of CO_2-reducers. However, the threshold for β-oxidation inhibition is not approached which allows propionate and longer VFA to be converted to acetate, and eventually methane gas.

Table 4.1: Average (n ≥ 5) Steady-state VFA and Alkalinity within laboratory reactors in order to give a relative measure of process stability. Ratio between Total VFA and Total Alkalinity (α-ratio) of 57.5°C digesters suggest process instability and potential failure.

Reactor	Total VFA (ppm as CH_3COO^-)	Total Alkalinity (ppm as $CaCO_3$)	**α-ratio**
49.0°C	1226	3391	**0.36**
51.0°C	1039	4180	**0.25**
53.0°C_1	943	4269	**0.22**
53.0°C_2	1194	4819	**0.25**
55.0°C	492	4695	**0.10**
57.5°C_1	4052	3722	**1.09**
57.5°C_2	3411	3965	**0.86**

It is important to note that decreased biosolids VOSC production was achieved by operating digestion systems at or above 51°C. This gives a reasonable range of thermophilic temperatures at which VOSC production can be controlled while ensuring sustainable methanogenesis during digestion and good process stability.

CONCLUSION

This study has clearly demonstrated that increased digestion temperature can be used as a feasible biosolids odor control method. Thermophilic digestion at 53°C yielded biosolids that produced 83% less VOSC than similarly treated mesophilic biosolids. Not only did increased digestion temperatures have an effect on the peak headspace VOSC, high temperature thermophilic digestion also decreased the potential for the production of biosolids sulfur odors in the absence of VOSC degradation. This result suggests that not only the activity, but also the

viability of VOSC producers can likely be manipulated by an easy to control operational parameter.

Biosolids digested at high temperatures are advantageous when considering land application and other storage disposal options. The large difference between digestion and ambient temperature causes thermal shock on the organisms involved in the production and degradation of VOSC. This causes the peak output of VOSC to occur up to a full month after dewatering. Exposure to air by windrowing or other agitation could likely increase this lag phase due to the introduction of oxygen into the anaerobic environment.

While digestion temperatures above 55°C have been shown to cause methanogenic inhibition leading to process instability, a reasonable range exists at which a reduction of biosolids VOSC production can be decreased while maintaining good process stability. In this study, the optimal digestion temperature that minimizes both the production of biosolids odor and the accumulation of H_2 is approximately 53°C.

In order to fully understand the effect that operational parameters, including mixing, dewatering and digestion temperature, have on the production of biosolids odor, further microbial characterization of the organisms responsible for the production and degradation of VOSC is needed. A better understanding of the mechanisms that govern odor production and control will aid treatment plant designers and operators in creating sludge digestion systems that will achieve a beneficial balance between system performance, stability, and a high quality biosolids output.

ACKNOWLEDGEMENTS

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Chapter 5

Conclusion

Thermophilic anaerobic digestion represents a complex microbial ecosystem which is inherently sensitive to its engineered environmental conditions. The operational parameters selected for a thermophilic system are often chosen on the basis of certain desired outcomes such as the deactivation of harmful pathogens, with little attention being paid to ancillary factors such as operational stability and biosolids odor production. However, past and current research provides a sound basis for the hypothesis that small changes in operational parameters such as solids retention time, mixing, and digestion temperature can have a great impact on these secondary measures of system performance.

Since the current time-temperature requirements for biosolids pathogen reduction are often most easily met by simply affecting the steady-state digestion temperature of a digestion system, it is useful to examine the impact of these specific temperatures on such metrics as solids removal efficiency, methanogenesis, operational stability, and residual biosolids aesthetics.

The results of the first phase of this research suggest that the performance and stability of anaerobic digestion may be limited by digestion temperatures greatly exceeding 55°C. Above this temperature, decreases in methanogenic activity paired with the accumulation of short chain fatty acids and dissolved hydrogen were observed. These data suggest the thermal inhibition of an essential microbial community, the hydrogenotrophic (CO_2-reducing) methanogens at digestion temperatures above 55°C. Reactor operation at these temperatures were particularly damaging to the natural homeostasis between fermenting and methanogenic organisms, in lieu of calculations supporting a thermodynamically favorable condition for fatty acid oxidation. The thermal inhibition of this microbial community became the controlling factor in determining the

solids removal efficiency, methanogenesis, and operational stability of anaerobic digesters operated at 57.5℃.

The second phase of this research focused on the minimization of volatile organic sulfur compounds (VOSC) associated with digested and dewatered biosolids as an indicator of the aesthetic quality of this material. The data suggested that a sharp decrease in VOSC-production occurred between sludges digested at mesophilic and thermophilic temperatures. The data also showed that, to a point, elevated temperatures in the thermophilic range (53℃, 55℃, 57.5℃) produced less VOSC than biosolids digested at lower thermophilic temperatures (49℃ and 51℃). Since the biosolids methanogenic community in appeared to be equally capable of degrading VOSC across over the range of thermophilic temperatures, the conclusion is that the activity of VOSC producing organisms within digested and dewatered biosolids is greatly impaired by high-temperature thermophilic digestion.

These results exhibit the impact that small changes in an operationally defined parameter such as reactor temperature can have on essential microbial communities within an anaerobic reactor. The implication of these findings is that care must be taken when engineering a digestion process to achieve a particular outcome such as pathogen reduction such that core microbial communities are not negatively impacted by design decisions. While the aim of this research was to establish the effect of specific digestion temperatures on the performance, stability, and biosolids odor production associated with thermophilic anaerobic digestion, the following outcomes were also achieved:

1. This research introduces elevated digestion temperature within the thermophilic range as a viable method for the reduction of biosolids odors. In addition, the increased lag time between dewatering and the occurrence of peak headspace VOSC concentrations strongly favors high-temperature thermophilic anaerobic digestion for the management of on-site odors associated with biosolids storage.

2. The data in this research describes, in detail, a viable mechanism for temperature induced failure of thermophilic systems based on the temperature sensitivity of hydrogenotrophic methanogens.

3. The mechanism of temperature induced failure observed in this research helps to suggest methods for operating digestion systems at prohibitively high temperatures. Previous research has shown that enhanced microbial consortia proximity through the application of fixed biomass and/or non-mixed conditions is an effective method for maintaining low concentrations of short-chain fatty acids (Speece, 2006). This research and research by Scholten and Conrad (2000) suggest that the artificial removal H_2 from the digester headspace could effectively be used to supplant the role of hydrogenotrophic methanogens in the digester community.

In summary, careful selection of digestion temperature can not only be used to maintain regulatory compliance with biosolids pathogen reduction requirements, but also can be adjusted in order to affect the performance, stability, and aesthetic quality of a digestion system employing thermophilic anaerobic digestion.

Appendices

Appendix 1

Supplementary Data

Table A1.1: Nitrogen speciation in digester feed and thermophilic anaerobic digesters operated at steady state. Feed 1 was applied to reactors 53°C_1 and 57.5°C_1. Feed 2 was applied to reactors 51°C and 55°C. Feed 3 was applied to reactors 49°C, 53°C_2, and 57.5°C_2.

	Number of Samples at Steady-state	Total Ammonia	Total Kjeldahl Nitrogen	Total Organic Nitrogen
	n	mg/L, as Nitrogen average ± standard deviation		
Feed 1, 3.0% TS	**3**	**137 ± 31**	**1327 ± 176**	**1190 ± 185**
Feed 2, 3.0% TS	**3**	**243 ± 8**	**1419 ± 56**	**1175 ± 50**
Feed 3, 3.0% TS	**4**	**214 ± 18**	**1451 ± 49**	**1237 ± 61**
49°C	**4**	**766 ± 49**	**1251 ± 51**	**485 ± 18**
51°C	**3**	**754 ± 13**	**1253 ± 39**	**499 ± 35**
53°C_1	**3**	**851 ± 39**	**1503 ± 8**	**652 ± 46**
53°C_2	**4**	**786 ± 49**	**1531 ± 108**	**745 ± 103**
55°C	**3**	**780 ± 8**	**1273 ± 51**	**499 ± 46**
57.5°C_1	**3**	**908 ± 19**	**1481 ± 168**	**572 ± 160**
57.5°C_2	**4**	**941 ± 68**	**1476 ± 77**	**535 ± 107**

Notes:

Determinations of nitrogen species were carried out on whole sludge samples on the day of obtaining sample. Total ammonia and TKN were measured as specified in *Standard Methods* (APHA, 1995). Organic nitrogen defined as the difference between TKN and total ammonia measurements.

References:

American Public Health Association (APHA); American Water Works Association (AWWA); Water Environment Federation (WEF) (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Edition., Washington, D.C.

Table A1.2: Soluble protein analysis of blended raw sludge feed and thermophilic anaerobic digesters operated at steady-state. Feed 1 was applied to reactors 53°C_1 and 57.5°C_1. Feed 2 was applied to reactors 51°C and 55°C. Feed 3 was applied to reactors 49°C, 53°C_2, and 57.5°C_2.

	Number of Samples at Steady-state	Soluble Protein
	n	mg/L as BSA
Feed 1, 3.0% TS	4	177 ± 42
Feed 2, 3.0% TS	4	338 ± 90
Feed 3, 3.0% TS	4	436 ± 80
49°C	4	532 ± 120
51°C	4	486 ± 58
53°C_1	4	569 ± 29
53°C_2	4	572 ± 102
55°C	4	459 ± 80
57.5°C_1	4	687 ± 91
57.5°C_2	4	622 ± 97

Notes:

All samples were filtered through 0.45µm filters and frozen prior to analysis. Soluble protein concentration was analyzed using the Hartree (1972) modification of the Lowry et al. (1951) method.

References:

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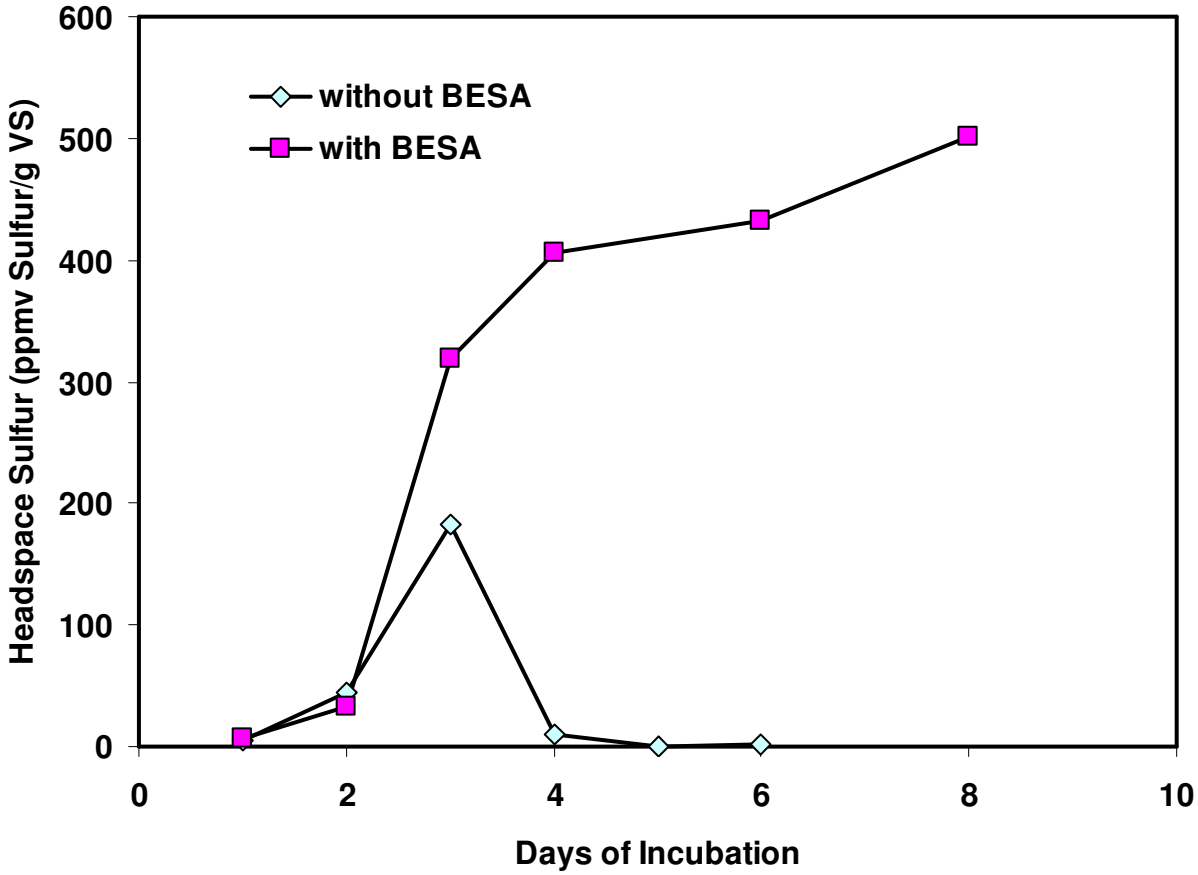


Figure A1.1: Trend of VOSC production and dissipation for biosolids digested at 35°C.

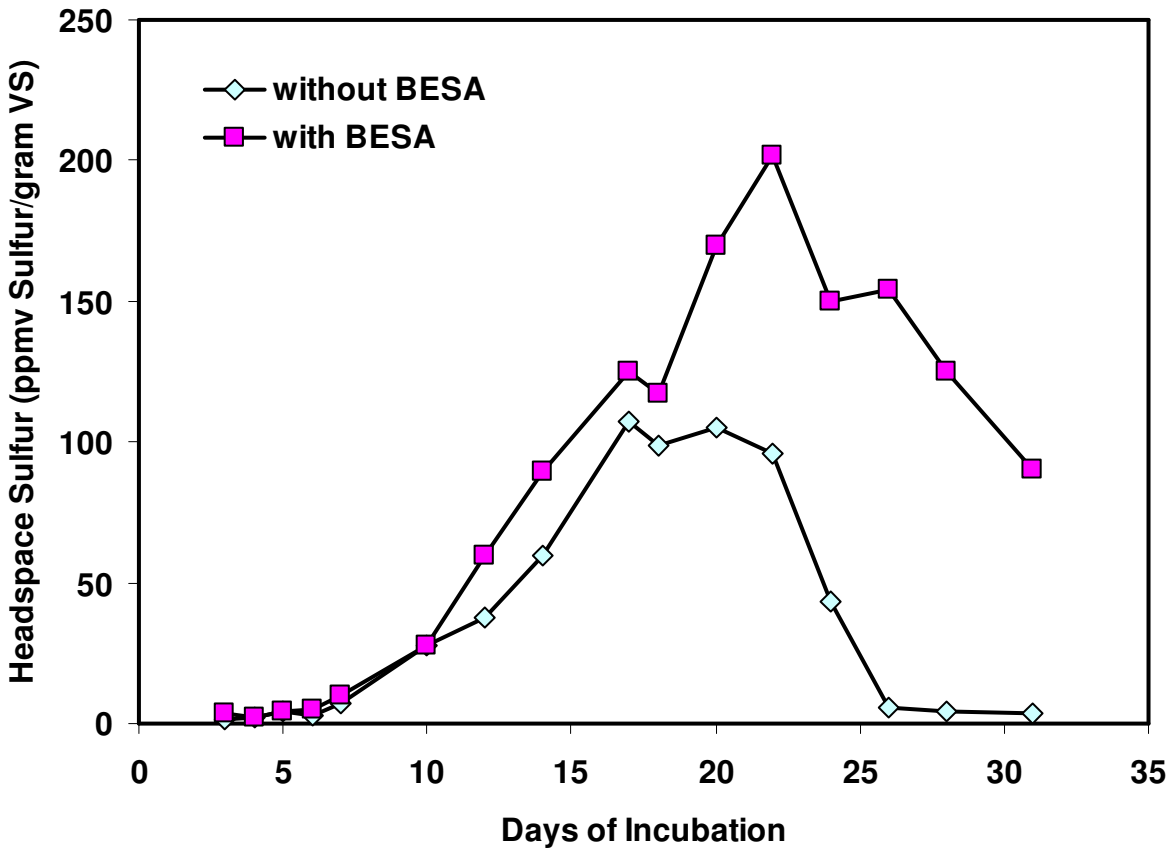


Figure A1.2: Trend of VOSC production and dissipation for biosolids digested at 49°C.

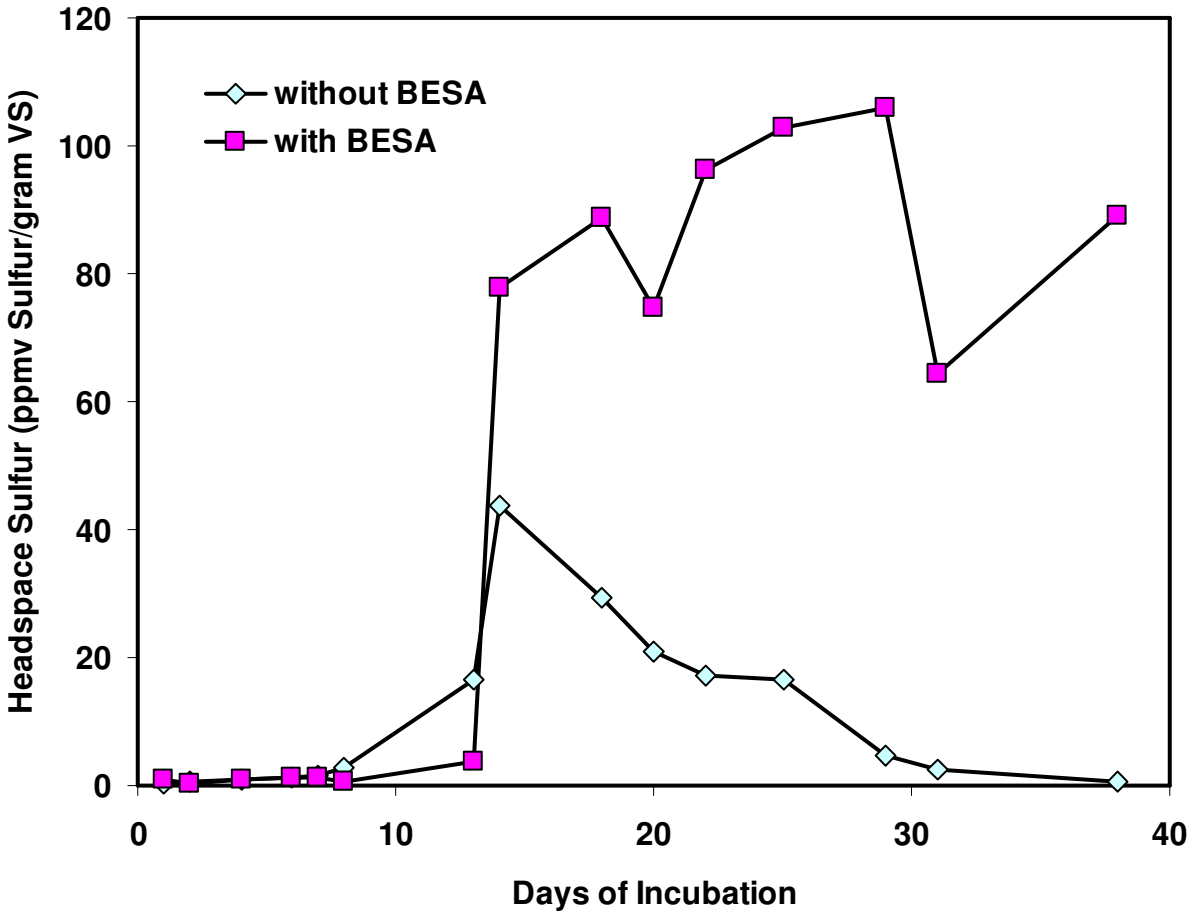


Figure A1.3: Trend of VOSC production and dissipation for biosolids digested at 51°C.

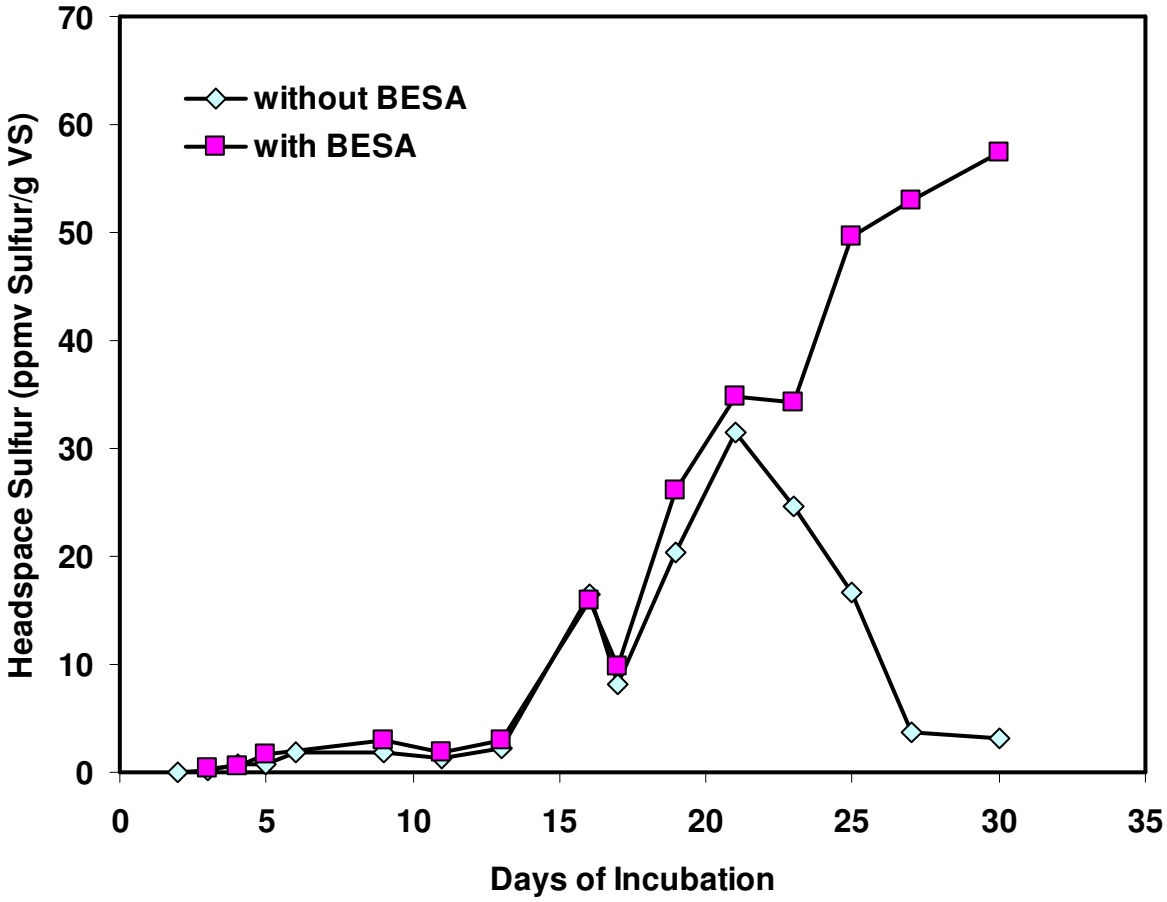


Figure A1.4: Trend of VOSC production and dissipation for biosolids digested at 53°C.

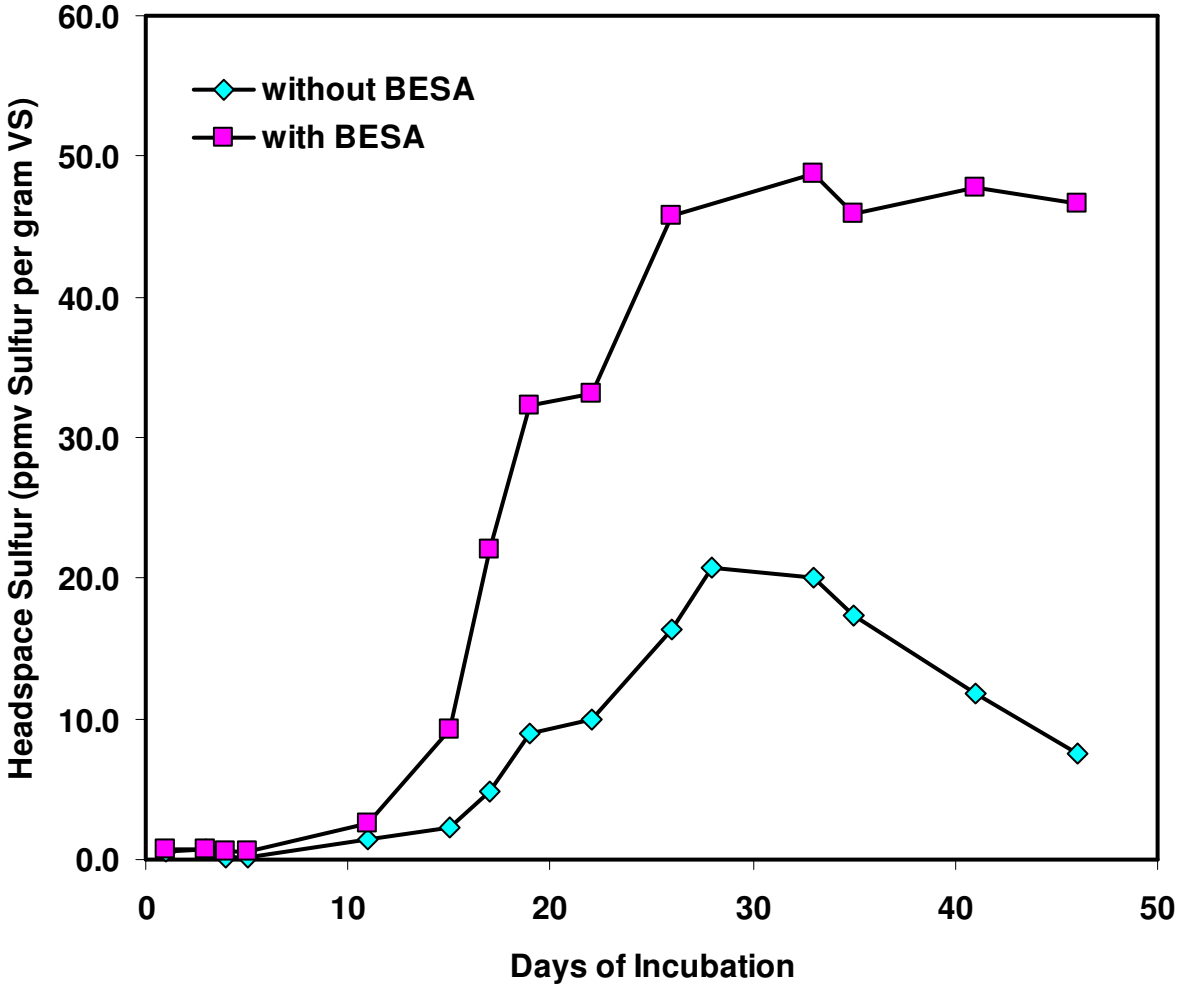


Figure A1.5: Trend of VOSC production and dissipation for biosolids digested at 55°C.

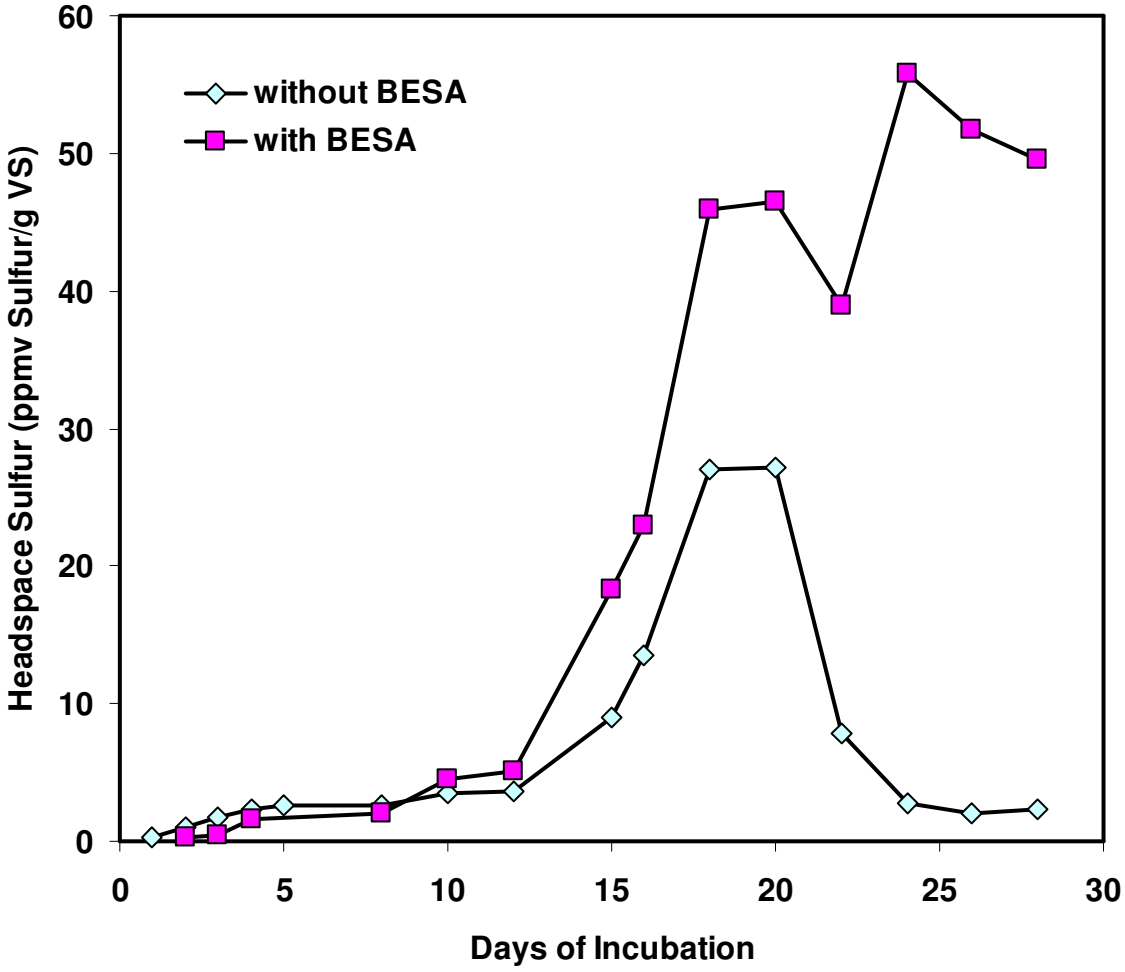


Figure A1.6: Trend of VOSC production and dissipation for biosolids digested at 57.5°C.

Appendices

Appendix 2

Statistical Analyses

The following pages include statistical anlyses perfromed on pH and CH_4 data across the range of thermophilic temperatures. While a robust statisitcal analysis of each data set collected during this study was not carried out, the parameters pH and CH_4 are considered sufficient for the establishment of repeatability of reactor perfromance as affeect solely by digestion temperature, as well as similarities or differences between reactor perfromance across the range of thermophilic temperatures.

The statistical analysis used in this study was the non-parametric Kruskal-Wallace one way analysis of variance based on ranks. The test was perfromed using the NCSS computer software package (Kaysville, Utah) at a confidence level of 95% ($\alpha = 0.05$).

Discussion of these analyses and reports is included in Chapter 3.

Analysis of pH Data **Attachment A2.1**
Analysis of CH_4 Data **Attachment A2.2**

Attachment A2.1

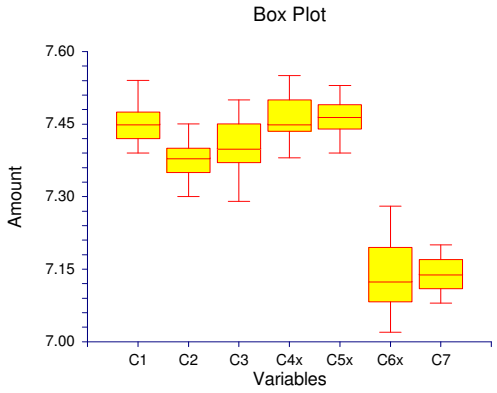
Statistical Analysis of pH Data

Analysis of Variance Report
Response 49°C, 51°C, 53°C_1, 53°C_2 ,55°C, 57.5°C_1, 57.5°C_2

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	0.4216	0.673335	Accept
Kurtosis Normality of Residuals	0.4223	0.672831	Accept
Omnibus Normality of Residuals	0.3560	0.836929	Accept
Modified-Levene Equal-Variance Test	4.7389	0.000159	Reject

Box Plot Section



Kruskal-Wallis One-Way ANOVA on Ranks
Hypotheses
H0: All medians are equal.
Ha: At least two medians are different.

Test Results

Method	DF	Chi-Square (H)	Prob Level	Decision(0.05)
Not Corrected for Ties	6	148.6355	0.000000	Reject H0
Corrected for Ties	6	148.8547	0.000000	Reject H0

Kruskal-Wallis Multiple-Comparison Z-Value Test

Variable	C1	C2	C3	C4x	C5x	C6x	C7x
C1	0.0000	3.6622	2.1490	0.4657	0.7694	7.3446	6.9521
C2	3.6622	0.0000	1.5108	4.1590	4.8032	4.0863	3.7539
C3	2.1490	1.5108	0.0000	2.6316	3.1221	5.4142	5.0560
C4x	0.4657	4.1590	2.6316	0.0000	0.2694	7.8233	7.4178
C5x	0.7694	4.8032	3.1221	0.2694	0.0000	8.7135	8.2329
C6x	7.3446	4.0863	5.4142	7.8233	8.7135	0.0000	0.1984
C7	6.9521	3.7539	5.0560	7.4178	8.2329	0.1984	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 3.0381

Attachment A2.2

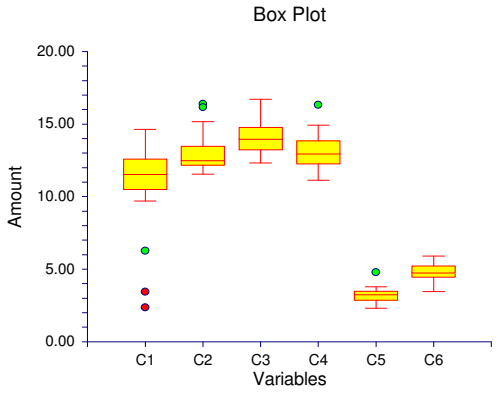
Statistical Analysis of CH_4 Data

Analysis of Variance Report
Response 51°C, 53°C_1, 53°C_2, 55°C, 57.5°C_1, 57.5°C_2

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	-6.8122	0.000000	Reject
Kurtosis Normality of Residuals	6.4758	0.000000	Reject
Omnibus Normality of Residuals	88.3426	0.000000	Reject
Modified-Levene Equal-Variance Test	5.3901	0.000140	Reject

Box Plot Section



Kruskal-Wallis One-Way ANOVA on Ranks
Hypotheses
H0: All medians are equal.
Ha: At least two medians are different.

Test Results

Method	DF	Chi-Square (H)	Prob Level	Decision(0.05)
Not Corrected for Ties	5	118.96	0.000000	Reject H0
Corrected for Ties	5	118.9645	0.000000	Reject H0

Kruskal-Wallis Multiple-Comparison Z-Value Test

Variable	C1	C2	C3	C4	C5	C6
C1	0.0000	2.0201	4.1238	2.2107	4.7372	2.6380
C2	2.0201	0.0000	2.1932	0.3232	7.0450	4.8564
C3	4.1238	2.1932	0.0000	1.7284	9.2382	7.0496
C4	2.2107	0.3232	1.7284	0.0000	6.9132	4.8659
C5	4.7372	7.0450	9.2382	6.9132	0.0000	2.1886
C6	2.6380	4.8564	7.0496	4.8659	2.1886	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.9352