Shear Forces, Floc Structure and their Impact on Anaerobic Digestion and Biosolids Stability

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ABSTRACT

This study was conducted to address the controlling factors of biosolids stability as they relate to mesophilic anaerobic digestion, dewatering processes and digestion enhancement by wet sludge disintegration technologies. The working hypothesis of this study is that digestion performance; nuisance odor generation and the degree of digestion enhancement by wet sludge disintegration are directly related to anaerobic floc structure and its interaction with shearing forces. Mesophilic digestion was studied in two modes of operation, convention high rate and internal recycle mode to enhanced digestion using a wet sludge disintegration device. The internal recycle system operated on the premise that stabilized sludge would be removed from the digester disintegrated, either by mechanical shear or ultrasonic disintegration for this study, and returned it for to the digester further for futher stabilization. Both benchscale and full-scale demonstrations found this mode of digestion enhancement to be effective for mechanical shear and ultrasonic disintegration.

It was also determined that volatile solids destruction in both conventional and enhanced mesophilic anaerobic digesters can be reasonably predicted by the concentration of cations in the sludge being treated. It was found that depending on the disintegration device used to enhance digestion performance was influenced by different cation associated fractions of the sludge floc.

Along with the improvement of digester performance, overall biosolids stability was investigated through of volatile organic sulfur emissions from dewatered biosolids. In doing so, a method to mimic high solids centrifugation in the laboratory was developed. The centrifugation method identified three major factors that contribute to the generation of odors from biosolids: shear, polymer dose, and cake dryness. The inclusion of shearings suggest that one means of reducing odors from biosolids generated by centrifugation is to use a shear enhanced digestion technology to degrade odor precursors, such as amino acids, within the digester prior to dewatering. Furthermore, the mechanical shearing within a digester is thought to be similar to that of mechanical shear enhanced digestion; therefore, the floc properties that control the digestion process would control observed odor generation.

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Chapter 1 : Literature Review

Biosolids Generation- What are they and where do they come from?

Biological wastewater treatment is the most common engineered means of treating municipal wastewater sources prior to discharge. The manipulation of the environmental conditions within the system promotes the growth of indigenous organisms to remove selected pollutants. These systems are effective at generating low nutrient and low carbon effluents but there are inherent costs associated with the use of microbiological systems for wastewater treatment.

One of the greatest costs associated with biological wastewater treatment is dealing with excess biomass or sludge. Sludge is commonly generated in two locations within the treatment train, during primary clarification and secondary clarification. Primary sludge is mostly comprised of large readily degradable particulate matter that is retained by gravity settling. Primary sludges while organically rich also carry the most significant pathogen load of all sludges since they are the least treated of all materials. Secondary sludges are generated from the biological treatment of the colloidal and soluble fraction of the wastewater stream. Typical unit operations that generate secondary sludges are carbonaceous BOD removal, nitrification and biological phosphorous removal. Depending on the design of the system, secondary sludges could be a collected as a mixed consortium or from individual unit operations. The removal of secondary sludges from the wastewater treatment train is necessary to maintain the organisms at an appropriate metabolic state. The typical means of secondary sludge wasting is by removal of solids from the under flow of the secondary clarifier.

The effluent from biological wastewater treatment systems is often treated with a disinfectant and discharged to a receiving body of water. However, the sludges produced cannot be simply

returned to the environment. Depending on the location of the facility and local regulations, a variety of sludge disposal solutions are available, including land filling, incineration, land application or reprocessing for commercial sale.

Outside of facilities that use incineration as the means of sludge disposal most facilities use an anaerobic digestion technology prior to dewatering and disposal to treat both primary and secondary sludges. The primary objectives of anaerobic digestion are to reduce the mass of material, reduce pathogen loads, and minimize the vector attraction to the dewatered material. Following digestion, the sludge is usually dewatered and ready for ultimate disposal. The stabilization and dewatering of sewage sludge generates an organic and nutrient rich material commonly referred to as biosolids.

Current Regulatory Considerations and Engineering Practices in Sludge Stabilization

The U.S. Environmental Protection Agency[1] has identified a set of parameters to determine the suitability of biosolids for land application. These parameters are:

- Limits on pollutant concentrations within biosolids
- Management practices
- Limits on pathogen loads
- Limits on vector attraction

While contamination of biosolids with hazardous chemicals, metals and other compounds such as endocrine disruptors are important and are a concern. Most domestic wastewater treatment plants are more concerned with meeting pathogen reductions and reducing vector attraction in

order to allow for land application. The degree to which a facility can achieve pathogen reduction and reductions in vector attraction will determine the classification of the sludge for land application. Table 1-1 list the specific requirements for pathogen reduction in Class A biosolids while Table 1-2 lists the pathogen reduction requirements for Class B biosolids.

Table 1-1: U.S. EPA Pathogen Reduction Requirement for Class A Biosolids

Pathogen	**Reduction**	**Source**
Salmonella sp.[1]	< 3 MPN per 4 grams total solids biosolids	[1]
Enteric Viruses[2]	< 1 PFU per 4 grams total solids biosolids	[1]
Viable helminth ova	<1 viable helminth ova per 4 grams total solids biosolids	[1]

N = most probable number

2 PFU = phage forming units

Table 1-2: U.S. EPA Pathogen Reduction Requirements for Class B Biosolids

Pathogen	**Reduction**	**Source**
Fecal Coliform Density	< 2 x 10^6 MPN[1] or CFU[2] per gram total solids biosolids	[1]
Salmonella sp.	Not Monitored	[1]
Enteric Viruses	Not Monitored	[1]
Viable helminth ova	Not Monitored	[1]

1 MPN = most probable number

2 CFU = colony forming units

As can be seen in Table 1-1 and Table 1-2 there are significant differences in the pathogen reduction requirements for Class A and Class B biosolids. These differences lead to restrictions on where the material can be applied as well as restriction on access and site management

practices. However, whether a material is Class A or Class B, the requirements for reducing vector attraction are the same.

Vector attraction regulations are in place to reduce the likelihood that disease carrying organisms, “vectors”, will not congregate and proliferate in areas which biosolids are stored or land applied. These organisms serve as a mechanism for transporting infectious material or organisms to humans. Disease vectors include certain insects such as flies, and mammals such as rats. Currently the U.S. EPA does not have standards for determining biosolids stability and thus vector attraction, rather, they employ a group of treatment option that have specific performance citeria, in which if the criteria are met, then reduction in vector attraction is achieved. The one of the most commonly used citeria for sufficiently reducing vector attraction is the demonstration of 38% volatile solids reduction in a digester. The digester used is typically a mesophilic anaerobic digester.

Mesophilic anaerobic digesters typically can produce Class B biosolids by reducing the fecal coliform load below the 2 million MPN requirements and reducing the volatile solids concentration by at least 38%. This technology is one of the most common means for not only stabilizing biosolids but also reducing the mass of material by mineralizing both primary and secondary sludges to methane and carbon dioxide.

Solids disposal represents a very significant cost to wastewater treatment facilities. Some estimates have solids management representing as much as 50% of the annual operating budget of wastewater treatment facilities. Most of this cost is associated with the final disposal process,

hauling and tipping fees at landfills or land application sites. While other disposal methods may be economically viable for smaller utilities, for very large facilities land application represents a significant cost savings.

In recent years there has been a move toward enhancing the digestion process. The primary focus of digestion enhancement is improved solids destruction to reduce the mass wasted. With enhanced digestion there is an increase gas production, deterioration of dewatering properties and potential for enhanced pathogen destruction. The mechanisms for enhancing anaerobic digestion can be summarized into two general categories. The first being ecological changes and the second being physio/chemical changes to the digestion system.

The ecological changes are those in which the digestion environment is altered leading to functional changes in the physiology of the organisms present as well as structural shifts in the entire microbial community. The most common means of achieving these ecological changes is though varying the solids retention time and/or increases in operational temperatures.

Acid/Gas digestion is a good example of how shifts in solids retention time can effect a community. In the acid phase thesolids are introduced at a high loading rate resulting in a low SRT resulting in particulate hydrolysis and the production of volatile fatty acids. At low SRTs hydrolyitic and acidogenic organisms dominate and the slow growing methanogens are washed out. As a result, volatile fatty acids accumulate and organic matter is solublized. In the second phase of the Acid/Gas system, the SRT is increased and the effluent of the Acid phase is introduced. The high concentration of VFA's and solubilzed material serves to give

methanogenic bacteria a competitive advantage, resulting the conversion of the VFA's to methane gas. By operating in this manner it is thought that hydrolysis and methanogenesis are maximized.

Thermophilic digestion is an example of how temperature can effect digestion performance. At elevated temperatures (~55^{o}C) select hydrolytic and acidogenic organisms as well as select methanogens dominate resulting in good solids destruction. But also the elevated temperature leads to increased deactivation of pathogenic organisms. If the system meets the time and temperature requirements for Class A solids, significantly more disposal options become available to the utility due to its pathogen free classification.

Shifting or manipulating the ecology of sludge digestion systems has produced some very efficient and successful commercial technologies. It has also allowed for the more liberal use of biosolids as a soil amendment due to its pathogen free classification. While these technologies are effective there is a very significant drawback in terms of up front capital. The largest utilities usually can afford such changes with careful planning. However, mid size to small utilities may not have sufficient access to the capital need for such expansion and thus whole process changes are not econcomically viabl. Another problem for all utilities is land. Most own a finite amount of land and thus have a finite capacity for expansion. Thus investing in large solids handling and disposal facilities could consume this precious resource.

Based on these observations there has been a steady movement toward using physio/chemical processes to enhance existing mesophilic digestion systems. These processes are categorically

known as wet sludge disintegration technologies. The primary objective of these technologies is to apply physical forces, chemical, radiological, or oxidative processes or promote chemical alterations to sludge to enhance the bioavailability of sludge. The approaches taken to achieve this goal are extremely diverse. Each of these technologies looks to exploit some weakness or characteristic of sludge to enhance the digestion process. Thus a through understanding of the technologies can result in the introduction of a novel and effective treatment option for wastewater treatment facilities. It could also lead to a deeper understanding of sludge floc structure, community structure, and microbial interactions.

Wet Sludge Disintegration Technologies

One means of enhancing the digestion of sewage sludge and potentially the stability of the biosolids is the used of wet sludge disintegration technologies. Sludge disintegration has been defined in the literature as "the destruction of sludge by external forces"[2]. The term external forces can take shape as one of or a combination of many technologies. These technologies include, mechanical shear, chemical oxidation, sonication, γ-irradiation, electroporation, and thermal disintegration. Each of these technologies has been investigated and reported some degree of digestion enhancement. The most common technologies investigated to date are:

- sonication
- mechanical shear
- thermal processes
- oxidative technologies

Each will be discussed below to give an overview of the state of the art of wet sludge disintegration technologies.

Ultrasonics and Sonication Enhanced Digestion

Sonication has been one of the most studied methods of WSD technology; in particular, the use of sonication to disrupt waste activated sludge (WAS) has been investigated throughly. Sonication has been found to increase the negative charge associated with the floc material[3] and disrupt flocs. The process of sludge floc disruption is thought to be due to high temperature (5000K) and hydrodynamic shear generated from the collapse of cavitation bubbles within the area surrounding the ultrasonic probe[4].

The application of this technology has mainly focused on the preprocessing of WAS prior to anaerobic digestion using frequencies between 20 kHz and as high as 3217 kHz[4]. Frequencies greater than 500 kHz have been found to disrupt materials to a lesser extent as radical generation predominates over mechanical forces[5].

The effects of sonication on mesophilic anaerobic digestion have varied. Research suggests that enhanced digestion is a function of power level, solids concentration[6], sample volume, vessel geometry, probe position[7], and exposure time. Increased gas production and volatile solids reductions are commonly observed. Reductions in pathogens resulting from the use of sonication has not been consistent; some research has suggested a reduction[7] while other research showed no change[8].

Mechanical Shear Enhanced Anaerobic Digestion

Mechanical shear has also received some attention but not to the same extent as sonication. The mechanical devices that have been studied include rotor-stator shear devices [9], Dyno Mills[10], jetting and colliding technologies[11] and lysate-thickening centrifuges [12].

As with the sonication technologies, the results vary from technology to technology with regard to their ability to enhance anaerobic digestion. With the application mechanical shear, other interesting benefits have been noted including reduction in digester foaming [13, 14] and increased protease activity [10]. An increase in protease activity could improve the digestion process since it is believed that hydrolysis of proteins is the rate-limiting step in the process and most of the residual material associated with anaerobic sludge is proteinaceous in nature [15].

Increased protease activity, which is critical to enhancing the hydrolysis step of digestion, was observed using mechanical shear, but may not be observed in sludges treated with other WSD technologies such as thermal destruction, chemical destruction or sonication. Thermal and chemical processes can potentially denature proteases. Sonication can potentially destroy the protease structure since the shear forces asscociated with the collapse of a cavitation bubble have been shown to effect materials with molecular weights as low as 40,000 daltons [5]. Many proteases and other enzymes have molecular weights greater than 40,000 daltons. The effect of the WSD technology on extracellular enzymes, particularly proteases, must be considered when looking at overall performance and considering increased performance per unit of applied energy. Greater floc destruction achieved with thermal, chemical or ultrasonic technologies compared to mechanical shear may be offset by a reduction in the biodegradation rate if enzymes

are destroyed. The enhanced biochemical activity associated with mechanical shear technologies may give these a distinct advantage at a lower energy cost.

Thermal Sludge Disintegration Processes

Thermal pretreatment of sludge for anaerobic digestion is one of the few technologies that are being operated full-scale. The Cambi process was installed in Hamar, Norway using temperatures between 130 and 180 ^{o}C to thermally hydrolyze feed sludge prior to digestion. This process produces an agriculturally suitable biosolids and a 50% reduction in digester size[16]. Though full-scale systems have been installed at several locations and research continues in the Cambi process to improve this process and better understand its limitations.

The application of thermal technology is not limited to the Cambi process. Thermal disintegration has been applied to waste activated sludge prior to digestion [17] as well as dewatered sludges after which the liquefied material is reintroduced to the digester for further degradation [18, 19]. Similar to the Cambi process, both of these processes use operating temperatures of 175 ^{o}C, and in the case of dewatered sludge elevated pressure is used as well (4 Mpa)[19].

The high specific heat of water is an obvious draw back to thermal systems. Large quantities of energy are utilized in heating water rather than disintegrating biosolids. Sawayama et al. [19] reported that better thermal energy use disintegrating dewatered sludge because it is more concentrated and thus energy does not have to be expended heating water. Also, depending on location and available heating sources, thermal disintegration can be expensive.

Oxidative Technologies (Ozone)

Ozone has been used in the water industry for disinfection due to its oxidative properties. Ozone has gained attention as a means to increase the bioavailability of sewage sludges for enhanced anaerobic digestion. Scheminski et al. [20] investigated the use of ozone to oxidize digested biosolids and return them for additional degradation. When compared to other disintegration technologies (sonication, high pressure homogenization, thermal, and combined thermal chemical) only thermal chemical, the addition of a chemical treatment and heat in combination, had a greater degree of solubilization (55%) as compared to ozonation (40%).

Ozonation has also been used as a means to pretreat biosolids prior to digestion. Weemaes et al. [21] found that a maximum 64% reduction in volatile materials based on COD could be achieved with pre-ozonation, as compared to a 38% reduction for non-ozonized biosolids. Pre-ozonation also increased methane production by a factor of 1.8 at a dose of 0.1gO3/g-COD but at higher doses, the lag phase prior to gas production increased.

Ultrasonics and Sonication Principles and Uses

Principals of Sonication

Ultrasonication generates cavitation acoustically at frequencies of 20 kHz or greater, beyond the range of human hearing. The process involves alternating periods of compression and rarefaction, during which if the negative pressure generated is greater than the tensile strength of the fluid and a small bubble or void will form[22]. Once formed the cavitation bubble will expand and in then rapidly implode generating an extreme microenvironment having pressures of

~1000 atmospheres and temperatures of >4000 ^{o}C [23] Under these intense conditions both mechanical and chemical degradation of organic matter can occur. The collapsing bubble causes shock waves to be emitted to the surrounding fluid[22] resulting in the high mechanical shear. Concurrent with the high shear forces material within the collapsing bubble can under go pyrolysis. Depending on the dissolved gas content and solution composition different chemicals can be formed. Makino *et al*. [24] demonstrated that the pyrolytic decomposition of water generates ·OH and ·H radicals.

Acoustic cavitation can be impacted by environmental factors, which may limit its effectiveness as a sludge disintegration technology. Tuziuti *et.al*. [25] demonstrated that the efficiency of sonochemical reactions is heavily impacted by the size and number of particles in solution. Aluminum oxide particles greater than 10 μm in size showed an increase in potassium iodine oxidation while a maximum rate was observed at a concentration of 20 mg-alumina/mL with the rate decreasing at higher and lower concentrations. A recent study by Mao *et.al*.[26] reported that during the ultrasonic disintegration of primary and secondary sludge there was an optimum concentration for maximum disintegration, 0.98-2.6 %TS and 1.02-2.88% TS respectively. The significance of an optimum concentration has a direct impact on the operational considerations of a sonication unit. Prior to digestion it is common practice to thicken sludge, primary 5-10% and secondary 4-6%[27], significantly more than the value reported by Mao *et.al*.[26].

Other solution parameters have been shown to impact the ultrasonic efficiency including, viscosity, initial temperature and dissolved gas composition. Mason and Lorimer[22] reported that with increasing solution temperature there is a decrease in the pressure and temperature

within the caviation bubble, thus lessening its capacity for physical and chemical changes upon collapse.

Quantification of Power Input to Samples

One of the most difficult items when comparing ultrasonic technologies to each other is the quantification of energy input to the sample. Within the literature the level of sonication applied to a sample varies from author to author. Tiehm *et.al*.[4] proposed uniform terminology for the application of ultrasonic energy to sewage sludge. Table 1-3 summarizes the proposed terms presented in Tiehm's work. While the terms proposed by the Tiehm study are effective, a forth descriptor should be added, Specific Ultrasonic Dose (SUD). Multiple studies have expressed the amount of energy applied to a system as a function of the concentration of material being disintegrated on a mass basis, SUD. Given that both particle size and concentration effect ultrasonic reactions[25] and the material being catalyzed is the sludge itself then power input may be best described by this measure.

Table 1-3: Terminology for the Description of Ultrasonic Energy Input to a Sample

Terminology	Description	Units	Citation
Ultrasonic Intensity	power supplied per unit area of transducer	W/cm^2	[4]
Ultrasonic Density	power supplied per unit volume of sample	W/cm^3	[4]
Ultrasonic Dose	total power supplied per unit volume of sample for a specific exposure time	$W\text{-}s/cm^3$	[4]
Specific Ultrasonic Dose	total power supplied per unit volume of sample for a specific exposure time per unit mass of material being disintegrated	W-s/kg-solids	

Having uniform descriptors for the different measures of ultrasonic power input a means of calculating actual electrical power used and applied can be discussed. Löning et.al.[28] reported

three locations of power measurement allows for a total systems efficiency analysis, power to the unit, power to the transducer and the power imparted to the sample. The ultrasonic dose reported in the literature is extremely variable. Table 1-4 summarizes the ultrasonic dose for a variety of studies. The values reported are based on the power input given, the time of operation and the volume of sample used. In general little or no information relating to samples solids concentration is given.

Table 1-4: Ultrasonic Dose Values Calculated based on Literature Parameters for Wastewater Processes

Source	Ultrasonic Dose (W-min/ml)	Frequency (kHz)	Location of Sonicator
Muller *et al*. (2006)	2.82	20	Pretreatment
	1.79	20	Recycle
	1.85	20	Recycle
*Chu et al.[7]	6.6		Pretreatment
*Yoon et al. [29]	36	20	Recycle-MBR Aerobic
*Laffite-Trouque and Forester [8]	47	23	Pretreatment
*Wang et. al. [30]	20,000-80,000	9	Pretreatment

* assumed full sonicator wattage was applied

What is evident from Table 1-4 is that there are a wide variety of ultrasonic doses reported in the literature. These values are based the reported wattages which may or be the rated wattage of the unit. In some cases the total power of the unit is not applied and thus the power input or utilization is grossly over estimated. For the purposes of comparing disintegration efficiencies of different sludge samples and under different conditions power equivalency is critical. Löning et.al.[28] utilized a calorimetric method relating the change in temperature over time to the specific heat and mass of solution exposed to sonication. Christi[23] reported the same approach to determining energy input to a sample from an ultrasonic probe with the power input being calculated using Equation 1-1.

Equation 1-1, utilizes the heat energy evolved from the collapsing cavitation bubbles to determine the amount of power applied. However, you must assume that endothermic and exothermic sonochemical reactions are negligible within the system and the only heat is associated with the collapsing bubbles[31]. Hua and Hoffmann[32] demonstrated that the amount of heat released upon bubble collapse is a function ultrasonic frequency and saturating gasses, thus power input to sludge could be dependent upon the sludge type as well as equipment used.

Equation 1-1: Calculation of Energy input to a Liquid Sample by an Ultrasonic Probe

$$E(W) = \frac{\Delta T}{\Delta t} * C_p * m$$

Where : **E** = Power in Watts (W)

C_p= Specific heat of the solution (Water) (J/g-°C)

m= mass of solution (g)

Ultrasound and Bacterial Interactions

The primary use of ultrasonic equipment with bacteria is to either damage or to lyse the cells in suspension. In wastewater applications it is commonly thought that a majority of the material associated with enhanced solids destruction is due to cellular lysis. Based on this assumption/ assertion a review of the capacity of ultrasonic radiation to lyse bacterial or archeal cell is warranted along with the factors that affect it.

Within the wastewater literature there is little discussion on the quantification of cellular lysis due to ultrasonic cavitation. Tiehm *et al*.[4] reported cellular lysis of ultrasonically treated WAS based on the increase in soluble chemical oxygen demand (COD). While the release of COD is associated with the rupture of bacterial cells there is significant COD associated with the extracellular polymeric substances (EPS) surrounding them. With out verification by a lysis indicators such as Glucose-6-Phosphate Dehydrogenase (G-6-PDH)[33], alkaline phosphatase or free DNA [34] in solution it is premature to say lysis is the main mechanism of sludge reduction.

The capacity of ultrasonic radiation to cause lysis in biological cell is well documented. Scherba et al.[35] demonstrated that the shear forces generated from the collapse of caviation bubbles generated at 26 kHz could lyse gram postive bacteria (*Staphylococus aureus* and *Bacillus subtillus*) as well as gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). This study also noted that it did not appear that the increased peptidoglycan layer associated with the gram positive organisms provide any enhanced protection to lysis relative to the gram negative organisms.

While the morphology of the bacteria may not impact the lytic capacity of ultrasound the environmental conditions under which it occurs has a significant impact. Raso *et al*.[36] reported that the food pathogen *Yersinia enterocolitica* under ambient conditions showed a 1-log reduction per cycle after 1.5 minutes of ultrasonic exposure under ambient pressure and temperature. However, when pressure was increased to a maximum of 600 kPa the time to equivalent removal was reduced to 0.2 minutes. The impact of elevated temperature on lysis was

realized only in a narrow range of 50-58 °C below which no change was observed and above which temperature changes alone had the same impact as ultrasonic treatment.

Along with the physical environment in which the ultrasonic device is operating in the type of device has a direct impact on cell lysis. Ultrasound generates cavitation by operating at a specific frequency and having the probe oscillate certain amplitudes. At low frequencies (20 and 38 kHz) dispersion and lysis are maximized while at higher frequencies (512 and 850 kHz) only dispersion occurs[37]. Increasing the amplitude of low frequency ultrasounds can increases the kill rate of the device as well[36].

The use of high frequency ultrasound, while not as effective as low frequency at immediate cell lysis, does have specific advantages. At high frequencies the generation of radicals and oxidative chemicals increases. Hua and Hoffman[32] showed that the production rate (k_{freq}) (frequency in kHz) of H_2O_2 and ·OH from sonication was $k_{500}>k_{80}>k_{40}>k_{20}$. The increase in H_2O_2 and ·OH formation was attributed to increased implosion temperatures and pressures and the relative increased stability of cavitation bubble generated at higher frequencies. The formation of oxidative chemical such as H_2O_2 and radicals ·H and ·OH can have toxic effects of microorganisms in close proximity. However, the impact of radicals and oxidative chemicals of organisms can be diminished by the environment they are generated in. Mišík and Riesz[38], demonstrated that glucose and hydrophobic amino acids (Tryptophan, Phenylalanine, Tyrosine, Leucine, Valine and Methionine) significantly reduce the yield of ·H and ·OH from sonochemical reactions. Other reported radical (·OH) scavengers include bicarbonate, bromide and benzoate[24]. Many of these compounds are commonly found with in the wastewater

environment. Thus it is likely that free radical damage and oxidative chemical damage to cells will be minimal relative to the mechanical stresses associated with a collapsing cavitation bubble.

Other Applications of Ultrasound

The application of ultrasonic radiation for environmental purposes is a growing field. Beyond its application for the enhancement of sludge digestion, applications are being developed for the removal of organic contaminants from a variety of industrial effluents as well a remediate-contaminated ground water.

A recent study by Xu *et al.*[39] investigated using 20 kHz ultrasound to remove organics and ammonium-N from the effluent of a Coke gas making plant. What was found was that the removal of the COD fraction and ammonium-N fraction was influence by the saturating gas, pH and the intensity of ultrasound. The main mechanism for removal hypothesized by Xu *et al*. was the sonochemical decomposition of ammonium-N within cavitation bubble and the organics along the fringes of the bubbles.

Mesophilic Anaerobic Digestion

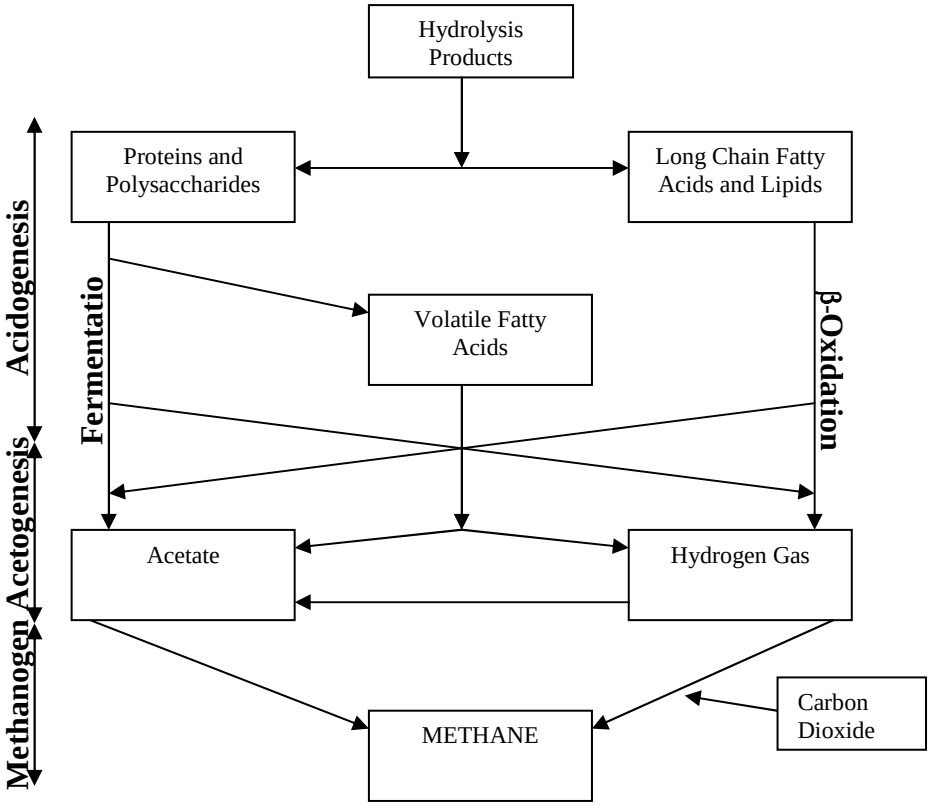
Mesophilic anaerobic digestion has been used for decades as a means of reducing biosolids mass, vector attraction and generating methane. Given its long history of use in the wastewater industry, a great number of small and large utilities utilize it for biosolids stabilization. Given this it is the primary target market of the emerging wet sludge disintegration technology market. However, for these technologies to be successful and to fully understand how they impact the

digestion process a considerable understanding of the ecology, biology and biochemical reactions which constitute the process of mesophilic digestion must be gained.

The Microbiology of Anaerobic Digestion

Anaerobic digestion is a biologically complicated process. There are a variety of metabolic processes, inhibitory compounds, hydrolytic activities and syntrophic associations that need to be considered. In general the progression of anaerobic degradation of organic matter moves through four principal reaction steps, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These processes are shown in Figure 1-1.

Figure 1-1: Major Metabolisms Involved in Mesophilic Anaerobic Digestion



Copied From: Grady, Diagger and Lim [40]

The progression of organic matter through the digestion process can be understood by looking at two fundamental kinetics descriptions or models, Michaelis-Menton and Monod kinetics. Each of these models produces a similar concentration response curve, a rectangular hyperbola. However the fundamental underlying assumptions for each model vary greatly. The following review describes the use of each applicable kinetic model for describing anaerobic digestion kinetics.

Hydrolysis of Organic Matter (Michaelis-Menton)

The hydrolysis of organic matter is considered by some to be the rate-limiting step in the anaerobic digestion process[41]. Extracellular enzymes located in the EPS of sludge floc [38] catalyze the break down of high molecular weight organic material to low molecular weight compounds. Because this is a specific enzyme-related processes, Michaelis-Menton kinetics are used to describe the kinetics. The composition and specific activities of extracellular enzymes have been shown to be a function of influent characteristic [42].

Hydrolytic enzyme activity can be located within the bulk solution, incorporated within the EPS matrix or bound to cell surfaces. Studies have shown that most hydrolytic enzymes are closely associated with the cell [43] (ectoenzymes) and EPS[28] while little is found in the bulk solution[44] (exoenzymes).

The observed rate of hydrolytic enzyme activity is affected by a variety of parameters. Temperature[45], redox condition[46], particle size[47], available surface area[48] and presence

of specific divalent metals[49] all have been shown to effect the observed degradation rates of extracellular hydrolytic enzymes.

Table 1-5 summarizes various specific enzyme activities reported in the literature. What is readily apparent is the fluctuation in values observed, even for the same enzyme. Some of the fluctuations are due to variations in the methodology in determining a unit of activity for an enzyme while others are due to the normalizing value (mg-protein or g-VS).

An important change in enzyme activity noted in the data of Table 1-5 is the effect that floc disruption has on the reported value. The mixed results for L-Leu aminopeptidase, α-glucosidase and lipase suggest that the increase in digestion efficiency observed with wet sludge disintegration technology is potentially due to changes in hydrolytic activity but increases in specific enzyme activities can also be expected to be sludge specific.

The traditional method for investigating hydrolytic activity in sludges is to model hydrolysis using first order kinetics. Recent research has shown that first order kinetics for modeling hydrolysis may not be the best fit for all sludges. Sanders et al. [48] and Vavilin et al. [50] have put forth two different models for hydrolysis both of which incorporate surface area into the hydrolysis rate equation. These researchers believe that the available surface area for hydrolytic bacterial growth and substrate access has a direct impact on the observed hydrolysis rate, increased available surface area increases the rate. Others have noted the applicability of surface area based hydrolysis kinetics for anaerobic degradation processes as well[45].

Table 1-5: Summary of Hydrolytic Specific Enzyme Activities Reported in the Literature

Enzyme Group	Activity	Units	Conditions	Source
Lipase	-0.036	U/mg-Protein	Methanogenic 20-	[51]
	1.11[1]	U/mg-Protein	22°C	
Protease	6700[1]	U/mg-Protein	WAS 20 °C, Plant 1	[52]
	4.177+/-0.447[1]	U/mg-Protein	WAS 40 °C, Plant 1	[52]
	75.8+/-10.5[1]	U/mg-Protein	WAS 50 °C, Plant 2	[52]
	8.00+/-0.83	ΔAbs/min/g-VS	Act. Sludge 1	[43]
	5.54+/-0.24	ΔAbs/min/g-VS	Act. Sludge 2	[43]
	5.44 +/-0.15	ΔAbs/min/g-VS	Act. Sludge 3	[43]
	5.21+/-0.13[1]	ΔAbs/min/g-VS	Act. Sludge 2	[43]
	5.33+/–0.10[1]	ΔAbs/min/g-VS	Act. Sludge 3	[43]
L-ala-aminopeptidase	10.5-57.8	U/mg-Protein	Act.Sludge Floc	[44]
L-Leu-aminopeptidase	17.3-38.0	U/mg-Protein	Act.Sludge Floc	[44]
	9.04+/-0.18	μmol/min/g-VS	Act. Sludge 1	[43]
	7.90+/-0.15	μmol/min/g-VS	Act. Sludge 2	[43]
	5.06+/-0.08	μmol/min/g-VS	Act. Sludge 3	[43]
	5.21+/-.013[1]	μmol/min/g-VS	Act. Sludge 2	[43]
	5.33+/-0.10[1]	μmol/min/g-VS	Act. Sludge 3	[43]
L-lys-aminopeptidase	10.0-18.5	U/mg-Protein	Act.Sludge Floc	[44]
L-glu-aminopeptidase	3.0-9.0	U/mg-Protein	Act.Sludge Floc	[44]
L-phe-aminopeptidase	1.0-5.5	U/mg-Protein	Act.Sludge Floc	[44]
Alkaline Phosphatase	19.5-47.3	U/mg-Protein	Act.Sludge Floc	[44]
α-glucosidase	5.0-13.0	U/mg-Protein	Act.Sludge Floc	[44]
	0.95+/-0.02	μmol/min/g-VS	Act. Sludge 1	[43]
	2.52+/-0.19	μmol/min/g-VS	Act. Sludge 2	[43]
	2.33+/-0.09	μmol/min/g-VS	Act. Sludge 3	[43]
	0.74+/-0.02[1]	μmol/min/g-VS	Act. Sludge 1	[43]
	1.85+/-0.11[1]	μmol/min/g-VS	Act. Sludge 2	[43]
	2.00+/-0.07[1]	μmol/min/g-VS	Act. Sludge 3	[43]
β-glucosidase	5.0-9.3	U/mg-Protein	Act.Sludge Floc	[44]
α-galactosidase	3.0-8.8	U/mg-Protein	Act.Sludge Floc	[44]
β-galactosidase	1.5-5.3	U/mg-Protein	Act.Sludge Floc	[44]

1: sludge was disrupted with physical process, sonication or Dyno Mill

Acidogenesis, Acetogenesis and Methanogenesis (Monod Kinetics)

Following the hydrolytic breakdown of particulate organic matter via hydrolysis, the observed kinetics of anaerobic digestion is usually described by Monod kinetics[40]. Monod kinetics are

used to describe overall rates without regard for specific enzyme activity. Figure 1-1 illustrates the various degradation steps involved in mesophilic anaerobic digestion.

Each of the major metabolic process in Figure 1-1 is carried out by a mixture of bacterial species whose presence will depend on the retention time [53] and influent characteristics [54] of the digester. Due to the potential number of organisms involved in each metabolic step through digestion, kinetic parameters are generally reported for each type of metabolism rather than individual organisms. Table 1-6 summarizes the Monod Kinetic parameters for each step of the anaerobic digestion process.

From the data presented in Table 1-6 it is possible to hypothesize which metabolic processes have the potential to operate at a higher specific growth rate with the addition of a WSD technology. The very high half saturation coefficient (K_s) values associated with *Methanosarcina* spp (300 mg/L) and Long Chain Fatty Acid oxidation (800 mg/L) suggest that an increase in acidogenesis and acetoclastic methanogenesis is possible with increased substrate availability. The relatively lower K_s values of the other metabolic processes suggest that the organisms involved are already operating at their maximum specific growth rate. Thought this approach is simplistic it does allow for a start to understanding how anaerobic metabolic processes are affected by WSD technology.

Table 1-6: Summary of Monod Kinetic Parameters for Major Organisms of Mesophilic Anaerobic Digestion

Bacterial Group	μ_{max} (hr^{-1})	K_s (mg –substrate COD/L)	Source
Amino Acid and Sugar Fermenting Bacteria	0.25	20-25	[40]
Long Chain Fatty Acid Oxidation	0.01	800	[40]
Propionate Oxidation	0.0065	250	[55]
Propionate Oxidation	0.0033	800	[56]
Methanosarcina sp.	0.014	300	[40]
Methanosaete sp.	0.003	30-40	[40]
H_2 Oxidizing Methanogens	0.06	0.6	[40]
Acetoclastic Methanogenesis	0.0173	166	[57]

Inhibition and the Detection of Process Upset in Anaerobic Digestion

The complex nature of anaerobic digestion and the interdependence of the metabolic processes make process inhibition a concern. The inhibition mechanisms for anaerobic digestion include product inhibition and toxic inhibition from the influent. Several metabolic by-products have been found to be inhibitory to the digestion process including, butyric acid, ammonia, hydrogen (gas), and sulfide. Influent toxins include xenobiotic chemicals and heavy metals. The major inhibitory concerns related to the use of WSD technology are product inhibition, destruction of specific organisms, or physical separation of symbiotic organsims [58, 59].

Zoetemeyer et al[60] demonstrated that high organic loads as well as butyric acid (2500 mg-C/L) is inhibitory to the acidogenic process. Long chain fatty acids are present in digesters as either free phase of floc bound. Linoleic acid has been shown to strongly inhibit acetoclastic methanogensis (30 mg/L), while hydrogenotrophic methanogenesis are inhibited to a lesser

degree (50 mg/L) [61]. Shin et al [62] reported that unsaturated LCFA were more toxic than saturated fatty acids and that increased destaturation increased toxicity.

Increases in LCFA oxidation, β-oxidation, can also result in increased hydrogen production. Hydrogen partial pressures greater than 10^{-4} to 10^{-6} atm makes propionate oxidation thermodynamically unfavorable[58]. Enhanced hydrogen production could be detrimental to the system unless bacterial populations are present which consume the excess hydrogen.

Ammonia-N is the metabolic byproduct of amino acid degradation in the digestion process. Elevated levels of ammonia-N have elicited a general stress response from *Methanosarcina mazei* S-6 as indicated by an increase in the transcription of the *dna K* [63]. Methanogenic communities have been shown to be more sensitive to high ammonia concentration than hydrolytic or acetogenic communities [64]. Under themophilic conditions acetoclastic methanogens have been shown to be considerably more sensitive to ammonia-N than hydrogenotrophic methanogens[65] with an observed 50% decrease in growth rate at 4 g-N/L and 7.5 g-N/L respectively.

The stability and the potential for process upset can be monitored through a variety of substrates and system parameters. Volatile fatty acids have been suggested as a means to monitor process stability. Propionic and butyric acid have been both shown to accumulate in failing digesters[57]. Some research has suggested that monitoring propionic acid levels can be used to detect process upset while others have suggested that butyric acid is a better indicator[66]. Headspace gas composition can be utilized as a measurement of process stability and

performance. Methane usually comprises ~70% of headspace gasses while ~30% is carbon dioxide. Fluctuations away from this 70/30 ratio would indicate process instability or upset.

Wastewater Floc Structure

The formation of biological flocs within the wastewater environment has garnered significant attention in determining how it impacts solid liquid separation. An understanding of what the components are and how they are put together can have an impact beyond how well it will settle or dewater. For wet sludge disintegration techonologies to become commercially effective the engineers applying the technology must understand how floc structure will impact process performance.

The structure of the floc will be directly impacted by the influent characteristics, organic composition, cation concentration, metals concentrations as well as the unit operations within the plant itself. Thus one would expect that a technology could be very successfully applied at one wastewater treatment facility and then be a complete failure at another. Understanding what the floc material is composed of and how it is constructed should allow for an informed assessment of the applicability of a specific technology to a specific sludge.

Extracellular Polymeric Substances (EPS) Composition in Sludge Floc Matrices

Waste activated sludge is the most common material treated by wet sludge disintegration technologies to enhanced digestion. WAS is primarily comprised of extracellular polymeric substances (EPS), microbial cell (eukaryotic, prokaryotic and archea), and inerts (clays, sands and metal precipitates). The EPS fraction represents the most diverse material in the sludge floc

matrix. Depending on influent characteristics it can be composed of nucleic acids, proteins, polysaccharides, fats and humic and fulvic acids. Vallom and McLoughlin[67] reported that a majority of the material (88%) falls within three classes, DNA/RNA, proteins and polysaccharides.

Of the three major classes of material Bura *et al.*[68] reported that proteins are the most significant and play a major role in bioflocculation. It has been suggested that 15 kD "lectin-like" proteins secreted by the bacteria play an integral role in biofloccuation by using divalent cations to form cationic "bridges" between individual bacteria[69]. Görner *et al.*[70] reported finding protein material likely to be lipoproteins as well as glycoprotiens which could be part of the lectin structure in sludge EPS. The presence of glycoproteins in EPS was reported by Jorand et al.[71] as indicated by 77% of total protein precipitating at pH 2, and also that a majority of the hydrophobic material associated with sludge flocs was protein in nature.

Cations and Metals in Flocculation

Cations and trivalent metals are critical components of wastewater floc structure. The interaction of different cationic species with the microogansisms and the organic matter within the wastewater environment has been shown to impact a variety of wastewater unit operations.

Higgins and Novak[72] first demonstrated the significance of cations in floc structure by demonstrating that activated sludge settling and dewatering was strongly influenced by the ratio of monovalent to divalent cations (M/D) in solution. The M/D model helped explain why activated sludge flocs deflocculated when exposed to electrophilic toxins which trigger the

Glutathione Gated Potassium Efflux stress response described by Bott et al.[73]. However, the model was incomplete. Park *et al*.[74] demonstrated that not only are monovalent and divalent cations critical to activated sludge flocculation but trivalent metals, iron and aluminum in particular, play a significant role in the observed sludge properties. The work by Park *et al*.[74] suggested a modification of the M/D ratio to include trivalent metals in the form of M/(D+T) where T represents the equivalence contribution of trivalent metals.

Monovalent, divalent and trivalent cations are incorporated into floc and impact observed sludge floc properties such as settling and dewaterability. The material associated with each of the classes differs. Higgins and Novak[69] reported that monvalent and divalent cations interact competitively binding to "lectin-like" proteins attached to the cell surface. However, when activated sludge is exposed to sulfide, iron sulfide is formed and there is a significant release of soluble protein but little polysaccharide[75].

The observation that cations selectively bind to different materials also has implications on how sludge flocs behave in different unit operations. Until recently most floc modeling has focused on the activated sludge basin and aerobic condition. However, it has been demonstrated that trivalent metals also impact the digestibility of waste activated sludges. Park et al.[76] reported that the digestion of waste activated sludge was positively correlated with the amount of sludge associated iron on a mg-Fe/g-ash basis. However, Park *et al*.[76] further reported a release of calcium and magnesium during the aerobic digestion of WAS, which also correlated with increases in solution nitrogen. What this data shows is that depending on the unit operation the

cation composition of the sludge being treated can have a significant impact on observed performance.

Trivalent Metal Sources in Wastewater

Based on the observations of Park et al.[76] it would appear that the material primarily associated with anaerobic digestion is that bound to iron. However, another metal trivalent metal that is a known coagulant and is present in wastewater sludges is aluminum. It is believed that by understanding the interactions of aluminum and iron with organic matter it should be possible to predict digestion and enhanced anaerobic digestion performance.

Sources of Iron in Wastewater Treatment Systems

Iron is commonly used in drinking water as well as wastewater treatment systems for a variety of purposes. Ferric chloride is the most common type of iron chemical used in wastewater treatment systems. It has been shown to be effective at chemical precipitation of phosphate for to help meet phosphorous limits. It is also an effective coagulatant and can be used in solid liquid separation process alone or in conjunction with polymer[77, 78]. Iron has also been added in the sewer system as a means of controlling sulfide emissions. [79] reported that drinking water sludges precipitated with ferric chloride could be discharged to wastewater facilities for sulfide control. Also iron rich effluents could be from a point source industrial discharge to the sewer system.

Beyond the anthropogenic sources of iron, there a variety of sources mechanisms by which iron can enter the wastewater treatment system. Biological iron reduction has been shown to

solubilize ferric iron to ferrous from otherwise stable sources. Based on the variety of sources it is not unreasonable to expect varying iron concentration from utility to utility.

Aluminum Sources in Wastewater Treatment Facilities

Unlike iron, aluminum is not used as much in wastewater treatment facilities. Aluminum, is used in drink water coagulation and flocculation processes and some utilities accept the resulting sludge either through the sewer or have it trucked to the site. There has been recent work looking into improving activated sludge floc settling by the addition of aluminum to the treatment process[80]. Other anthropogenic sources of aluminum are industrial discharges as well as domestic discharges. Aluminum is a common ingredient in many shampoos and grooming products. Thus event domestic discharges contribute to the aluminum load observed at the plant.

Iron Chemistry in Wastewater Systems

There are both anthropogenic and natural sources of iron in the wastewater treatment system. Regardless of the source iron chemistry, particularly redox chemistry, has been found to play a critical role in the observed sludge properties. Understanding how iron chemically/biochemically changes under different conditions found in unit operations is critical to predicting process performance.

The binding of iron species to organic matter has been extensively studied in natural wasters, focusing on natural organic matter. Luider *et al*.[81] showed that the binding affinity of dissolved

organic carbon to ferric hydroxide was dependent on the source, allochthonous or autochthonous, and how much biodegradation had occurred. When iron hydroxide binding was studied in wastewater systems similar results were found. El Samrani *et al*.[82] found that not only is the primary mechanism of iron colloid interaction charge neutralization but there was a greater affinity for long aliphatic structures over more branched structures at low iron doses. The apparent selectivity of iron hydroxide for specific structures can be translated into classes of materials. Novak et al. [83] showed that ferric chloride as a coagulant selectively removes solution protein prior to solution polysaccharide. Only after the optimum dose was achieved was polysaccharide removed.

The selectivity of iron hydroxide for protein can have an impact on the material associated with sludge flocs. Analysis of activated sludge[68] and anaerobic sludge[15] EPS has shown that it is primarily is protein. Furthermore, Cetin and Erdincler [84] found that the protein content of the EPS is a function of the influent carbon to nitrogen ratio. It is reasonable to believe that not only will the iron then interact with free colloids but also the floc matrix, especially at low C/N influents.

The coagulation of organic colloids by ferric iron and their subsequent incorporation into the sludge floc creates an environment that is favorable not only for chemical iron reduction but biological reduction. In wastewater systems it was commonly thought that the chemical reduction of ferric iron to ferrous iron was the predominant mechanism for floc disintegration[85]. However, recent research has shown that not only is biological iron reduction

possible but a significant number of the organisms persist in the wastewater environment and they may impact particular unit operations.

Caccavo Jr. et al.[86] reported that when autoclaved sludge was incubated with the iron reducing bacteria *Shewanell alga* BrY there was significant deflocculation, which was attributed to biological iron reduction. Rasmussen and Nielsen [87] estimated that 70-90% of the iron present in activated sludge is in the ferric form. Ferric iron is the terminal electron acceptor in biological iron reduction. When Fluorescence In Situ Hybridization –Microautoradiography (FISH-MAR) was applied to activated sludge under iron reducing conditions it was found that approximately 3% of the total microbial population in activated sludge was capable of dissimilatory iron reduction [88]. Nielsen *et al*.[89] reported the isolation of *Geobacter sulfurreducens* strain SL-1 from activated sludge from the Aalborg West wastewater treatment facility.

The biological reduction of iron may have greater significance to the observed performance of anaerobic digestion systems beyond floc disintegration. Recently Li *et al*.[90] reported that the addition of ferric hydroxide to anaerobic sludge alleviated the toxicity associated with high concentrations of long chain fatty acids within the digester due in part to the activity of iron reducing bacteria. While this study could not sufficiently define the phyisochemcal from the biological aspects it provides insight to an under studied community in wastewater treatment.

Just as biological iron reduction is possible Nielsen *et al*.[91] demonstrated biological iron oxidation in activated sludge. While this metabolism remains relatively undefined it does illustrate the complex nature of the wastewater ecosystem. Thus not only should physical and

chemical processes be investigated in the iron colloid interactions but the biological impacts must be considered as well.

Aluminum Chemistry in Wastewater Systems

Aluminum is another trivalent metal that has a significant history in the water treatment industry in terms of use as a coagulant of organic matter. The removal of organic matter by aluminum hydroxide shows similar patterns of behavior to ferric hydroxide, interms of charge nuetralization. Pommerenk and Schafran [92] investigated to the binding of inorganic and organic ligands to hydrous aluminum oxide. This work demonstrated that binding affinity was compound specific for both organic and inorganic ligands. Of particular interest in this study was that humic acids bound had a greater binding affinity to aluminum oxide than NOM. The authors attributed this variability to charge density.

Holbrook *et al*.[93] reported that aluminum significantly removes polysaccharides from solution in membrane bioreactors treating wastewater. The same study reported the change in protein as not significant for the aluminum doses tested.

While a majority of aluminum hydroxide adsorption studies have focused on NOM in natural waters. The field of vaccine delivery does provide some insight into the interaction of aluminum species with proteins. Aluminum adjuvants are highly purified aluminum species which are added to vaccines to increase the immune response by stabilizing the antigen in-vivo. Shi *et al*.[94] studied the impact of an aluminum hydroxide and aluminum phosphate adjuvants on the adsorption of model vaccines, lysozyme and ovalbumin, in interstial fluids. Shi *et al*.[94]

reported that not only did protein type impact the adsorption/desorption but the type of adjuvant as well. The strongest binding affinity was for ovalbumin with aluminum hydroxide, and the binding strength increased with aging of the protein-aluminum complex.

Biosolids Stability-Nuisance Odors

Anaerobic or aerobic digestion reduces the mass of solids and inactivates pathogens associated with primary and secondary sludge. Vector attraction requirements are commonly met by demonstrating a 38% reduction in volatile solids or by using one of the alternatives required by the EPA. However, these regulations do not directly address the issue of biosolids stability in the environment. It is possible that both pathogen and vector attraction requirements can be met and class B biosolids can be land applied yet still have significant odor issues.

One of the potential benefits of enhanced digestion is the possibility that the technology will not only increase solids destruction but also reduce the pool of material associated with the generation of nuisance odors from dewatered biosolids. However, in order to determine if enhanced digestion technologies are going to be effective at reducing odor generation, the source, sink and physical/chemical reactions that result in odor generation must be understood.

Classification of Odiferous Compounds from Biosolids

There are many volatile organic compounds emitted from biosolids following dewatering. These compound include sulfur-based, amine-based, aromatic hydrocarbons, and volatile fatty acids. Each of these compounds generates a unique olfactory response and the detection threshold for each varies from compound to compound.

Table 1-7, lists some of the compounds commonly found associated with biosolids and the wastewater environment. What is apparent from the data in Table 1-7 is that different compounds have very different detection thresholds. The variability in detection thresholds can result in a strong malodor response to even very low levels of odorant.

Table 1-7: Threshold Odor Concentration and Olfactory Response Common Odor Causing Compounds Associated with Biosolids

Compound Name	Formula	Detection Threshold ppmv		Olfactory Response	Ref.
		Low	High		
3-methyl-1H-indole	C_9H_9N	0.00000009	0.00119369	Fecal nauseating	[95]
indole	C_8H_7N	x	0.00039682	Fecal	[95]
Trimethylamine	$(CH_3)_3NH$	0.00033111	0.00033111	Fishy, pungent	[96]
Hydrogen Sulfide	H_2S		0.10050176	rotten eggs	[95]
		0.00050251	0.01005018	rotton eggs	[96]
Methanethiol	CH_3SH	0.00002034	0.04169882	sulfidy	[96]
Dimethyl Sulfide	CH_3SCH_3	0.00098433	0.02000164	Decayed cabbage	[96]
Dimethyl Disulfide	CH_3SSCH_3	0.00028568	0.15842180	sharp, pungent, putrid, decayed vegetables	[95]
Dimethyl Trisulfide	CH_3SSSCH_3	0.00139510	0.00445657	nauseating	[95]
Carbon Disulfide	CS_2	0.00781003	7.42434971	Disagreeable, pungent	[96]
Carbonyl Sulfide	COS				
Phenol	C_6H_5OH	0.05979120	0.09878546	Aromatic	[95]
4 methyl phenol (ρ-cresol)	$CH_3C_6H_4OH$	0.00047514	0.00203630	Fecal	[95]

* All values assumed to be at 1 atm, 25 ^oC

Sulfur-based odors and amine-based odors have been the focus of most biosolids odor research[97]. The precursors of these compound in biosolids has been identified as from either biogenic material, sulfur and heterocyclic amines, or from manufactured cationic polymers, aliphatic amines [98-100]. While the prescusors have been identified an understanding of biology behind the production and consumption of odorants is required.

Biological Generation of Odor Causing Compounds from Biosolids

The generation of organo-sulfurs, reduced sulfurs and heterocyclic amines has been linked the to amino acid metabolism. Specifically the functional groups associated with amino acids are removed and the product is either an odorant directly or undergoes chemical reactions to form one.

Significant dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) production from the biological degradation of methionine, methyl methionine, cysteine, and S-methyl-cysteine has been reported [101]. Higgins *et al*. [102] reported production of headspace dimethylsulfide (DMS) after the addition of 0.01-mmol methionine to dewatered biosolids. The same study showed that the DMS production was not equivalent when same amount of cysteine was added. The difference in production was attributed to the methane-thiol group present in methionine but absent in cysteine. Cysteine degradation generally produces reduced sulfur compounds, hydrogen sulfide, when degraded [103].

The apparent precursors to odor causing compounds are amino acids. Organisms associated with amino acid catabolism have been isolated from wastewater sludges. *Aminobacterium colombiense* isoliated from anaerobic sludge, and was found to be capable of fermenting, serine, threonine, glycine[104]. When this anaerobe was cultured with *Methanobacterium formicicum*, *A. colobiense* could metabolize, alanine, glutamate, leucine, isolueucine, valine, aspartate and methionine.

The catabolic enzymes linked to sulfur based amino acid metabolism include: cysteine lyase [105], S-alkyl cysteine lyase [105, 106], cysteine desulfhydrase [105] and L-methionine γ-lyase [106]. The general products and reactants of L-methionine-lyase and S-methyl cysteinase are given in Table 1-8. What is readily apparent from Table 1-8 is that the degradation of methionine and S-methyl-cysteine result in not only the production of methanethiol, but also critical metabolic intermediates, α-ketogluterate and pyruvate. The activities, while beneficial to the microbe, can serve to produce a significant problem for wastewater treatment plants.

Table 1-8: Enzymatic Reaction Catalyzed by L-methionine-lyase and S-methyl cysteinase

Enzyme	Reaction Catalyzed
L-Methionine-Lyase	Methionine → α-ketogluterate + ammonia + methanethiol
S-Methyl Cysteinase	S-Methyl Cysteine →pyruvate + ammonia +methanethiol

The production of methanethiol, DMS, DMTS and hydrogen sulfide is from the degradation of common amino acids but other sulfur species are common odorants associated with biosolids. Dimethyl sulfide and methanethiol can also be produced throught the methlyation of hydrogen sulfide. Lomans *et al*. [107] stated methlyation occurs when organisms are in contact with high concentrations of reduced sulfur to mimize toxicity effects. Stets *et al*. [108] also reported the methylation of methanthiol to dimethyl sulfide, but suggested that it could also be a cometabolic process due to a lack of specificity of a methyltransferase enzyme, mistaking methanethiol for HS-CoM. Given the high concentrations of methylated and reduced sulfur species associated

with centrifugally dewatered biosolids it is likely that both toxicity reduction and cometabolism are occurring resulting in a greater abundance of methlyated VOSCs.

Biological methylation is likely the most common mechanism for methlyation to be observed in biosolids. However, there are compound which can served to methylate reduced sulfur species chemically. The abiotic methylation of sulfur species can occur when methyl-p-toluene sulphonate, methyl iodide, methyl-methane sulphonate and methyl sulfate are present in solution[101].

Sulfur related odors are the primary odorant associated with biosolids other classes of compounds are known to generate problems for utilities. One group in particular is the aliphatic amines. Trimethylamine, which has a distinct fishy odor, has been linked to biosolids due to the cationic polymers used for coagulation. Chang *et al*. [100] studied the structure and release of aliphatic amines from biosolids with different polymers and concluded that the associated fishy odor is due to the quaternary amine functions used to give polymer it charge. Similar findings were reported by Novak et al. [99]. Subramanian [98] determined that the liming of sludges after conditioning with cationic polymers significantly increase TMA release due to the pH of the sludge exceeding the pKa of TMA.

The degradation of proteins and their associated amino acid residues along with cationic polymer can result in the release of malodorous compounds from sludge as previously described. Sulfur compounds and alphatic amines are common odorants of sludge other classes of odorous compounds can be released, especially from proteins, during respriration. *Clostridium spp.* are

common hydrolytic organisms associated with sludges. Spray[109] reported that *Clostridium nauseum* could produce both mercaptan and 3-methyl indole as a result of metabolic activity. However, two other species studied by Spray[109] did not produce indole, *Clostridium microsporum* and *Clostridium gummosum*. Stadtman *et al*. [110] reported that *Clostridium barkeri* also did not release indole during respiration. What the data shows is that simply presence of hydrolytic bacteria may not be sufficient to biologically produce odor compounds, the correct species must be present.

While it is primarily thought that the production of nuisance odors from biosolids is associated with amino acid metabolism recent work shows that other organisms can produce odorous compound from non-protein sources. Yokoyama et al.[111] reported isolating a *Lactobacillus sp.* which could generate skatole by the decarboxylation of indoleacetic acid. Yokayama *et al*.[111] reported literature findings that report that *Escherichia coli* was capable of generating indoleacetic acid from tryptophan degradation. Rumen *Lactobacillus sp*. were also shown to produce *p*-cresol, a common odorant in biosolids, through the decarboxylation of *p*-hydroxyphenylacetic acid[112]. While the presence or absence of *Lactobacillus sp*. in sewage sludge systems is not known, this type of metabolisms provides evidence that odors can be generated from the combined metabolic processes of multiple organisms and may not be due to a single species or group. Also this suggests that understanding and identifying the chemical composition of industrial discharges to utilities may be critical to understanding odor problems.

Biological Degradation of Odor Causing Compounds from Biosolids

The generation of nuisance odors from sludges is generally a biologically mediated process. The reduction or removal can be biological but also physiochemical, through the addition of chemical absorbents or masking agents. However, understanding the biological aspects of nuisance odor consumption is advantageous since it may be the most cost effective means of sequestration.

The metabolic decomposition of sulfur based odorants can be both aerobic and anaerobic. Aerobic processes take advantage of organisms such as *Thiobacillus novellas* SRM [113] which can be used in biological air scrubbing units to control off gas odors from dewatering facilities. However, most odor problems associated with biosolids come after dewatering during land application and storage. The metabolisms mediating the degradation of compound during application and storage are thought to be primarily anaerobic in nature.

The anaerobic degradation of methylated sulfurs and amines are thought to be primarily due to methanogenic activity. Higgins *et al*. [102] as well as Chen *et al*. [114] reported that when bromoethane sulfonic acid, a strong methanogenic inhibitor, was added to biosolids the degradation of methlylated sulfur compound did not occur. Zinder and Brock[115] attributed the degradation of methanethiol in fresh water sediments to methanogenic activity. The Zinder and Brock study determined the likely mechanism to be the conversion of methionine to homoserine and methanethiol followed by the conversion of methanethiol to methane, carbon dioxide and hydrogen sulfide. Recently, progress has been made into understanding the communities involved with the reduction of methlylated sulfur species. Lomans et al. [116] isolated *Methanomethylovorans hollandica* gen. nov., sp. nov. from the sediments of an activated sludge

storage basin. *M. hollandica* is capable of growth solely on either dimethyl sulfide as well as methanethiol, both common odorants from biosolids.

Heterocyclic amines have also been shown to be metabolized by methanogens. Gu and Berry [117] demonstrated that an enrichment of sewages sludge methanogens was capable of degrading indole as well as 3-methyl indole. Later work by Gu and Berry [118] showed that wetland soils also had organisms with the similar capacity, suggesting that land application could mitigate the related odors.

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Chapter 2 :The Application of Mechanical Shear in an Internal-Recycle for the Enhancement of Mesophilic Anaerobic Digestion

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Chapter 3 : A Comparative Study of Ultrasonic Pretreatment and an Internal Recycle for the Enhancement of Mesophilic Anaerobic Digestion

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Chapter 4 : The Laboratory Simulation of a High Solids Centrifuge for the Generation of Volatile Organic Sulfur Compounds from Biosolids

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The Laboratory Simulation of a High Solids Centrifuge for the Generation of Volatile Organic Sulfur Compounds from Biosolids

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ABSTRACT

Centrifugal dewatering of anaerobically digested biosolids has been shown to generate odor causing volatile organic sulfur compounds (VOSCs) from stored dewatered cake (Novak *et al.*, 2006). In this study it is shown that the production of these odorous compounds is directly related to the shear imparted to the sludge in the presence of polymer. The proposed mechanism for volatile organic sulfur generation is that shear causes floc disintegration and denatures floc-associated proteins. The denatured proteins interact with cationic polymer to form a readily biodegradable protein-polymer complex. Biodegradation of the protein-polymer complex during cake storage results in the release of VOSCs. Increasing cake solids content and increased cationic polymer doses were found to increase peak sulfur generation under a static headspace. However, when the polymer conditioning dose exceeded the optimum for conditioning, no additional VOSC was produced. Using this information, a laboratory method to mimic the generation of volatile organic compounds from centrifuges was developed for the study of VOSC emissions from dewatered sludges.

KEYWORDS

high solids centrifuge, volatile sulfur compounds, biosolids, shearing, odor

INTRODUCTION

Volatile sulfur species are a major contributor to the odors from dewatered biosolids (Novak *et al.*, 2006) and have been shown to be emitted to a much greater extent when sludges are dewatered by centrifugation rather than belt press filtration (Figure 4-1). A laboratory method to mimic the action of the dewatering process can be used to determine how various types of

dewatering equipment, digestion alternatives, conditioning practices and predigestion processes are likely to impact odor generation. It will also allow for a more refined definition of biosolids "stability" in terms of odor generation or vector attraction. Currently the USEPA regulations state that in order for mesophilic anaerobic digestion to meet vector attraction requirements, which are primarily determined by odor, one of the following must be met: a 38% reduction in volatile solids within the digester, less than 15% additional volatile solids destruction in a 30 day post digestion test, or an oxygen uptake rate of less than 1.5 mg-O2/hr-g-TS (USEPA, 1999). These regulations do not consider the type of dewatering device used and the factors during dewatering that can lead to significant odor generation from "stabilized" biosolids. An understanding of the physical, chemical and biological processes that occur during dewatering and lead to odor generation can determine if current regulations for vector attraction are appropriate or should be revised.

The generation of sulfur compounds

The production of VSCs has been linked to the degradation of proteins. It has been shown that dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) are generated from the degradation of the amino acids, methionine, methyl methionine, cysteine, and S-methyl-cysteine, and it thought that it is primarily due to the presence of a methane thiol group within each of the structures (Franzmann *et al.*, 2001). Higgins *et al*. (Higgins *et al.*, 2006b) reported a significant increase in dimethyl sulfide (DMS) production with the addition of 0.01 mmol of methionine to dewatered biosolids while cysteine, added at the same concentration, produced only a slight increase in DMS. Several enzymes have been linked to sulfur-containing amino acid metabolism; cysteine lyase(Derbali *et al.*, 1998), S-alkyl cysteine lyase(Derbali *et al.*, 1998;

Lomans *et al.*, 2002a), cysteine desulfhydrase (Derbali *et al.*, 1998) and L-methionine γ-lyase(Lomans *et al.*, 2002a).

While it is thought that the generation of nuisance odors is primarily due to the biological degradation of amino acids, other reactions can occur that result in the generation of nuisance odors (Higgins *et al.*, 2006b). Biological methylation of sulfide occurs when microorganisms are exposed to high levels of sulfide, resulting in an increase in methanethiol (MT) and DMS (Lomans *et al.*, 2002b). Abiotic methylation can occur when compounds such as methyl-p-toluene sulphonate, methyl iodide, methyl-methane sulphonate and methyl sulfate are present (Franzmann *et al.*, 2001). It is believed that of the two classes of methylation reactions, biological methylation is of greater importance in biosolids handling due the high sulfide levels in anaerobic sludge and the low probability of methyl donating chemicals being present in wastewater.

An understanding of the potential mechanisms for odor production must also be complimented with an understanding of the potential degradation pathways for odor causing volatile organic compounds (VOCs). Aerobic as well as anaerobic degradation of methylated sulfur compounds has been reported (Lomans *et al.*, 1999). It is thought that anaerobic degradation to hydrogen sulfide is the primary mechanism for the degradation of organic sulfur compounds, especially when dewatered biosolids are stored for extended periods thereby limiting oxygen transport to the inner portions of the pile. Anaerobic degradation of methylated sulfur compounds to sulfide is accomplished through the activity of methylotrophic methanogens. Higgins *et al*. (Higgins *et al.*, 2006b) demonstrated that when bromo ethane sulphonic acid (BESA), a strong inhibitor of

methanogenic activity, is added to dewatered biosolids, methanethiol persists in the static headspace. This is in contrast to the dewatered solids without BESA where headspace MT first increases then declines over time. Similar results have been reported for the degradation of DMS by Lomans *et al*. (Lomans *et al.*, 1999) in freshwater sediments.

Objectives of this study

Given the complexity of source and sink reactions for sulfur based odor compounds, it is likely that a variety of competing factors impact the generation rate of odors from dewatered biosolids. It has been found that high shear dewatering devices used for dewatering, such as high solids centrifuges, generate greater amounts of odor than low solids centrifuges and belt presses (Novak *et al.*, 2006) (Adams *et al.*, 2003). Furthermore, Muller *et al*. (Muller *et al.*, 2006) have shown that the mechanical shearing of anaerobically digested biosolids generates biodegradable protein. Based on these observations it is thought that shearing that occurs during centrifugation generates bioavailable proteins and this leads to formation of nuisance odors. The objective of this study was to determine the factors that lead to the generation of volatile organic sulfur compounds from dewatered sludge cakes and use this to develop a laboratory method to simulate the processes that occur during centrifugal dewatering for the study of odor causing compounds from dewatered biosolids.

MATERIALS AND METHODS

Shear Device: Two different shear devices were used during this study. A KADY Model-L Laboratory Mill, (KADY International, Scarborough ME) a rotor-stator device which produces an estimated velocity gradient (G) of ~11,000 s^{-1} and a 1/5 H.P. Waring Blender with a G of ~5,500 s^{-1}. The mean velocity gradient for the KADY mill was calculated based on its rotational

speed and theoretical maximum torque applied at the tip of the rotor. The velocity gradient for the Waring blender was calculated using an inline-torque meter and a stroboscope to obtain a relationship between the torque and the paddle rotational speed (Novak and Bandak, 1989). Based on a plot of torque verses rpm, the G value for the Waring Blender was estimated from the rpm of the blender.

Sludge Characterization: Total and volatile solids of liquid sludge samples as well as dewatered cakes were analyzed according to Standard Methods (APHA, 1995).

Optimum Polymer Dose: The optimum polymer dose was determined by adding different doses of polymer to 100 ml aliquots of the sample sludge and measuring the capillary suction time (CST). The polymer and sludge were sheared for a selected time and intensity. The polymer dose that produced the minimum CST was considered to be the optimum dose (Baskerville and Gale, 1968). The CST was measured using either a Triton Type 304-M or Triton Type 165 CST with Whatman 17-CHR as the chromatography paper.

Sludge Dewatering: The primary method of solid liquid separation in the laboratory was centrifugation using either a Beckman J2-HS Centrifuge or a Beckman-Coulter Avanti-JE Centrifuge, operated at 17,400 x g for 15 minutes at room temperature. The cake solids content of the sludge pellet was increased when necessary by using a hydraulic piston press (Subramanian, 2004). The piston press is capable of applying pressures up to 100 psi using compressed air. Variations in cake dryness were accomplished by changing either the mass of solids to be pressed or the applied pressure. When less sludge mass was used, the cake was

thinner and had a higher solids content. Whatman No. 41 or 42 filter paper was placed on porous metal frit to act as the filter media and to prevent fouling of the metal frit.

Sample Preparation and Characterization for VSCs: Biosolids were analyzed for the presence of VSCs in the headspace of an incubation vial using a modified version of the method described by Novak *et al*. (Novak *et al.*, 2002). The method described by Novak *et* al. (Novak *et al.*, 2002) was modified due to limitations in the amount of sludge available. The polyethylene terephthalate bottle was replaced to with a 250 ml borosilicate glass serum bottle with a polytetrafluoroethylene lined septa. The VSCs measured in this study include MT, DMS, DMDS and DMTS and hydrogen sulfide (H_2S). The methylated compounds MT, DMS, DMDS and DMTS are referred to as volatile organo-sulfurs or VOSC's. H_2S is the product of both cystiene metabolism and the metabolism of VOSC's by methylotrophic methanogens and is usually considered separately from the volatile organic sulfur compounds. The sulfur species concentrations reported in this paper are in terms of ppmv (1 atm and 25 ^{o}C) and are normalized to volatile solids when appropriate. The area response for each sample was compared to a standard of known concentration to calibrate the CG/MS response.

The VSC profile of the biosolids samples typically produce a pattern in which two distinct biological activities could be observed. During the early phase of incubation, the VSCs in the headspace increase. In the second phase, methylotrophic methanogens metabolize the methylated sulfur species resulting in a decrease in VOSCs and generation of methane and hydrogen sulfide (Higgins, et al, 2006). Hydrogen sulfide was typically low because sulfide precipitated with iron to form ferrous sulfide solids. If the sulfide binding capacity of iron is

exceeded, H_2S will persist in the headspace. A typical VOSC profile for biosolids under a static headspace is shown in Figure 4-1.

Error Bars: All error bars within figures represent +/- one standard deviation unless otherwise stated.

RESULTS AND DISCUSSION

Centrifugal dewatering results in greater VOSC generation from dewatered biosolids when compared to belt filter presses (Figure 4-1). The shear forces associated with the operation of centrifuges has been found to result in the production of VOSC from dewatered sludge cakes (Figure 4-2). The data in Figure 4-2 are for dewatered sludge cakes incubated in closed 40 ml containers incubated at room temperature. The data in Figure 4-2 show that the two continuous centrifuges, low and high solids, generated significantly more VOSC in the headspace than did the laboratory centrifuge (Beckman J2-HS). The high solids centrifuge generated more VOSC than the low solids centrifuge. The characteristics of the centrifuges are presented in Table 4-1.

The data in Table 4-1 show that a standard laboratory centrifuge does not adequately mimic its full-scale counterpart. The cake solids in the lab centrifuge are lower and the peak VOSC emissions are less than 10% of that observed in the low and high solids centrifuges. One reason for the observed differences in the VOSC emissions is that shear occurs in the entrance to continuous centrifuges, but not in a laboratory centrifuge (Boychyn *et al.*, 2001). Another possible reason for the observed difference is that the cake solids concentration is lower for the lab centrifuge. The final factor could be the polymer dose that effects the observed VOSC emissions from dewatered biosolids. Table 4-1 shows that polymer dose varies between high

solids, low solids and laboratory centrifuges. What is not known is the role the cake solids or polymer dose plays in VOSC generation. By understanding the forces within a centrifuge and how changes in cake dryness and polymer dose impact the production of VOSCs, steps to mitigate odor generation might be possible.

A premise of this research is that sludge entering a centrifuge is exposed to physical forces and changes in environmental conditions during dewatering. These forces include, but are not limited to, shear, increased thermal energy, changes in pH, changes in moisture and changes in oxidation /reduction potential.

Sludge, Shear and Polymer Interaction for VOSC Generation

Cationic polymers are commonly employed at wastewater plants to enhance the dewatering rate of sludge during centrifugation. Polymer coagulates the organic colloids and allows water to be released at a greater rate. Polymer is typically introduced just before sludge, containing a high proportion of protein (Morgan *et al.*, 1990), enters the centrifuge. The primary forces within this environment are shear and heat. Given the short exposure time of the polymer and solids at the entrance of the bowl area and the high specific heat of water, heat transfer was thought to be minimal. However, the shearing forces on the sludge are thought to be significant and the mechanical action could alter the sludge even within the short retention time of the bowl entrance. Higgins *et al*. (REF) reported shear intensities, the combination of the mean velocity gradient, G and the shear time, t, within centrifuges of G(t) =30,000-120,000. Therefore, in this study the significance of the interaction of shear, sludge and polymer was investigated.

Biosolids were collected from both a high and low solids centrifuge at the Philadelphia Water Department Biosolids Recycling Center (PWDBRC) treating the same sludge stream. Dewatered cakes from both centrifuges along with polymer and undewatered sludge was collected and shipped overnight to Virginia Tech. Approximately 8 grams of biosolids from each centrifuge was placed in a 40 ml EPA vial for VSC analysis. The undewatered sludge and polymer were exposed to a course of shear regimes in the KADY mill as outlined in Table 4-2. Polymer was added in two ways, directly into the sludge prior to shearing and after shearing was completed. For addition after shearing, sludge was removed from the mixer and polymer was added under gently mixing conditions using a stirring rod. The shear and polymer dose were varied in order to determine which condition best describes the action of a full-scale centrifuge. Polymer was added to the sludge at 0, 0.5, 1 and 1.5 times the average optimum dose reported by the plant for the high and low solids centrifuges. The sludge samples were exposed to the following shear regimes: no shear, shear in the presence of polymer and shearing followed by polymer addition after the shearing ended. Following shearing and conditioning of the sludge, samples were centrifuged in the laboratory to produce dewatered cake.

The dewatered cake (~8 grams) was added to EPA vials and incubated at room temperature, and the headspace was periodically sampled to determine VOSC concentrations of the experimental samples and the field centrifuge samples. Once the VOSC concentration peaked, the analysis was ended. Figure 4-3, shows the peak VOSC concentration for each of the experimental conditions. VOSC values observed for dewatered cakes from the field centrifuges are included in the note on the figure.

The high and low solids centrifuges had peak VOSC concentrations of 307 +/-24 and 112+/-6 ppmv/g-VS respectively. The laboratory samples did not attain peak VOSC concentration equal to either of the full-scale centrifuges. The only experimental set to produce VOSCs at levels approaching the full-scale system was the one in which the polymer was added while shearing occurred. This sample produced a VOSC peak concentration of 97 +/-4 ppmv/g-VS at the highest polymer dose. The headspace VOSC profiles for the laboratory generated samples are compared to the high and low solids centrifuge profiles in Figure 4-4 and the impact of shearing in the presence of polymer is evident. The only lab sample which generated VOSC concentrations close to the full-scale centrifuges was when shear occurred in the presence of polymer.

The shearing of sludge and polymer together generates significantly greater amounts of VOSC than the no-shear or post polymer addition conditions. However, the data suggest that factors other than shear also play a significant role in VOSC generation. Based on the observed trends in Figures 4-3, 4-4 and Table 4-1, three factors were identified as potential contributors to headspace VOSC generation. These are G(t), cake dryness and polymer dose. Each of these was assessed separately and those data are provided in the following sections of the paper.

Impact of Cake Dryness on VOSC Emissions

To investigate the impact of cake dryness on VOSC emissions from biosolids, sludge was collected from two different wastewater plants. The optimum polymer dose was selected with mixing conducted using a Waring blender and a shear time of 5 seconds (G(t) =27,500) and 30 seconds [G(t) =165,000]. The sheared and optimally conditioned sludge was centrifuged in the lab the sludge pellet was removed and then varying quantities of sludge were added to a piston

press for further dewatering. To increase cake solids the using the piston press, a range of applied pressures (30-90 psi) and compression times (10 to 100 minutes) were used. This produced dewatered cakes ranging in solids concentration from 16.8 to 29.6%, which is the reported range for centrifuges(USEPA, 2000).

The data in Figure 4-5 show the peak VOSC emissions as a function of cake dryness for two sludges, one with a very high odor potential and one with a very low odor potential. For both sludges there was a strong linear trend between increasing cake dryness and increasing peak VOSC, even though the odor potentials were very different. The data also show that when the cake solids are less than 15 to 17 percent solids, little total VOSC is generated, even for an odorous sludge with a high applied shear.

Effect of Polymer Dose on VOSC Production

Data in Figure 4-3, showed that sludge and polymer must be contacted in the presence of shear to produce VOSC's at levels comparable to those from centrifuges. The data in Figure 4-3 also indicate that more polymer results in higher VOSCs from dewatered cakes. To further clarify the relationship between polymer dose and VOSC emissions, the optimum dose of the sludge was determined using 5 and 30 seconds of shear time, dewatered using the laboratory centrifuge, followed by additional dewatering using the laboratory piston press.

The VOSC emissions were monitored over a period of time to determine the peak value. Figures 4-6a and 4-6b show the effect of polymer dose on the VOSC emissions from dewatered cakes with different polymer doses at shearing intensities of G(t) = 165,000 and G(t) = 27,500. The data in Figure 4-6a shows that polymer dose has 2 modes of action centered on the optimum

dose of 89 lbs-polymer/ton-TS. When conditioner was added at a dose less than 89 lbs-polymer/ton-TS there is a reduction in VOSC emissions. When polymer was added at doses greater than 89 lbs-polymer/ton-TS, the peak VOSC remained relatively constant. When the experiment was repeated at a lower shear intensity (G(t) = 27,500) the same result was observed (Figure 4-6b).

The interaction between polymer and sludge is thought to occur as follows. Shear releases colloidal organics from the floc matrix. Much of this organic material is exocellular polymeric material and a major fraction is protein (Morgan *et al.*, 1990; Muller, 2001). The colloidal material, especially protein, is coagulated by cationic polymer and reincorporated into the sludge matrix. The reincorporated protein is then degraded, even though it has been reincorporated into the sludge mass. It is thought that the protein-polymer complex retains the protein in a denatured state and microbes in the sludge can easily degrade both the protein and the cationic polymer. If underdosing occurs then there is insufficient polymer to coagulate all the released organics. As a result the ucoagulated protein material is lost in the centrate and does not serve as a source for VOSC production. When overdosing occurs, all of the released organics are recogaulated to the floc while the excess polymer goes unutilized. Since cationic polymer does not contain sulfur groups, the excess polymer will not contribute to VOSC emissions of the biosolids

The Effect of Shear Intensity on VOSC Emissions for Biosolids

Shearing of sludge and polymer together is critical to the production of VOSC's from biosolids. However, little is known about what impact various shear intensities have on VOSC production. To investigate the significance of shearing intensity on VOSC production a standardized shearing test procedure was used. A 1/5 H.P. Waring Blender with a velocity gradient (G)

estimated at 5500 s^{-1} was used to provide the necessary shear to a 100 ml standard sample size. The shearing intensity was altered by varying the shear time from 0 to 45 seconds, producing a G(t) range from 0 to 247,500. The optimum dose was determined at each shear intensity and the cakes were dewatered using the laboratory centrifuge and piston press. The VOSC emissions from the laboratory-generated biosolids were measured to determine the peak concentration.

Figure 4-7, shows the peak concentration of VOSC's from digested sludges exposed to different shearing intensities followed by dewatering. There is a strong increasing linear trend of VOSC peak generation with increasing G(t), R^2= 0.97. This test was repeated using 3 other sludges and the results are summarized in Table 4-4.

All of the sludges tested showed a similar peak VOSC response to increasing shear intensity except for Sludge B, which generated very little volatile organic sulfur at any shear intensity. The lack of correlation in this sample is due to very low VOSC levels and this is likely due specific sludge properties that result in low odors (Verma, 2006). The slope of each regression line is given in Table 4-3. The slope represents the change in peak VOSC with each change in shear intensity. What is apparent from the data is that each of the slopes are different, 0.006, 0.000006, 0.021 and 0.18 (ppmv)/G(t) for Sludge A, B, C, and D respectively. The data show that while increased shear intensity increases VOSC emissions the emissions from each are sludge specific.

The data in Figure 4-7 and Table 4-4 show that increasing shear intensity increases VOSC emissions from dewatered biosolids. The same effect was observed when the cake dryness was

increased at a single shear intensity (Figure 4-5). To determine the relative impact of shear intensity and cake dryness on VOSC emissions from dewatered biosolids the cake dryness experiment was repeated at 3 different shear intensities, G(t) = 0, 82,500 and 165,000.

Figure 4-8, shows the peak VOSC emissions for dewatered cakes at increasing G(t) and cake solids concentrations. The data show that with increasing cakes solids there is an increase in peak VOSC. However, the magnitude of peak VOSC generation is influenced more by the intensity of the shear than cake solids concentration. The maximum VOSC generation in the no shear condition, which represents the impact of cake solids concentration alone, occurred at the highest cake solids 25.2 ppmv/g-VS and 24.9 % TS respectively. Approximately the same VOSC emission (35.3 ppmv/g-VS) was observed at a cake solids concentration of 22.3 and a shearing intensity of G(t) = 82,500. When the shearing intensity was further increased (G(t)= 165,000) a peak VOSC of 31.1 ppmv/g-VS was observed at a cake solids concentration of 17.7 %. While both shearing intensity and cake solids impact VOS emissions, shearing intensity has a greater impact on VOSC emissions than cake solids concentration. One would then expect that high shear combined with a high cake solids concentration would produce the highest volatile sulfur concentration. This is exactly the condition created in a high solids centrifuge.

The data from this study show that each element, shear, polymer and cake dryness play a specific role in the observed generation of VOSC's from biosolids. The application of shear in the presence of sufficient polymer appears to be the most critical aspect in VOSC generation from biosolids. Boychyn *et al.*(2001) showed the greatest energy dissipation into liquids within a centrifuge occurs when the liquid enters and contacts the bowl surface. Furthermore, Boychyn *et*

al. (2001) found that the energy dissipation into the fluid doubled when the centrifuge was not operated under flooded conditions. Maa and Hsu (1996) showed that shearing can cause conformational changes in some proteins. The impact of the shear and changes in protein structure, including peptide breakage, is exacerbated when shear is applied at an air-liquid interface (Maa and Hsu, 1997). Furthermore, high intensity shear forces have been shown to release significant amounts of biodegradable protein from anaerobically stabilized sludge (Basu *et al.*, 2004; Muller *et al.*, 2006).

It appears that sludge and polymer entering a centrifuge is sheared, resulting in the release and denaturation of proteins bound within the floc matrix. While in a denatured state the cationic polymer interacts with exposed anionic sites on the protein, retaining the protein in a denatured state even when reincorporated back into the floc. Maa and Hsu(1997) attributed a similar model of protein-protein interaction in which hydrophobic regions of denatured proteins interacted resulting in aggregation of recombinant human growth hormone under shear conditions.

Once shear causes the denaturation of protein and binds to cationic polymer, it is open to enzymatic attack and biodegradation. This model of protein and polymer interaction also explains why post shear polymer addition and polymer doses below optimum result in a reduction in VOSC generation The absence of polymer during shearing likely allowed some proteins to regain their native conformation or at least fold in a manner that they are not as open to enzymatic attack. When there is insufficient polymer, uncoagulated proteins are lost in the centrate.

Higgins *et al*.(2006b) demonstrated that the inhibition of methanogenesis results in the persistence of VOSC's from biosolids under a static headspace. Stroot *et. al*. (2001)and McCarty and Smith (1986), have suggested methanogens exists in a syntrophic relationship, requiring close proximity to other organisms to persist. Therefore, it is expected that the dispersion of the floc by shearing not only generates biodegradable protein-polymer structures but also result in an inhibition of methanogenesis by separating the syntrophic methanogenic organisms.

In addition to shear in the presence of polymer, the dryness of the cake also has a strong influence over the observed VOSC emissions. Figure 4-5, showed that for the same shearing intensity and polymer dose, the observed peak VOSC concentration was directly dependent upon the cake solids concentration. Increasing the cake dryness from 16-30%, a range of cake solids achievable by centrifuges, resulted in increased peak VOSC concentrations. The reduction of water within the cake mass has two effects that may promote VOSC generation/emission. First, increased cake dryness could result an in increased flux of oxygen into the cake during scrolling and at the cake surface during storage, resulting in a reduction in methanogenic activity. Second, the reduction in water volume associated with the biosolids means that there is less solvent in which to dissolve sulfur species. As a result, saturation would be reached earlier and the excess mass of material would volatilize into the headspace.

A Procedure to Mimic the VOSC Generation by a Centrifuge

What this study has shown is that the cake solids concentration, shear intensity and polymer dose all play a critical role in the level of observed VOSC generation. Using this information, a method to mimic the forces within a centrifuge was developed and is shown in Figure 4-9.

Shearing of the polymer and sludge is the initial step to mimic the high shear forces that have been reported at the entrance of bowl centrifuges (Boychyn *et al.*, 2001). Given the limited shear intensity of small volume laboratory shear devices, extended shear times are used to get a G(t) similar to a centrifuge. Higgins *et al*.(2006a) reported that G(t) ranged from 30,000 to 120,000 for a variety of centrifuges based the G(t) from a calibrated shearing device which produced an equivalent polymer dose. Novak and Bandak (1989) reported that unconditioned anaerobic sludge was not as sensitive to shearing time as it was to G with regard to dewatering. As was shown in Figure 4-8, varying the time of shearing can result in changes in VOSC generation. However, increased mixing or shear times to compensate for the lower G value in laboratory shear devices compared to field centrifuges will have some limitations.

Once the shear time is selected, the optimum polymer dose must be determined using the lab shear device and selected mixing time. The sheared sludge-polymer mixture is then dewatered using a laboratory centrifuge. However, further dewatering is necessary to achieve the higher cake solids of industrial centrifuges. Higher cake solids are achieved in our lab by adding sufficient mechanical pressure for a set period of time using a piston press. Depending on the cake solids desired, different loadings, pressures and compression times can be used. The maximum cake solids concentration achieved by this method was ~32%.

After the completion of the dewatering step the cakes are homogenized by hand, cutting the cake into small pieces and mixing them. This ensures a similar surface area exposure observed with centrifuge cake pellets and allows for the mixing of several press runs into one

representative sample. Following homogenization, a known mass of cake is added to a 250 ml serum bottle. Typically a 10% mass to volume ratio is used which results a load of 25 g-TS.

The method outlined in Figure 4-9 was used to generate dewatered biosolids sludge collected from the PWDBRC. The dewatered cakes were monitored from VOSC production and the profiles were compared to their full-scale counter parts. The data in Figure 4-10 show the relationship between the peak VOSC emissions from the lab simulation cakes compared to those from full-scale centrifuge cakes. The excellent correlation between the VOSC emissions (R^2= 0.93) demonstrates that the method proposed in Figure 4-9 can adequately simulate a centrifuge for VOSC monitoring. Although the predictions based on the lab method were somewhat lower than for the field data, by altering the shearing time, the predictability can be improved.

The proposed method provides a means to investigate the causative agents of VOSC emissions from biosolids. This method would allow for evaluation of the effect of operational or technological changes that could result in lower odor generation from sludges. For example, the effect of anaerobic digestion pretreatment processes or chemical addition that would lead to the degradation of VOSC precursors or a reduction in their release could be studied in the laboratory using this method.

This method provides an important tool to study the release of VOSC's from biosolids. However, VOSC's while a major odorant are not the sole compound associated with dewatered biosolids. The method has not been validated for aromatic compounds, volatile fatty acids and heterocyclic amines, all of which emanate from biosolids. However, these compounds originate

from the biodegradation of amino acids and the source of amino acids in dewatered cakes is floc-associated protein so the method is expected to be applicable.

CONCLUSIONS

The overall objective of this study was to develop a method for simulating a full-scale centrifuge in the laboratory for the analysis of VOSC emissions from biosolids. The proposed method outlined in Figure 4-9, when compared to biosolids from full-scale centrifuges, showed an excellent capacity to produce VOSC emissions similar to full-scale centrifuges. Moreover, of equal importance, this study identified 3 major factors controlling VOSC generation from biosolids, shear intensity, moisture content and polymer dose.

Shear intensity, cake dryness and polymer dose all have a direct impact on the level of VOSC emissions from cakes. However, it is the unique interaction between polymer, shear, and sludge that produces the necessary substrate for VOSC generation. Understanding this relationship may result in the identification of sludge properties beyond protein content that are responsible for making a sludge high or low odor. As a result digester operation may be modified to minimize or predigest this fraction of material, resulting in lower odor sludge. Also by further understanding this relationship it maybe possible to redesign centrifuges such the necessary polymer-shear-sludge combination can be avoided or minimized, resulting a high solids-low odor centrifuge.

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Table 4-1: Sample Characteristics of High, Low and Laboratory Centrifuge Dewatered Sludge Cakes

Parameter	High Solids Centrifuge	Low Solids Centrifuge	Laboratory Centrifuge
Shearing	High	Low	None
Cake Solids (%)	30.8+/-.05	25.5 +/-0.09	16.2 +/-0.4
Peak VOSC ppmv/g-VS	307 +/- 24	130 +/- 6	9.7 +/- 0.4
Polymer Dose (g-polymer/kg-TS)	27	9	18
Relative Centrifugal Force (x G)	2200	N/A	17,400

Table 4-2: Shear, Polymer and Sludge combinations used to mimic High Solids Centrifuge forces.

Sample Label	Experimental Treatment
No Shear	Polymer + Sludge → Laboratory Centrifugation→VSC Analysis
Shear with Polymer	Polymer + Sludge + Shear (30 sec) → Laboratory Centrifugation → VSC Analysis
Shear with Post Polymer Addition	Shear (30 sec) + Sludge → Polymer Addition→ Laboratory Centrifugation→ VSC Analysis

Table 4-3: Summary of Impact of Shear Intensity on VOSC Production from Digested Sludge

	Shear Intensity G(t)	Sludge A	Sludge B	Sludge C	Sludge D
Peak VOSC (ppmv)	0	0.9	0.8	17.4	15.6
	27500	x	X	66.3	x
	55000	72.2	0.7	103.5	x
	82500	x	X	x	128.8
	165000	903.4	0.5	364.8	306.0
	247500	1391.5	2.7	x	x
	R-square	0.97	0.49	0.99	0.98
	Slope (ppmv)/G(t)	0.006	0.000006	0.0021	0.0018

Figure 4-1: VOSC Production from Biosolids Dewatered by Centrifugation and a Belt Filter Press

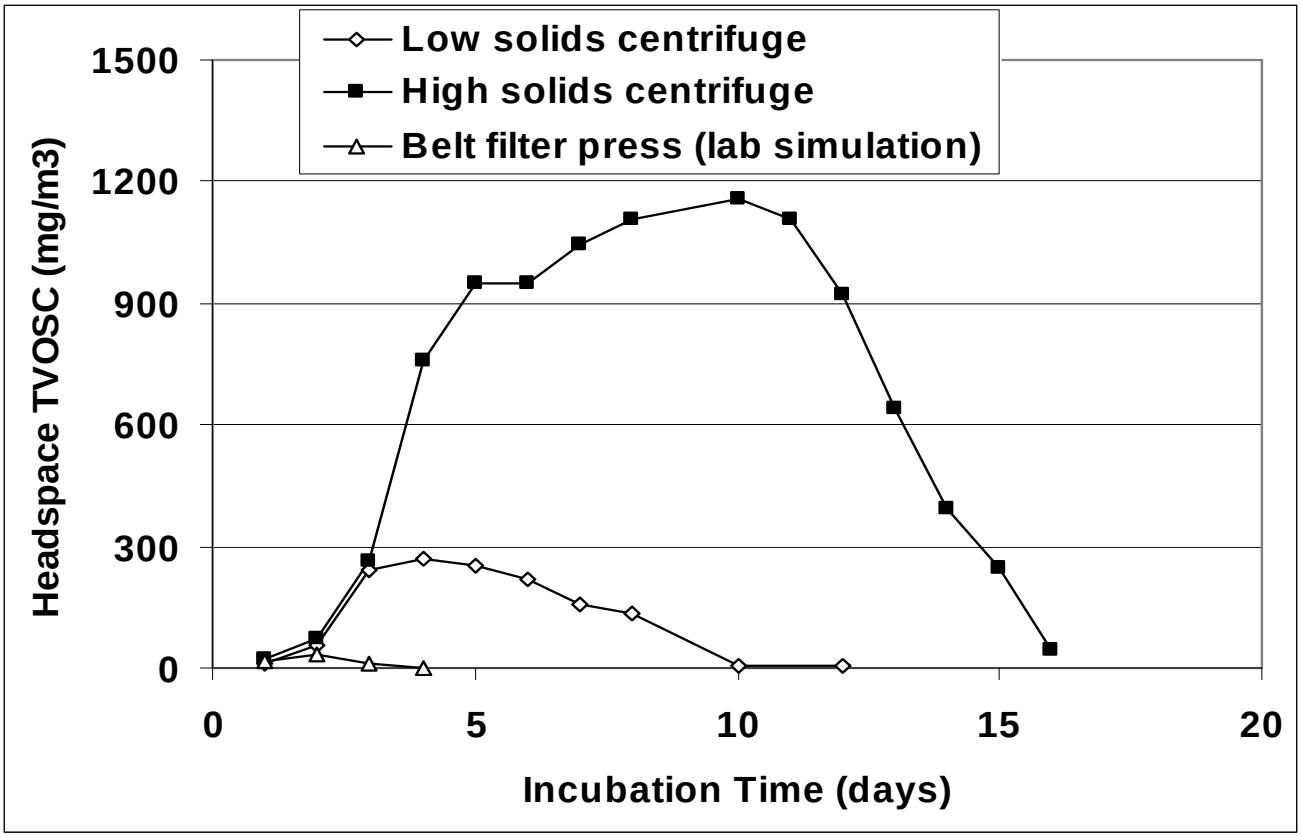


Figure 4-2: VOSC Profiles for High, Low and Laboratory Centrifuges Dewatering the Same Sludge

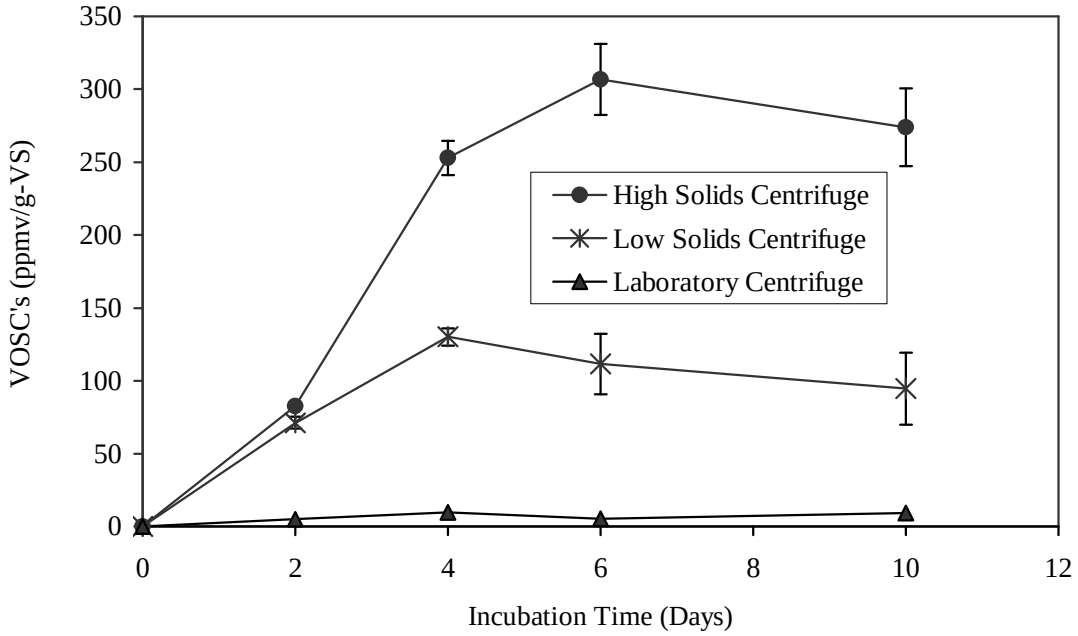


Figure 4-3: The Impact of Shear and Polymer Interactions on VOSC Emissions from Dewatered Biosolids

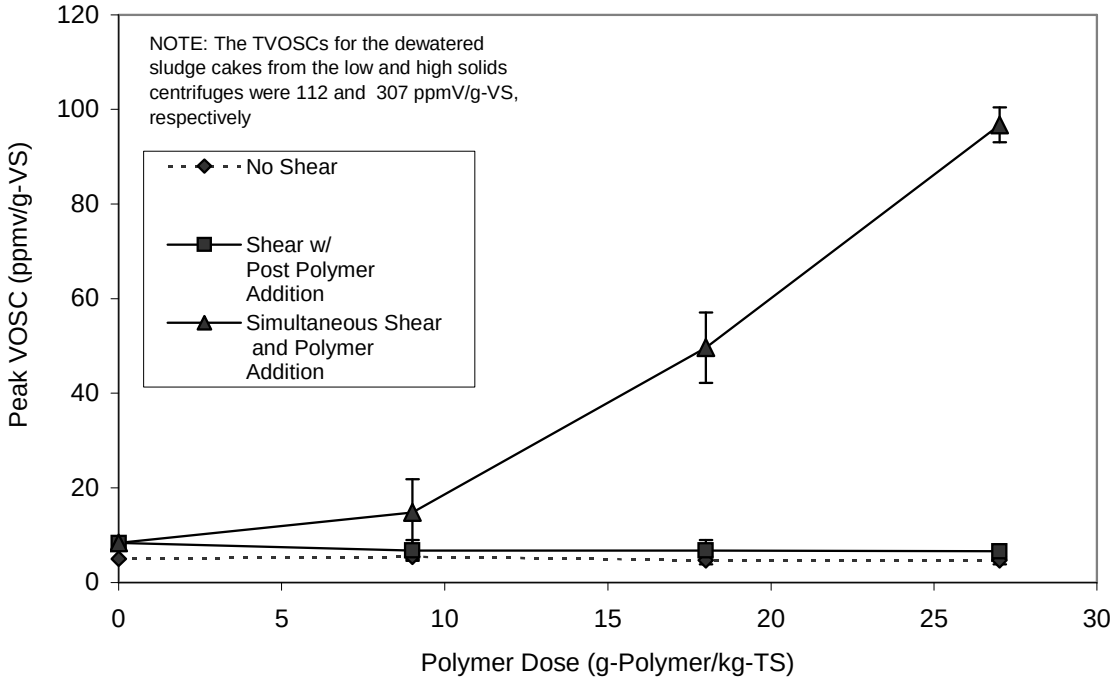


Figure 4-4: Headspace VOSC Profiles for High and Low Solids Centrifuges Relative to Different Laboratory Shear Regimes

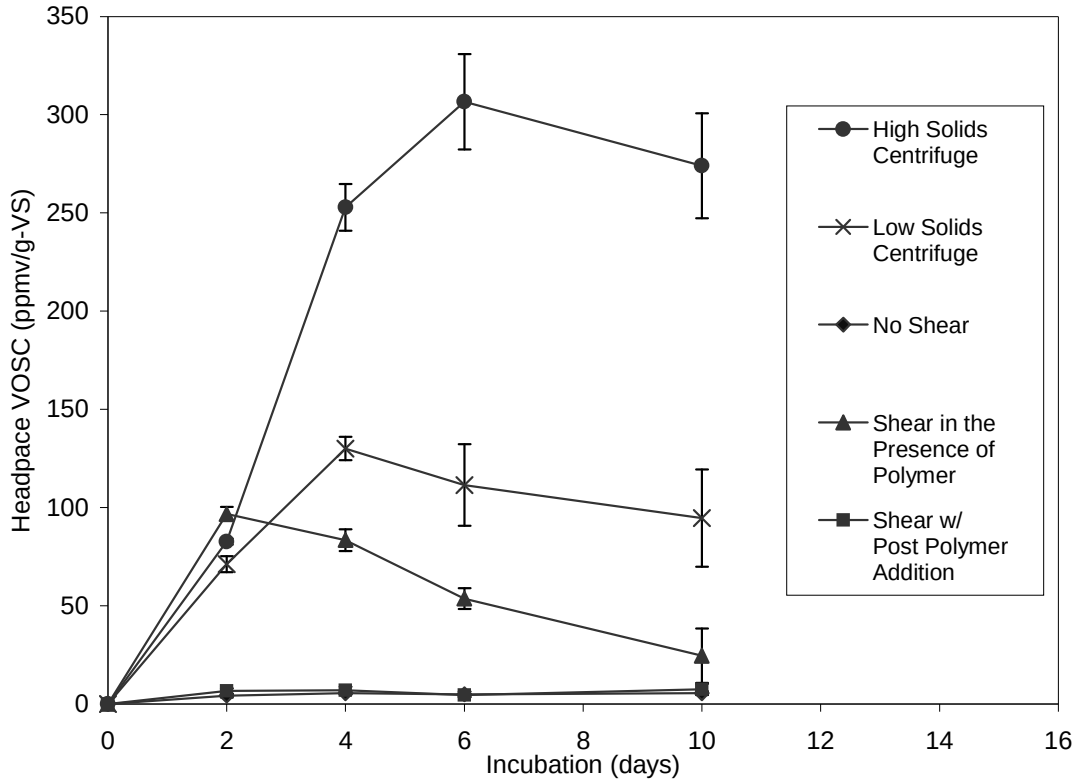


Figure 4-5: The Impact of Cake Dryness on Peak VOSC Emissions from Biosolids

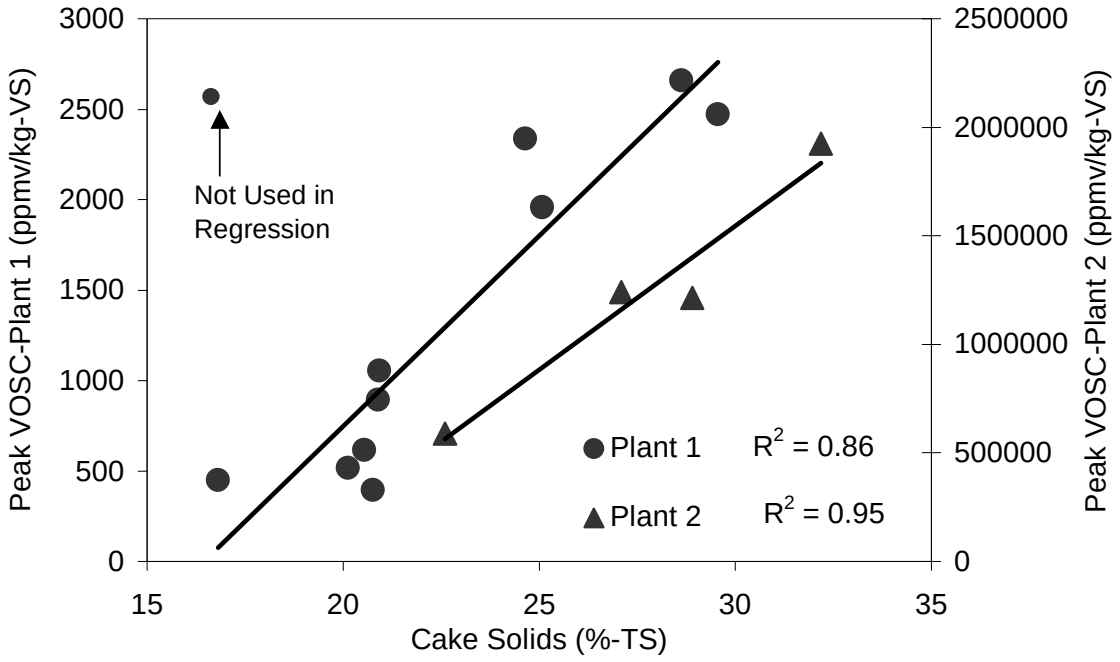
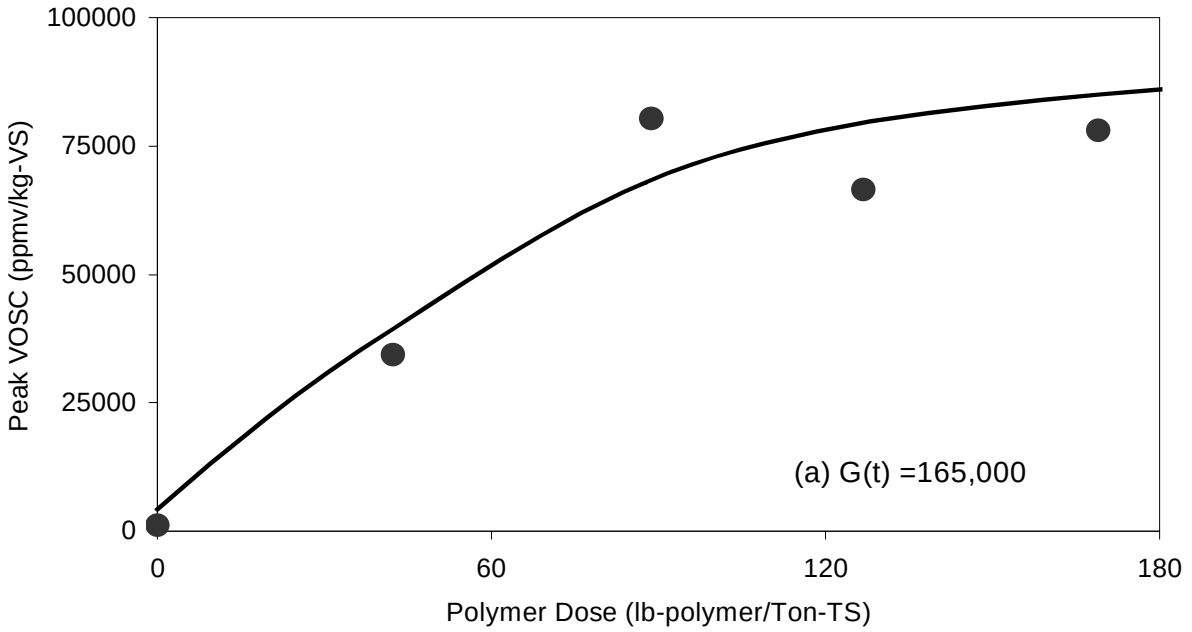


Figure 4-6: Impact of Polymer Dose on Observed Peak VOSC Emissions from Biosolids



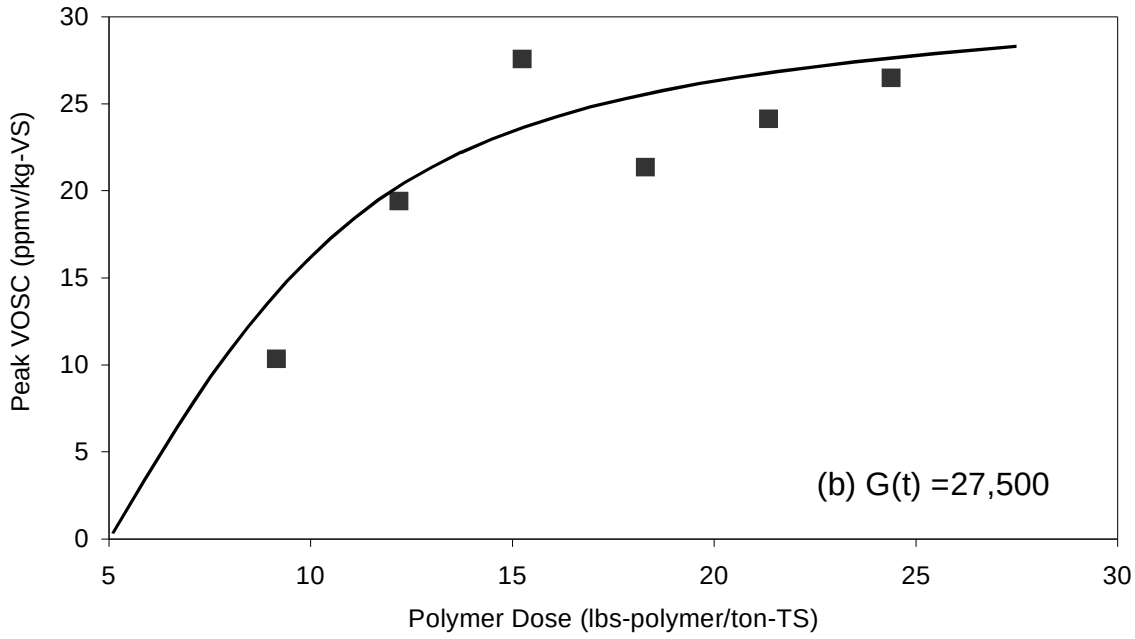


Figure 4-7: The Impact of Shearing Intensity on Peak VOSC Emissions from Biosolids

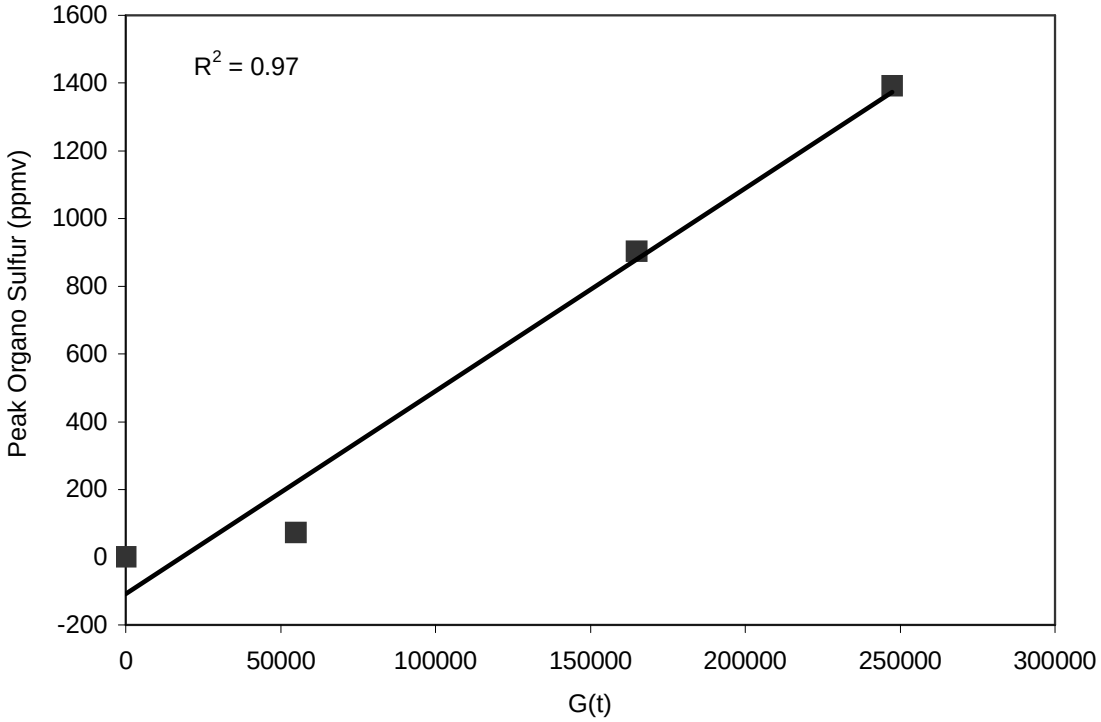


Figure 4-8: Contribution of Cake Dryness and Shear Intensity to Peak VOSC from Dewatered Biosolids

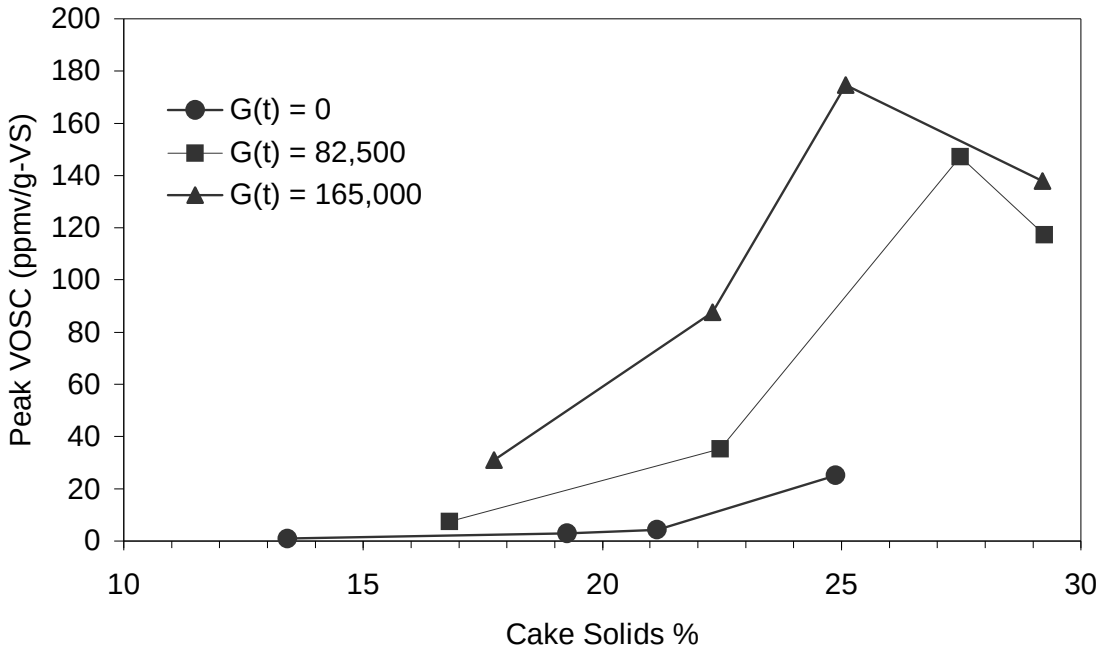


Figure 4-9: Outline of Proposed Method for the Simulation of a Centrifuge in the Laboratory

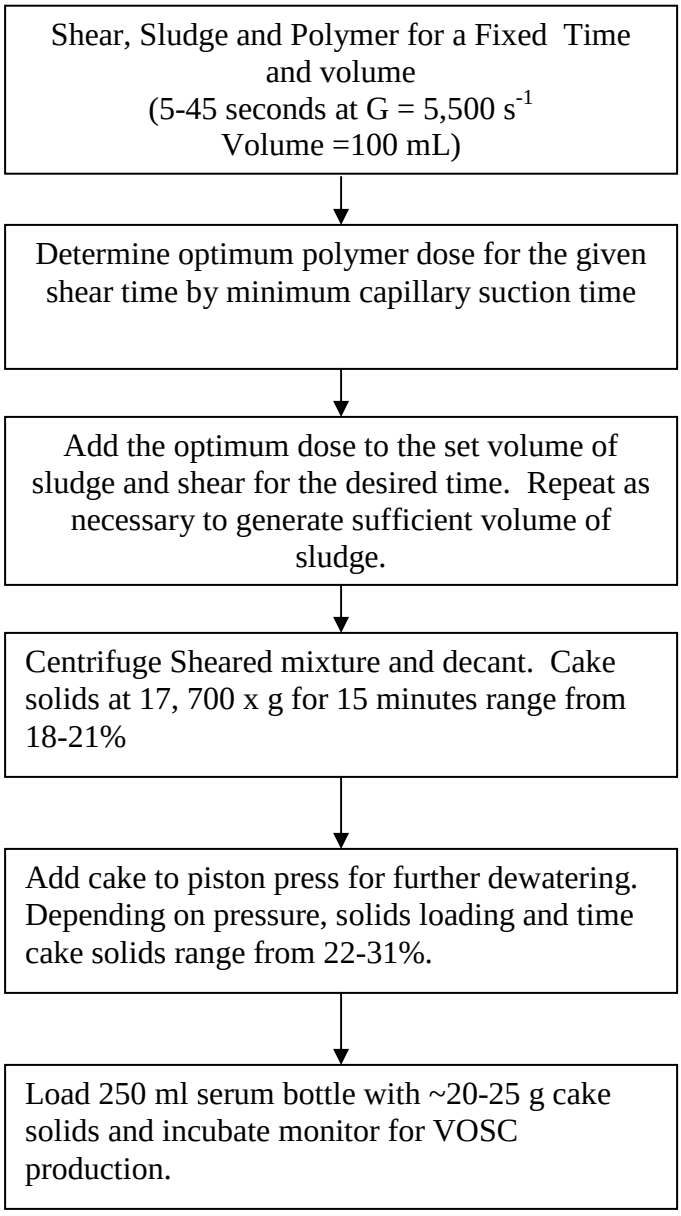
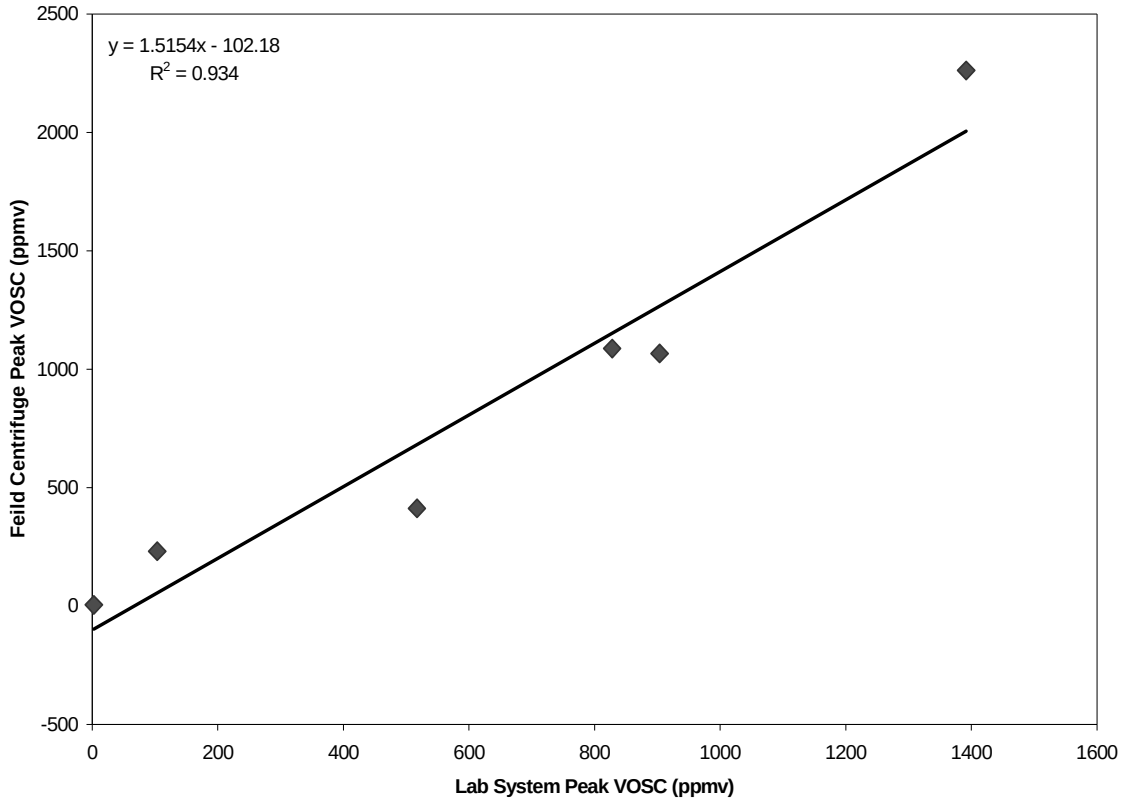


Figure 4-10: Comparison of the Laboratory Centrifuge Simulation with Full Scale Centrifuge Data for the Determination of Peak VOSC Concentrations



Chapter 5 : The Impact of Floc Metals on Enhanced Mesophilic Anaerobic Digestion Technologies

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The Impact of Floc Metals on Conventional and Enhanced Mesophilic Anaerobic Digestion Technologies

Christopher D. Muller and John T. Novak

ABSTRACT

In this investigation the impact of floc associated cations on enhanced anaerobic digestion, and the enhancement of anaerobic digestion using mechanical and ultrasonic disintegration in an internal recycle were studied. The data show that the degree of digestion enhancement in terms of volatile solids destruction varies, 2.6 to 68.9% and 12% to 80.4% for mechanical and ultrasonic disintegration, respectively. Mechanical shear disintegration operates primarily through particle size reduction and sulfide-enhanced disintegration. However, mechanical shear was strongly influenced by the iron and aluminum content of the floc. Ultrasonic disintegration was found to target material primarily associated with divalent cations. Trivalent metals iron and aluminum were found to have a strong negative impact on ultrasonic disintegration, likely due to wave attenuation and particle recoagulation. This study shows that the metal composition in floc and thus the biochemical composition of sludge has an impact on the degree of digestion enhancement. Therefore, the applicability of specific disintegration technologies can be estimated from measurements of floc metal content.

KEYWORDS: sludge disintegration, floc structure, cations, iron, aluminum, sonication, mechanical shear, anaerobic digestion

INTRODUCTION

The enhancement of mesophilic anaerobic digestion by sludge disintegration technologies is thought to be a cost effective means of increasing digester performance Increases in biogas production and volatile solids destruction associated with enhanced digestion can result in cost savings to the utility and the degree of savings is specific to the technology used for disintegration (Müller 2001).

The available technologies for enhanced anaerobic digestion are diverse. These technologies include ultrasonics (Tiehm *et al.* 2001), thermal processes (Li and Noike 1992), oxidative processes (Weemmaes *et al.* 2000), mechanical shear processes (Muller *et al.* 2006b), lysate centrifuges (Dohányos *et al.* 2004), and jet-ram technologies. What is apparent from the literature is that no single technology has become the standard means of process enhancement. One reason for this is that there is variability in the performance in these systems from facility to facility. One explanation for this is that the material being treated is not uniform in composition between wastewater treatment plants. Therefore certain sludges may be more or less amenable to disintegration by specific technologies.

The biological and biochemical composition of wastewater sludges is dictated by the conditions within the treatment plant, along with the composition of the raw wastewater being treated. The variability in treatment options and wastewater characteristics has lead to several different models being proposed for activated sludge floc structure. Within the floc structure a variety of organic compounds have been reported to be present in varying concentrations. The major organic fractions found in activated sludge flocs are proteins, polysaccharides, nucleic acids, (Vallom and McLoughlin 1984) and microoorganisms. All of these components can potentially serve as sources of biodegradable material following treatment by a disintegration technology.

Extracellular polymeric substances (EPS) are likely to be the primary source of biodegradable substrate that is generated during sludge disintegration. Andreottola *et al.*(2006) determined that for energy inputs less than 130 kJ/L, which is economically viable, the main impact of ultrasonic disintegration was floc dispersion and particle size reduction, not cellular lysis. Cellular lysis

was found to occur at energy inputs >130 kJ/L which was considered by Andreottola *et al.* (2006) to be economically unfeasible. EPS is primarily comprised of three classes of materials, proteins, polysaccharides and nucleic acids (Vallom and McLoughlin 1984). Proteins are a likely source of biodegradable substrate generated by sludge disintegration since they are the largest fraction of material associated with the EPS of both anaerobic sludge (Morgan *et al.* 1990) and activated sludge (Bura *et al.* 1998). Increases in digester ammonium-N, a waste product of protein metabolism, has been reported in enhanced anaerobic digestion systems using both ultrasonic disintegration (Muller *et al.* 2006a) and mechanical shear (Muller et al. 2006b).

Activated Sludge Floc Stucture

In order to understand how activated sludge flocs disintegrate, a fundamental understanding of the chemical and biological makeup of floc is needed. The agglomeration of microorganisms and EPS is thought to be mediated by floc-associated metal cations (Novak *et al.* 2001). Novak *et al.* (2003) theorized that there are at least two types of EPS within the floc matrix, one amenable to aerobic digestion linked to divalent cations and another, amenable to anaerobic digestion and associated with iron. However, the composition of the floc EPS is not just determined by the metals content, but also the biochemical composition of the organics in the influent. Cetin and Erdincler (2004) reported that at low carbon to nitrogen ratios, floc contained higher concentrations of proteins compared to sludges with low carbon to nitrogen ratios. Therefore the accumulation and retention of different organic fractions within the floc matrix is a function of the presence and concentration of specific cation species in solution as well as the influent wastewater chemical composition.

Monvalent and divalent cations are thought to act as cationic "bridges" between bacterial species, which are reported to be comprised of 15 kilodalton "lectin-like" proteins as the terminus of this "bridge" structure (Higgins and Novak 1997a). Increasing concentrations of monovalent cations in solution relative to divalent cations have been shown to weaken and disrupt the floc structure (Higgins and Novak 1997b; Murthy *et al.* 1998). This occurs by displacement of divalent cations within the "lectin-bridge" structure with weaker binding monovalent cations, leading to deflocculation.

The role of trivalent metals is thought to be different than that of monovalent and divalent metals. Iron and aluminum are the common trivalent metals associated with the sludge floc matrix. These metals are thought to act as scavengers of free organic materials from solution, which then become incorporated into the EPS matrix of the sludge floc (Novak et al. 2003). Iron and aluminum hydroxide are responsible for the collection of organic debris contained in primary effluent and lysis products produced by cell decay. Novak *et al.* (2001) and Murthy *et al.* (2000) reported that iron binds solution proteins preferentially over polysaccharides in the wastewater environment. Park *et al.* (2006) recently reported lectin activity in iron associated EPS. What these studies suggest is that trivalent metals may also support bridging between organisms or surface attachment of bacteria to solid iron surfaces. Recent work by Abu-Orf *et al.* (2004a) also demonstrated that aluminum has similar properties to iron in improving sludge settling and effluent quality by coagulation of solution organic material. Holbrook *et al.* (2004) reported that the addition of aluminum hydroxide significantly reduced solution polysaccharides in a membrane bioreactor system while no significant decrease in solution protein was detected.

Based on these observations the following structural model is proposed for sludges. Sludge flocs are composed of EPS, bacteria and particulate debris from varying sources. The adhesion of flocculant bacterial species to each other and to organic macromolecules is mediated by excreted "lectin-like" proteins on the cell surface to which monvalent and divalent cations serve as a "bridge" between the surface proteins. Colloidal material becomes associated with the floc matrix through entrapment within the gelatinous EPS matrix,by hydrophobic interactions, or by coagulation with iron and aluminum hydroxides. The source of organic colloids is either from the primary effluent or from cell lysis products. Figure 5-1a shows a theoretical sludge floc and the cations associated with the different organic fractions.

RESEARCH OBJECTIVES

The objective of this study was to determine how the performance of wet sludge disintegration technologies are impacted by the composition of the sludge being treated. It is hypothesized that the concentrations of floc-associated cations will impact the performance of individual sludge disintegration technologies. Furthermore it is thought that the metal content of flocs could be used as a means of screening sludges for their suitability for digestion enhancement.

RESEARCH APPROACH

This study deals with the effectiveness of two disintegration technologies, mechanical shear and ultrasonics, and their capacity to generate biodegradable organic matter. For this study, the unit operation selected was an internal recycle mode of operation rather than more commonly studied pretreatment of feed waste activated sludge. The internal recycle enhanced digestion is a process in which digested sludge is removed from the digester, treated with the appropriate disintegration technology and then returned to the digester for further stabilization. It is thought that not only

would the shearing of digested sludge enhance the stabilization process in terms of volatile solids destruction, but could also reduce nuisance odor generation

The internal recycle digestion system was used in this study as part of a larger investigation into its potential as a commercially viable technology (Muller et al. 2006a; Muller et al. 2006b). Given the variability of the current pretreatment systems, this study was conducted to help determine the scope of application for this technology.

The experimental approach taken in this study focuses not only on gross floc properties and the degree of digestion enhancement but also investigates the mechanisms controlling the process. This is accomplished by using whole sludge samples from a variety of utilities as well as conducting experiments with laboratory-generated flocs to understand the process more completely.

MATERIALS AND METHODS

Sludge Disintegration Technologies: Two sludge disintegration technologies were used in this study. Mechanical shear was applied using a KADY Model-L laboratory mill (Kady International, Scarborough, ME). This device is uses a rotor-stator technology to apply mechanical shear to sludge. The velocity gradient was estimated to be 11,000 s^{-1} based on calculations of power input from the information provided on the equipment, motor power and rotational speed accounting for gear reductions. Ultrasonic disintegration was accomplished using a 20 kHz ultrasonic probe and process controller with the capacity of 1000 W from the Dukane Corporation, St. Charles, Il. Temperature control was accomplished using water jacketed sample vessels.

Floc Associated Metals: The concentrations of sodium, potassium, magnesium, calcium, iron and aluminum associated with sludge were measured using either a Jobin Yvon Ultima Inductively Coupled Plasma Atom Emission Spectrophotometer (Horiba Jobin Yvon, Edison, NJ) or a Thermo Electron X-Series inductively coupled plasma mass spectrophotometer (Thermo Electron Corp., Waltham, MA). Prior to analysis, sludge samples were dried at 103 ^{o}C in acid washed beakers. A known mass was then digested using EPA method 3050B (EPA 1996) that was modified by using 35% hydrogen peroxide rather than the prescribed 30%. Quantification of sample metal concentrations was accomplished by comparing the response from unknown samples to that of standards of known concentration.

Solution Cations: Solution concentrations of sodium, potassium, magnesium and calcium were measured by ion chromatography, on a Dionex DX-120 ion chromatograph (Sunnyvale, CA) equipped with a Dionex AS-40 autosampler. Fifteen mM sulfuric acid was used as the eluent at a flow rate of 1 ml/min. The chromatography column was a Dionex CS-12 proceeded by a guard column packed with Dionex CS-14 resin. The concentrations of samples were quantified by comparison to the dose response of standards of known concentration. Data were collected and analyzed using Chromeleon 4.0 chromatography software.

Analytical Tests: Solids concentrations, total and volatile, and chemical oxygen demand (COD) were measured using the procedures described in Standard Methods. Solution protein content was measured using either the Frølund modification (1996) of the Lowry method (Lowry *et al.* 1951) or the Hartree modification (Hartree 1972) of the Lowry method. Bovine serum albumin

was used as the standard protein. Solution carbohydrate concentrations were measured using the method described by Dubois *et al.*,(1956) with the modification of using 5% wt/vol phenol reagent (Lab Chem Inc. Pittsburgh, PA) rather than the prescribed 5% wt-phenol/wt-water. Assuming standard temperature and pressure there should be little variation in the reagent. Dextrose was used as the standard carbohydrate.

Particle Size Analysis: Particle size analysis was conducted using a Horiba Laser Scattering Particle Distribution Analyzer Model LA-300 (Horiba Instruments Inc., Irvine CA). Samples were diluted to a concentration that produced a laser transmittance between 85-92% prior to analysis. Following each analysis the sample chamber was sonicated and washed 3-5 times with distilled water to ensure that residuals from the previous samples were removed. The median particle size was determined and the particle size distribution was constructed using the LA-300 for Windows Version 3.57 software package provided with the equipment.

Synthetic Floc Sulfide Disintegration Studies: This study was carried out to investigate the specific behaviors of iron and aluminum and their associated ligands in the presence of a chemical reductant without the complications related to whole sludge systems. The protein, Bovine Serum Albumin (BSA) (Powder description, Fisher Scientific, Pittsburgh PA), was used as the model ligand for the tests. Ferric chloride and aluminum chloride were used as the chemical coagulants. The optimum dose of coagulant was determined as the dose of coagulant (41 mg-Fe/g-BSA and 34 mg-Al/g-BSA) that produced the minimum centrate BSA concentration of a solution for 1000 mg-BSA/L. Once the optimum dose was determined, a stock mixture of metal-BSA flocs was generated and split into four sub samples. Sodium sulfide was added at doses of 0 to 0.0124 meq-S and 0 to 0.024 meq-S for the aluminum and iron

systems respectively. Sodium sulfide was chosen as the chemical reductant since reduced sulfur in the form of hydrogen sulfide is nearly ubiquitous in the digester environment either as a product of sulfate reduction or amino acid metabolism. The mixture was then centrifuged and the centrate was analyzed for solution BSA.

Synthetic Floc Ultrasonic Disintegration Studies: This study was carried out to determine if the disintegration of iron- and aluminum-bound proteins resulted in a change in the capacity of the trivalent metal to recoagulate the released proteins. Iron and aluminum were used to coagulated BSA (1070 mg-BSA/L) with the optimum dose being the minimum solution BSA concentration achieved. Solution BSA was defined as the concentration of BSA remaining in solution following centrifugation at 2500 rpm for 2 minutes as measured by the Frølund *et al*. (1996) method. The mixture of iron or aluminum flocculated BSA was then exposed to ultrasonic disintegration (20 kHz, 1000W) for 30 seconds. Immediately following the disintegration event the solution BSA concentration was measured. Subsequent measurements were made as rapidly as possible.

Batch Digestion Studies: Batch digestion studies were conducted to determine the effect of floc metals concentrations on the performance of enhanced anaerobic digestion. Both mechanical shear and ultrasonic disintegration were tested. The batch digestion period was set at 7-days, since most of the additional volatile solids destruction was realized during that time (Figure 5-2). Mesophilic temperatures (~35 ^{o}C) were used to mimic the temperature conditions in the field. Only a fraction of the total volume of the batch digester was treated with a disintegration technology. It has been reported by McCarty and Smith (1986) and Stroot *et al*. (2001) that the spatial separation of methanogens from their syntrophic partners can result in process upset and

instability, and this can be caused by excessive shearing of the sludge. By shearing only a portion of the sludge, an undisturbed, actively respiring anaerobic community remains in the batch digester. Prior to treatment with mechanical shear the sludge was screened to remove hair and large grit particles.

Mechanical Shear-Testing: For the mechanical shear testing, 1/3 of the digester content were sheared for a period of 4 minutes which corresponds to a G (t) ~ 264,000, while the other 2/3 of the sludge was not sheared. Total and volatile solids were measured from the stock sludge at time zero and from each digester at the conclusion of the study. Six samples for total and volatile solids were taken from the digesters at the conclusion of the 7-day incubation period. The volatile solids destruction was calculated for each sample based on the mean initial volatile solids concentration. The calculated volatile solids destruction of each sample was then grouped into one of two categories control or mechanically treated. A t-test for differences in means was conducted to determine if a difference existed between the measured volatile solids destruction (VSD) between the control and treatment could be detected using an alpha of 0.05.

Ultrasonic Disintegration-Testing: The ultrasonic disintegration tests were conducted in a manner similar to the mechanical shear digestion studies. The same temperature and incubation time were used but the sludge had to be handled differently due to the impact of varying solids concentrations on the disintegration rate. Mao *et al*.(2004) reported that as the concentration of sludge increases, the disintegration efficiency by ultrasonics decreases. Tuziuti *et al*. (2005) observed that increasing particulate concentrations reduced the formation of sonication-induced cavitation bubbles in solution, attributing the reduction to wave attenuation by the particles. The results were verified for this study by testing the impact of sludge concentration on the

disintegration rate for 3 different waste activated sludges at varying solids concentrations. To minimize the impact of thermal decomposition of the sludge from the heat generated during bubble collapse all samples were treated while submerged in an ice bath. The disintegration rate was determined by finding the slope of the median particle size of the sludge for multiple exposure times (0, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 420 seconds) for each solids concentration tested. The change in particle size per unit time was normalized by the energy utilization of the system calculated from the displayed on the ultrasonic process control unit using Equation 5-1.

Equation 5-1: Power Utilization = P_{total}* (Percent Utilized)* t
P_{total} = total power of unit (1000W),
Percent Utilized = read from display
t = time of operation

A typical disintegration rate verses solids concentration profile is shown in Figure 5-3. From the data in Figure 5-3 and data for the other sludges tested, a solids concentration of 1 percent was selected for the sonication study. As can be seen in Figure 5-3, at 1% solids, the disintegradation was near its optimum. This was true for all of the sludges tested.

Once the initial solids concentration was determined and the sludge diluted to 1% total solids, duplicate batch anaerobic digesters were set up. The amount of material treated by the ultrasonic device was increased from 33% to 50% to make changes in volatile solids destruction more readily detectable at the reduced solids concentration. Ultrasonic disintegration was applied for a period of 3 minutes in a 500 ml glass-tempered beaker. Tap water was run through the water

jacket of the tempering beaker in order to minimize the impact of thermal decomposition on the disintegration process. Duplicate control and ultrasonic treated digesters were then sampled for total and volatile solids concentration in triplicate. Following 7-day mesophilic incubation, the total and volatile solids concentrations were measured in triplicate and the volatile solids destruction was calculated for each sample using the mean volatile solids concentration at time zero as the initial condition. The calculated VSD for the control and ultrasonically treated sludges were grouped to determine if the measured VSD for the treatment digesters are statistically different from the measured VSD of the control using the t-test and an alpha of 0.05.

Statistical Analysis: Descriptive statistics, mean and standard deviation, were calculated for all samples where appropriate. The differences in mean concentrations were detected by using the Student's T-test. Microsoft Excel 2000, (Microsoft Corporation, Redmond, WA) data analysis tool pack was used working under the assumption of equal variances. The confidence level was set at 95% by using an alpha of 0.05; therefore, any p-value less than 0.05 was considered significant.

Analysis of observed trends was conducted using the regression analysis function of Microsoft Excel 2000 to determine the fit of the regression curve and if the trend was greater than zero. Along with the regression analysis the residuals plot was checked to determine if there are underlying trends within the data, which could influence the analysis.

RESULTS AND DISCUSSION

Behavior of Iron and Aluminum Flocculated Proteins in Simulated Digester Environment

The anaerobic digestion of a portion of the sludge has been attributed to changes in the redox potential, resulting in the reduction of iron and the release and subsequent biodegradation of iron-bound organic material during digestion. The relationship between iron and digestible organics from waste activated sludge was noted by Park *et al.* (2006a). They showed that the concentration of floc-associated iron in waste activated sludge can be used to estimate volatile solids destruction during anaerobic digestion of waste activated sludge. Novak *et al.* (2003) attributed such behavior to the reduction of iron and release of organic material, specifically proteins, associated with the floc matrix.

The association of iron with proteins is not unique. Many enzymes and molecules associated with electron transport in microorganisms use iron-associated proteins for a variety of purposes in metabolic processes. Some of these proteins such as desulfoferrodoxin, found in *Desulfovibrio desulfuricans* ATCC 27774 can have multiple iron core sites (Devreese *et al.* 1996). Iron and sulfur can interact with amino acids such as cysteine to form iron sulfur clusters (Türker and Erkoç 2003). The formation of iron-sulfur structures in enzymes suggest that free sulfur groups associated with amino acids (cystiene and methionine) within protein fragments or lysis products released by cells could interact with iron in the wastewater or sludge. The iron-protein complexes would be relatively stable under oxic conditions, but destabilize or break down under anaerobic or reducing conditions.

The oxidation and reduction of iron is critical in biological systems for the transport of electrons and thought to be important for the release or organic matter. Iron reduces from a 3+ valence

state to a 2+ valence state under reducing conditions which are found in anaerobic digesters. The reduction of iron in the presence of sulfide results in the formation of ferrous sulfide (FeS) as well as other forms of iron sulfide, depending on environmental conditions (Rickard 1995).

To investigate the relationship between organic matter flocculated by trivalent metals under reducing conditions, a model floc system was used. Metal-protein flocs were generated by coagulating bovine serum albumin (BSA) using both iron and aluminum and to study its release under reducing conditions. This was done to investigate the release pattern of metal-protein associations within a reducing environment without the complications of protein release due to cellular lysis, biological metal reduction and hydrolytic activity that would occur with whole sludge experiments.

The data in Figure 5-4 shows the response of BSA associated with iron and aluminum in the presence of sulfide. BSA coagulated with iron was readily released into solution after the addition of sulfide, reaching the original solution concentration at the maximum sulfide dose tested. Even at the lowest sulfide dose (0.0060 meq-S) there was an increase in centrate BSA from 2 mg-BSA/L to 115 mg/L-BSA, indicating that iron bound protein will be released into solution when sulfide reduces and binds to iron. However, when similar sulfide doses (0 to 0.0124 meq-S) were added to BSA flocculated with aluminum, only a small amount of coagulated BSA was solublized. These data indicate that protein bound to aluminum is not likely to be chemically solubilized in an anaerobic digester environment while iron bound material is readily released.

Chemically mediated volatile solids destruction is likely to only account for a portion of the material released and degraded within digesters. Increases in volatile solids destruction observed with increasing SRT suggest there are other reactions involved in VSD. One potential source of further iron reduction is biological iron reduction.

Iron reducing bacteria have been found to be present in wastewater sludges and have been reported to potentially comprise > 3% of the total community (Nielsen *et al.* 2002). The species *Geobacter sulfurreducens* strain SL-1 was isolated from activated sludge (Nielsen *et al.* 1997) and Nielsen et al. (1997) also reported that iron reducing bacteria from the α, β and γ sub-classes of proteobacteria were present in waste activated sludge. The presence of active iron reducing organisms in digested sludge suggests that chemical reduction of iron alone may not entirely account for the release of iron-associated organics and their degradation. What this suggests is that under normal digestion conditions, only a portion of the oxidized iron is reduced by sulfide or changes redox state, leaving a fraction of iron-associated material available for biological iron reduction. This potentially explains why with longer SRTs there is a greater VS destruction, beyond just a decay of the biomass. At higher SRTs, the biological reduction of iron can continue and will result in increased VS destruction, whereas, if the change in redox potential accounted for the release, the release and biodegradation would be expected to be relatively rapid.

Aluminum-protein complexes were shown in this research to be stabile in the presence of sulfide (Figure 5-3). Similar results were reported by Edwards *et al.*(1997) for the addition of iron and aluminum hydroxide drinking waters sludges to wastewater plants for sulfide control . The

Edwards *et al*. (1997) study showed that addition of ferric hydroxide sludge reacted with free sulfide (4.5-7.2 mol-S/mol-Fe-dosed) while alum hydroxide sludges were relatively inactive, removing only 0.034 mol-S/mol-Al-dosed. The relative chemical inactivity of aluminum in a reduced environment and the lack of biological processes that include aluminum suggest that much of the aluminum bound organic material would be less bioavailable. Davydov *et al.* (1998) reported that iron oxide and amorphous iron hydroxide surfaces had 100% reactivity with hydrogen sulfide, while aluminum oxide only had 10% reactivity. While aluminum can react with sulfide to generate aluminum sulfide species, the solution concentration of sulfide required to release a similar amount as iron bound organics would be 10 fold. This could produce an environment that is chemically toxic to the microbial populations in anaerobically digesting sludge and thus is unlikely.

The significance of these findings are that there are potentially two types of material associated with the trivalent metal fraction in a sludge floc, an iron bound fraction in which redox chemistry plays a role in the release of organic material from the sludge structure and an aluminum bound material which is resistant to chemical reduction and biological degradation. The differences in biodegradability as well as the incomplete solubilization of organics by chemical reduction tells us little about the amenability of the iron and aluminum associated organic fraction to shear-enhanced anaerobic digestion. One or both pools of trivalent metal-associated organics could serve as a source of biodegradable substrate to be formed during wet sludge disintegration.

The Impact of Floc Cations on Mechanical Shear-Enhanced Anaerobic Digestion

The use of mechanical shear in an internal recycle has been demonstrated to be an effective means of enhancing mesophilic digester efficiency (Abu-Orf *et al.* 2004b; Basu *et al.* 2004; Muller et al. 2006b). While the process shows potential as commercially viable technology, understanding the mechanisms that control its performance is critical for proper application. Floc structure and composition are one of the factors that we think has an impact on the observed performance of enhanced digestion technologies. From the literature two groups of metals have been identified as important to flocculation, divalent cations (Higgins 1995) and trivalent cations (Park *et al.* 2006b). For the purposes of this paper the material associated with these metals will be simplified. Trivalent metals, are thought to act as scavengers within the wastewater system bycollecting debris and lysis products and incorporating them into the floc matrix. Divalent metals, specifically calcium and magnesium, are thought to be primarily associated with "lectin-like" proteins, which mediate microbial flocculation. To determine if floc associated cations impact the enhancement of digestion the following study was conducted.

Mesophilic anaerobically digested sludges were collected from different wastewater treatment facilities and tested to determine the degree of digestion enhancement that could be achieved for a given energy input. The results of batch testing shown in Figure 5-5 demonstrate that the degree of digestion enhancement is variable from sludge to sludge and also varies over time for sludge from a single plant as indicated by the numbering in each label. Depending on the sludge tested the increase in volatile solids relative to the control ranged from 2.6-68.9%. Signficant increases were observed in plants, A ($p = 0.00004$) ,C ($p = 0.001$), D ($p = 0.03$), and E ($p = 0.00007$) but no statistical difference was observed for plant B. The maximum increase observed

was 109% (sample D in Figure 5-5), but for this sample the volume treated by shear was increased to 50% and a digestion time of 8-days which will could account for the extra VSD. What the data show is that like conventional anaerobic digestion, shear recycle enhanced digestion is highly variable from one sludge to another.

Since we suspected that iron-associated organics might be weakly bound compared to aluminum and divalent cation-associated organics, volatile solids destruction was compared to various cations in the sludge. The percent increase in volatile solids destruction was plotted as a function of varying cation ratios that are thought to influence flocculation (Higgins and Novak 1997a; Park et al. 2006b). Figure 5-6 shows the correlation between the different floc ions and the percent increase in volatile solids destruction resulting from mechanical shear.

When the increase in additional volatile solids destruction (AVSD) was compared to floc ions suggested from the literature to be important in flocculation (Figure 5-6), it is apparent that the additional digestion was not influenced by the M/D ratio (Higgins and Novak 1997b) or M/(D+T) (Park et al. 2006b). The monovalent to divalent cation ratio (Figure 5-6a) shows a poor correlation with no significant trend between the sludge cation content and AVSD (R^2_{linear} = 0.40, p = 0.12) nor did the ratio of M/(D+T) (Figure 5-6b) (R^2_{linear} = 0.38, p = 0.14). Given the lack of correlation between digestion enhancement and the cation ratios that impact flocculation, it was thought that the shearing likely impacted only a fraction of the material associated with the sludge floc. Figure 5-6c shows that there was also little impact of the D/T ratio on AVSD (R^2_{linear} = 0.38, p = 0.14) nor did the (M+D)/T ratio (Figure 5-6d) (R^2_{linear} = 0.38, p = 0.14) The impact of trivalent metals alone, the fraction thought to be primarily coagulated debris and lysis

products showed no discernable impact on the degree of digestion enhancement (R^2_{linear} = 0.02, p = 0.75) (Figure 5-6e).

As observed under simulated conventional mesophilic anaerobic digestion conditions, iron and aluminum behave differently under a reducing environment. Given this difference in the response to iron and aluminum, the percent increase in AVSD was plotted as a function of the ratio of floc-associated iron to aluminum. A good correlation between the degree of digestion enhancement and the Fe/Al ratio was observed as shown in Figure 5-6f (R^2_{linear} = 0.84, p = 0.004). The data set is limited in samples, 7 points 2 from plant A, 3 from plant B and 1 each from C and D, which causes a weighting of the trend to the highest Fe/Al ratio but there is no legitimate reason to remove any of the values from the analysis. Variability in the metals concentration in samples from the same plant allows each to be considered a separate sample. Therefore it appears that the ratio of Fe/Al has a significant impact on the observed degree of digestion enhancement by mechanical shear.

The proposed mechanism of action for mechanical shear enhanced anaerobic digestion through the disintegration of digested sludge centers on particle disruption and protein denaturation. The disruption of the floc matrix can result in the exposure of iron-protein complexes within the gelatinous EPS to sulfide, which results in ferrous sulfide formation and protein release in a similar manner as was in from the data in Figure 5-4. Shear forces have been shown to cause protein denaturation (Maa and Hsu 1996), which could allow sulfide to access a portion of protein structure that was previously inaccessible. Maa and Hsu (1996) reported similar interactions for hydrophobic regions of denature proteins interacting impacting refolding. The

subsequent release and degradation of protein has been observed in the full-scale application of this technology (Muller et al. 2006a).

While iron interacts with the sulfide, resulting in a release of bound protein, aluminum appears to have the opposite effect. Aluminum has a negative impact on the degree of digestion enhancement, which may be due to the resistance of aluminum-bound organics to solubilization or if solublized, the released material could be rapidly recoagulated. For iron, when shear releases bound organics, the iron released into solution can be rapidly precipitated with sulfide (Rickard 1995). The denaturation or break down of trivalent metal protein complexes by shear forces is likely to be the similar for iron and aluminum bound proteins.

Along with the sulfide-enhanced floc disintegration there are other factors that will result in the mechanical enhancement of sludge digestion. The release of EPS bound extracellular enzymes from the floc matrix may account for some of the observed digestion enhancement. Dohányos *et al.* (2004) demonstrated that a lysate-centrifuge was capable of harvesting extracellular hydrolytic enzymes from digested sludge. The enzyme-laden centrate was then returned to the digester to increase the hydrolytic rate. Along with the release of hydrolytic enzymes from the floc matrix, there is an increase in particulate surface area due to particle dispersion. Sanders *et al.* (2000) and Vavilin *et al.* (1996) modeled the hydrolysis of particulate matter as not only a function of enzyme properties but included available surface area in determining the observed hydrolytic rate. These studies demonstrated that breaking down a large particle into smaller particles with increased surface area for bacterial adhesion or enzymatic attack resulted in an increase in the observed hydrolytic rate. The mechanical shearing of digested sludge may not

cause significant cell lysis (Muller 2001). However, it will reduce particle size and increases available surface area. Thus it is likely that not only is some organic matter immediately rendered readily bioavailable, but other material may also becomes degradable.

What is apparent from the data is that the addition of mechanical shear to an internal recycle stream enhances anaerobic digestion by acting upon the iron-associated fraction of the floc material. This is the same material that is reported to control conventional mesophilic anaerobic digestion and serve as the pool of precusors to organic sulfur odors from dewatered biosolids (Verma, 2005). While lysis may occur, there appears to be little indication that it is significant relative to particle disintegration.

Ultrasonic Disintegration-Enhanced Digestion is Impacted by the Divalent to Trivalent Cation Ratio

Based on the results of the mechanical shear study, the impact of sludge associated metals was studied in an internal recycle system that used ultrasonic energy rather than mechanical shear to disintegrate the sludge. Ultrasonics generate disintegrative forces mechanistically different than mechanical shear, but it is often reported as a mechanical disintegration process in the literature. The acoustic radiation only generates a cavitation bubbles in locations where there is inter-molecular weakness. These points of weakness could be on the floc surface or interior depending on its composition. Once a bubble forms and subsequently collapses the disintegrative force is applied to the surrounding material. Upon collapse there are significant mechanical shear forces associated with water jets that form during bubble collapse. It is likely that these jets work in a similar manner to a mechanical shear device and thus sonication is often included as a mechanical process. However, along with the shear forces there are significant

pressure and temperature gradients formed. Temperatures up to >4000 K and pressures up to 1000 atmospheres (Christi 2003) result in pyrolysis of organic matter. The generation of free radical species •H and •OH along with hydrogen peroxide from water within or intimately associated with the collapsing bubble has been reported (Hua and Hoffmann 1997). The combination of elevated temperatures and free radicals could serve to chemically enhance the digestibility of the organic material within the floc matrix or cause significant cell damage, death and lysis. To determine if mechanical shear and ultrasonic disintegration enhance digestion in the same manner, batch tests were conducted in a using a method similar to that described for the mechanical shear system.

The data in Figure 5-7 show how additional volatile solids destruction was impacted by ultrasonic disintegration relative to the control. As in Figure 5-6, with mechanical shear, the extent of enhancement was found to be sludge specific. The percent increase in volatile solids destruction relative to the control condition ranged from 12% to 80.4%. Significant differences in the AVSD were detected in 5 out of the 8 samples, A ($p= 0.0007$), A2 ($p = 0.00003$), B ($p = 0.003$), D2 ($p = 0.016$) and E ($p= 0.02$). The variability in digestion enhancement was not only spatial, but also temporal. These results mimic those observed with mechanical shear enhancement, suggesting that the degree of digestion enhancement is not only a function of energy input, but also depends on sludge composition.

The digestion enhancement data for ultrasonics was compared to the same floc ion ratios used in the mechanical shear analysis. Figure 5-8 shows the results of these comparisons. As with mechanical shear, the M/D (Figure 5-8a) ($R^2_{linear} = 0.15$, $p = 0.34$) and M/(D+T) (Figure 5-

8b)(R^2_{linear} = 0.04, p = 0.7) relationships did not correlate well with the changes in volatile solids destruction. Once again the data show that the cation ratios that are important in sludge flocculation do not predict how well they can be deflocculated by disintegration.

What the data in Figures 5-8a and 5-8b suggest is that like mechanical shear, ultrasonics acts upon a specific fraction of the material associated with the sludge floc. However, the material primarily disintegrated by ultrasonics is not the same as mechanical shear. Data in Figure 5-8c shows a significant correlation (R^2_{linear} = 0.67, p = 0.013) between the divalent cation-associated material relative to the trivalent associated material on additional volatile solids destruction.

Unlike mechanical shear, the trivalent bound organic matter has a strong negative impact on disintegration. As the concentration of both iron and aluminum increase per unit mass of sludge, the degree of digestion enhancement decreases as shown in Figure 5-8e (R^2_{power} = 0.72). Furthermore, the impact of trivalent metals on digestion enhancement does not appear to be related to the reduction in particle size, as was the case for mechanical shear. As the amount of iron increased relative to aluminum in the sludge floc, the degree of digestion enhancement decreased (R^2_{power} = 0.74) (Figure 5-8f). This suggests that iron has a negative impact on ultrasonic enhanced digestion that far outweighs any enhancement due to particle dispersion and sulfide interaction.

One potential mechanism for iron-mediated reduction in ultrasonic disintegration efficiency is due to the formation of ferrous sulfide precipitates and their attenuation of the ultrasonic waves. These particles accumulate within the floc matrix, giving the digested sludge its distinct black

color, as the ferrous iron reacts with reduced sulfide. Increasing concentrations of iron in the feed sludge resulted in increased concentrations of these particles within the digested floc matrix. Tuziuti *et al*. (2005) reported that with increasing solution particle concentrations (aluminum oxide) there is a decrease in the formation of cavitation bubbles due to the attenuation of sound waves by the particles. Therefore it may be possible that the ferrous sulfide particles within the floc matrix attenuate the ultrasonic waves sufficiently such that cavitation bubbles only forms on the surface of flocs rich in iron reducing disintegration efficiency.

The role of aluminum is likely the same in ultrasonic disintegration as is in mechanical shear systems, recoagulating released materials. The collapse of a cavitation bubble will produce liquid jet which can have a significant shearing effect and thus cause particle disruption. But just as theorized with mechanical shear, the release is short lived due to recoagulation by aluminum.

Divalent Cation Release Pattern Suggests Targeted Disintegration

The selective disintegration of sludge in rich in calcium and magnesium is supported by changes in solution cations monitored during sonication. Figure 5-9 shows the change in solution divalent cations in waste activated sludge (WAS) during sonication and when exposed to mechanical shear. The data in Figure 5-9 show that there is a rapid release (within 15 seconds) of divalent cations when WAS is exposed to ultrasonic radiation. After reaching a peak release the solution divalent cations decrease with increasing sonication time. The decrease is likely due to ionic interactions from the solubilized biopolymer which often caries a negative charge. The release of divalent cations is much lower and slower when WAS is exposed to high intensity

mechanical shear. Only after a period of 5 minutes of constant shearing is there a small increase in solution divalent cations.

The mechanism for this targeted disintegration within the floc matrix may be associated with the concentration of particles and dissolved gasses surrounding the respiring microorganism. Particulate matter, as well as high concentrations of dissolved gasses, promote the formation of acoustic cavitation bubbles. As the sound waves move from compression to rarefaction and the hydrogen bonds are placed in tension, if there is sufficient weakening a cavitation bubble will form. Particulate matter and microgas bubbles serve as weak points in the water matrix leading to cavitation in and around these anomylies in the matrix.

When cells respire there is a release of gas through the membrane and the cell wall, which could generate localized weak points in the floc structure. Thus the growth and collapse of the cavitation bubble would be sufficiently close to result in disintegration of the "lectin-like" structures and releasing the associated divalent cations to solution. The release of divalent cations from WAS exposed to ultrasonic disintegration has been reported elsewhere in the literature (Wang *et al.* 2006).

Characterization of Released Organic Matter Supports Targeted Disintegration

Waste activated sludge was processed using both mechanical shear and ultrasonic disintegration on a batch basis for 20 minutes. The samples were centrifuged and filtered through a 0.45 mm membrane filter. The filtrate was then analyzed for protein content as well as polysaccharide content. Given that the extent of release from each disintegration system may be different due

their individual modes of operation, absolute quantities of protein and polysaccharide were not used. Rather the ratio of polysaccharide to protein was used to determine if the composition of material released was different.

Figure 5-10, shows the change in the ratio of polysaccharides to protein on a mass ratio in the supernatant for both ultrasonic and mechanical shear disintegration. After about 5 minutes of operation the ratio for the ultrasonic disintegration process was about 0.66 while mechanical shear was much lower at 0.25. What the data suggest is that the material released during ultrasonic disintegration is chemically different that that of mechanical shear. Given the increase in polysaccharide content relative to protein with ultrasonic disintegration, and the release of divalent cations as well (Figure 5-9) it is possible that glycoproteins, which can be lectins, or lippopolysaccharides are being disintegrated and released from the cell surface.

The two major classes of organic matter associated with floc material are proteins and polysaccharides. Based on the literature, it appears that much of the material associated with iron and aluminum hydroxides are proteinaceous. The material associated with calcium and magnesium could be cell excreted "lectin-like" proteins (Higgins and Novak 1997a). While lectins are often referred to as proteins many are actually glycoprotein (Görner *et al.* 2003) having both protein and polysaccharide fractions. Lectins have been reported to be present in sludge flocs, and are thought to be glycoproteins in structure due to the precipitation at pH 2 (Jorand *et al.* 1998). Bura *et al.* (1998) reported that lectins in municipal sludges have α-D-mannosyl and α-D-glucosyl residues. Along with glycoproteins cell surfaces are often associated with lipolysaccharides that perform a number of functions.

Disintegration Impact on Trivalent Metal-Protein Coagulation

The capacity of sonicated iron-associated protein and aluminum-associated protein particles to recoagulate was studied in controlled batch tests using bovine serum albumin (BSA) as the model protein. The objective was to determine if disintegration had an impact on the refloccuation of released material. Figure 5-11, shows the behavior of the metal-protein flocs under these conditions. BSA was much more effectively coagulated in the iron containing solution, leaving only 9.82 mg/L of BSA in solution of the initial 1070 mg-BSA/L, at optimum dose. Aluminum hydroxide, removed 82.7% of the BSA at the optimum dose leaving 182 mg-BSA/L in solution. After ultrasonic exposure there was a net release of BSA into solution for both iron and aluminum hydroxide bound materials, 96 and 104 mg-BSA/L respectively. However, after approximately 10 minutes, the released material was almost completely recoagulated by both the iron and aluminum in solution.

The data show that the ultrasonic disintegration of iron and aluminum bound materials does not permanently release material associated with these metals. It is likely that mechanical shearing would show a similar behavior. The significance of these finds is that the benefits of either sonication or mechanical disintegration of iron and aluminum-associated organic materials is likely to be limited because of recoagulation of organic matter. Given these finding, it is likely that sludges rich in aluminum will not benefit much from disintegration since much of the material released will be recoagulated. The disintegration of iron-associated material under reducing condition will enhanced digester performance.

Considerations for Wet Sludge Disintegration Technologies and Applications beyond Enhanced Solids Destruction

The data presented in this study not only provides valuable insight into how floc structure impacts enhanced digestion technologies but also shows that the enhancement of mesophilic anaerobic digestion is both sludge and technology specific for systems using an internal recycle mode of operation. Simply adding energy to a system in the form of sludge disintegration is not necessarily the most efficient means of improving digester performance. Having an understanding of how a technology interacts with the sludge medium and how the sludge responds is critical to selecting the correct technology for each facility.

Mechanical shear appears to impact the whole sludge, working primarily on the mechanism of particle size reduction and exposure of unreduced iron protein complexes to sulfide. The limitations of this technology in an internal recycle mode of operation are that the shear device will only be able to impact particles down to a specific size and a sludge rich in aluminum will reduce the process efficiency by recoagulating released materials.

Ultrasonic disintegration in an internal recycle appears to be most effective at disintegrating sludges poor or devoid of trivalent metals. However, the impact of the trivalent pool of metals appears to be metal specific. The formation and accumulation of ferrous sulfide particles within the floc matrix may attenuate ultrasonic radiation, preventing it from entering the floc matrix reducing the effectiveness of the disintegration process. Aluminum bound material appears to impact ultrasonics in the same manner as mechanical shear in recoagulating released material.

The differences between the way in which mechanical shear and ultrasonic disintegration apply their destructive energies appears to make them individually suited for specific sludges. Although both produce shear forces, the mechanisms of disintegration are not the same. Therefore it is likely that no one single sludge disintegration technology will serve to enhance digestion for all sludges. Rather the selected application of a suite of technologies is need to provide effective and efficient digestion enhancement.

The data presented in this study suggest that no one technology is going to be a panacea for enhanced of solids destruction. However, understanding how these technologies interact with the sludge suggests that some technologies will have an added benefit beyond just enhanced solids destruction. Mechanical shear was shown to primarily impact the material associated with iron while being limited by increasing floc aluminum. Recent work by Muller *et al*. (2004) demonstrated that the shear associated with sludge introduction to a centrifuge is directly linked to volatile organic sulfur compound (VOSC) emissions from centrifugally dewatered biosolids. VOSC's have been reported to be one of the primary odors associated with dewatered anaerobically stabilized sludge(Adams *et al.* 2003). Verma (2006) reported that the emission of VOSC's from dewatered sludge had a positive correlation between the peak VOSC emission and the concentration of iron associated with the biosolids. What this suggests is that the material released and degraded by mechanical shear in an internal recycle may be acting upon the same pool of iron associated proteins which serve as precursors for VOSC emissions.

Given that mechanical shear and ultrasonic disintegration impact two different fractions of the sludge floc further suggests that the observed decrease in odor associated with each technology

will be different. It is likely that since each technology produces a shear effect there will be some reduction by either, when operated in an internal recycle, but the mechanical shear will out perform the ultrasonic device due the negative impact of trivalent metals for the ultrasonic process.

While further study is required and warranted to validate the impact of enhanced digestion technologies on VOSC emissions, the significance of understanding the interaction of sludge properties and disintegration technologies cannot be ignored. By understanding how sludges and disintegration technologies interact, not only can enhanced solids mass reduction be achieved but enhanced biosolids stability, in terms of VOSC emissions, as well. This could potentially allow for the coupling of enhanced mesophilic digestion with high solids centrifugation to produce a relatively small mass high cakes solids concentration of low odor (VOSC) biosolids.

CONCLUSIONS

The selection and application of any disintegration technology is going to be sludge specific. Digested sludges rich in iron and poor in aluminum are best suited from mechanical disintegration, in which it is thought that particle size is reduced and surface area is increased allowing for additional chemical iron reduction, organics release and areas for enzymatic attack and microbial adhesion. Unlike mechanical shear, ultrasonic disintegration is suited for sludge poor or devoid of trivalent metal. The recoagulation of released proteins by aluminum hydroxides and the potential for attenuation of ultrasonic irradiation by ferrous sulfides in the floc matrix results in a reduction in cavitation efficiency and thus disintegration. Thus, when selecting a wet sludge disintegration technology to improve digester performance some

knowledge of floc composition and structure will help identify which facilities are best suited for the technology under consideration.

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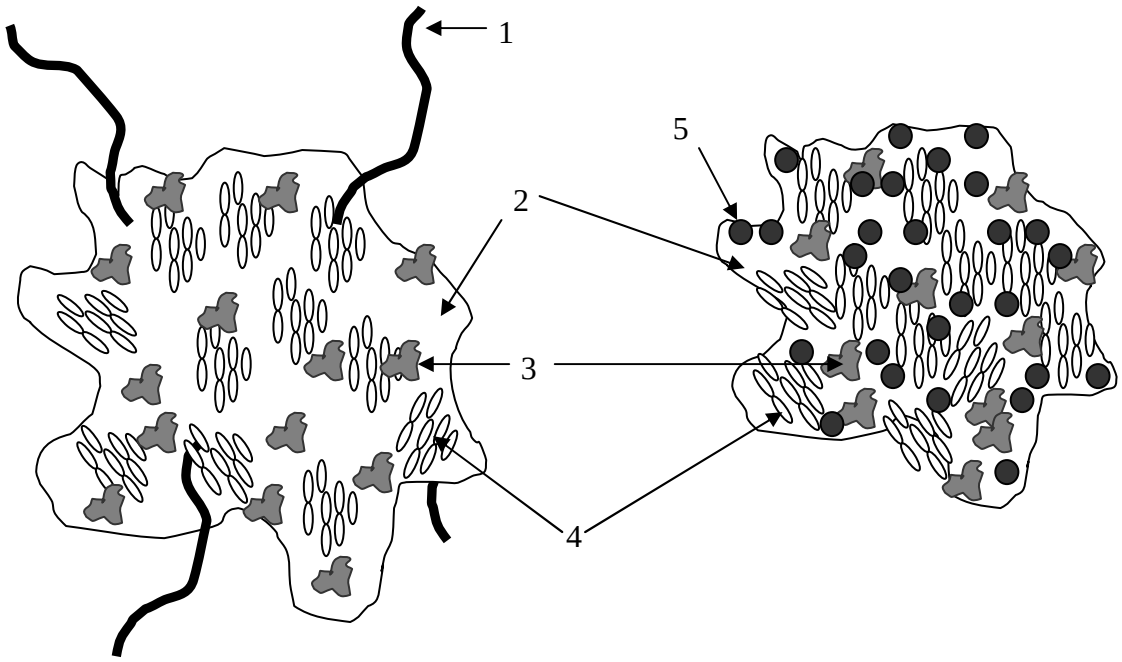


Aerobic Sludge Flocs (a)

Anaerobic Sludge Flocs (b)

LEGEND

1. Filamentous Bacteria
2. EPS (Monovalent and divalent cations)
3. Colloidal Organic Matter (iron and aluminum)
4. Flocculent Microorganisms (Monovalent and divalent cations
5. Ferrous Sulfide Precipitates (iron-anaerobic only)

Figure 5-1: The Relationship between Floc Associated Cations and Floc Structure Components in Activated Sludge and Anaerobic Sludge

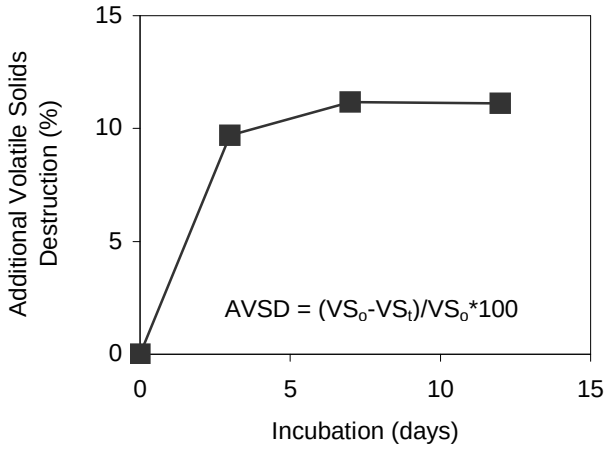


Figure 5-2: The Change in Additional Volatile Solids Destruction with Incubation Time at Mesophilic Temperatures

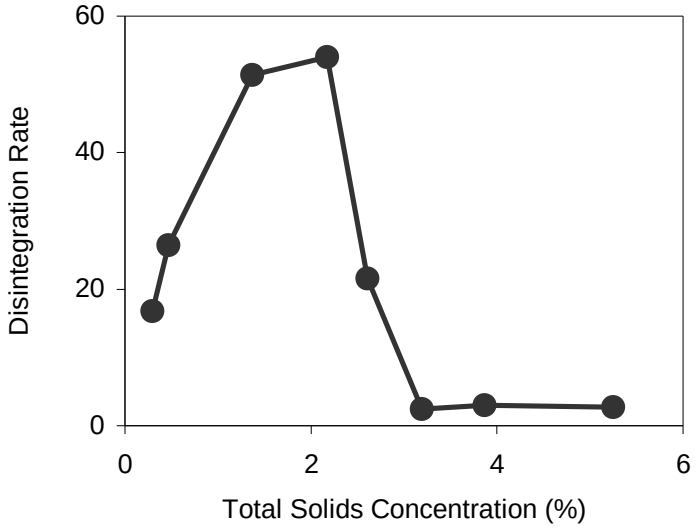


Figure 5-3: The Impact of Initial Solids Concentration on Ultrasonic Disintegration Rate

* Disintegration Rate = (PSR%/(kW-hr-kg-TS)); PSR =particle size reduction

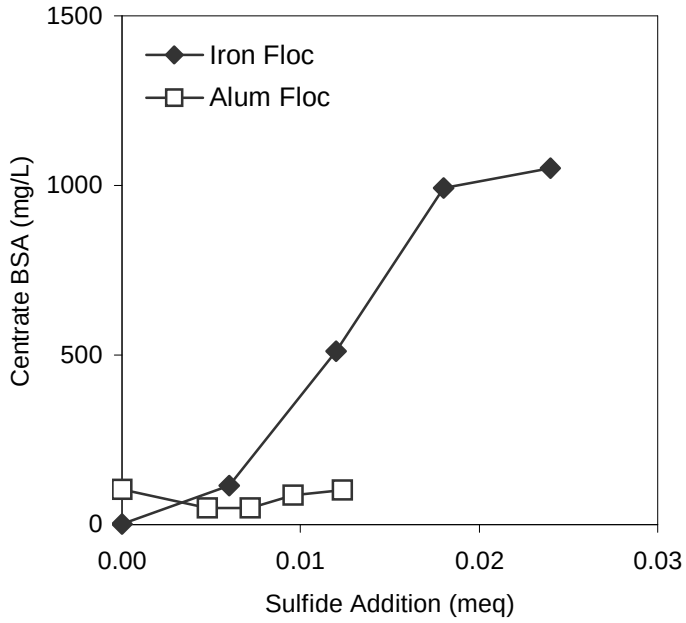


Figure 5-4: The Stability of Iron and Aluminum Hydroxide Associated Proteins in the Presence of Sulfide

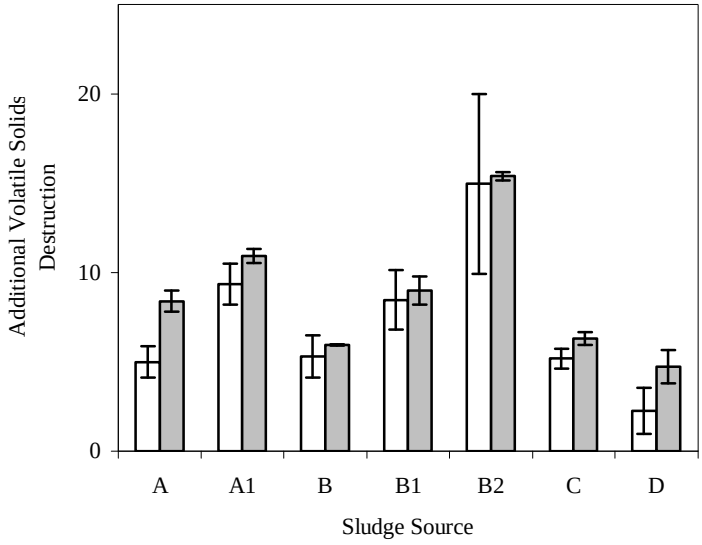


Figure 5-5: Change in Volatile Solids Destruction between Shear Enhanced Anaerobic Digestion and Traditional High Rate Anaerobic Digestion (Muller et al. 2006b)

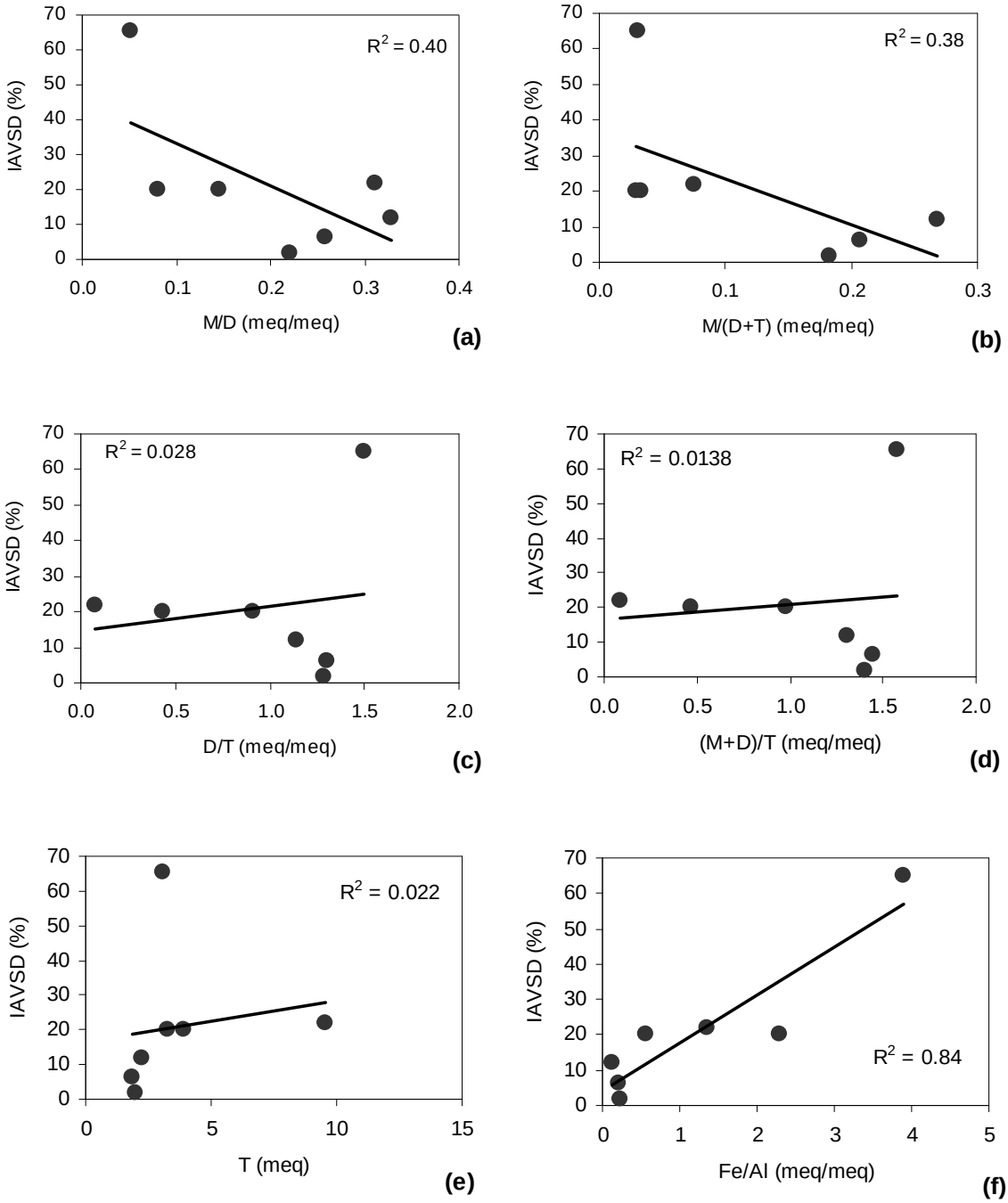


Figure 5-6: Correlation between Proposed Floc Models or Floc Components and the Degree of Digestion Enhancement Using Mechanical Shear in an Internal Recycle: (a) Monovalent to divalent cation ratio (Higgins and Novak 1997b), (b) Monovalent to divalent plus trivalent (Park et al. 2006b) (c) divalent to trivalent ratio (d) Monovalent plus Divalent to trivalent cations "lectin-like" material verse trivalents (e) tivalent bound organic matter only (f) Iron to Aluminum

(Abbrieviations (M = Monovalent cations, D = Divalent Cations, T= Trivalent Cations, Units = meq)

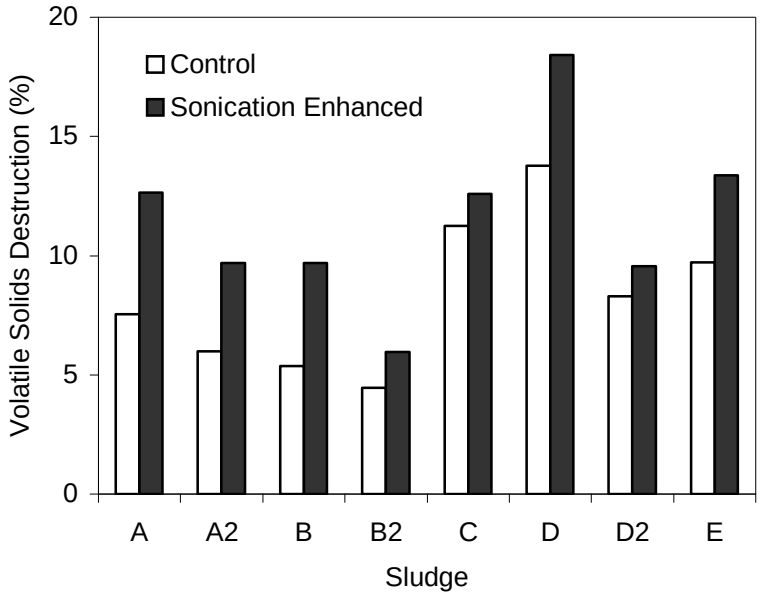


Figure 5-7: Additional Volatile Solids Destruction from Digested Sludge from Traditional Mesophilic Anaerobic Digestion and Internal Recycle Ultrasonic Enhanced Anaerobic Digestion

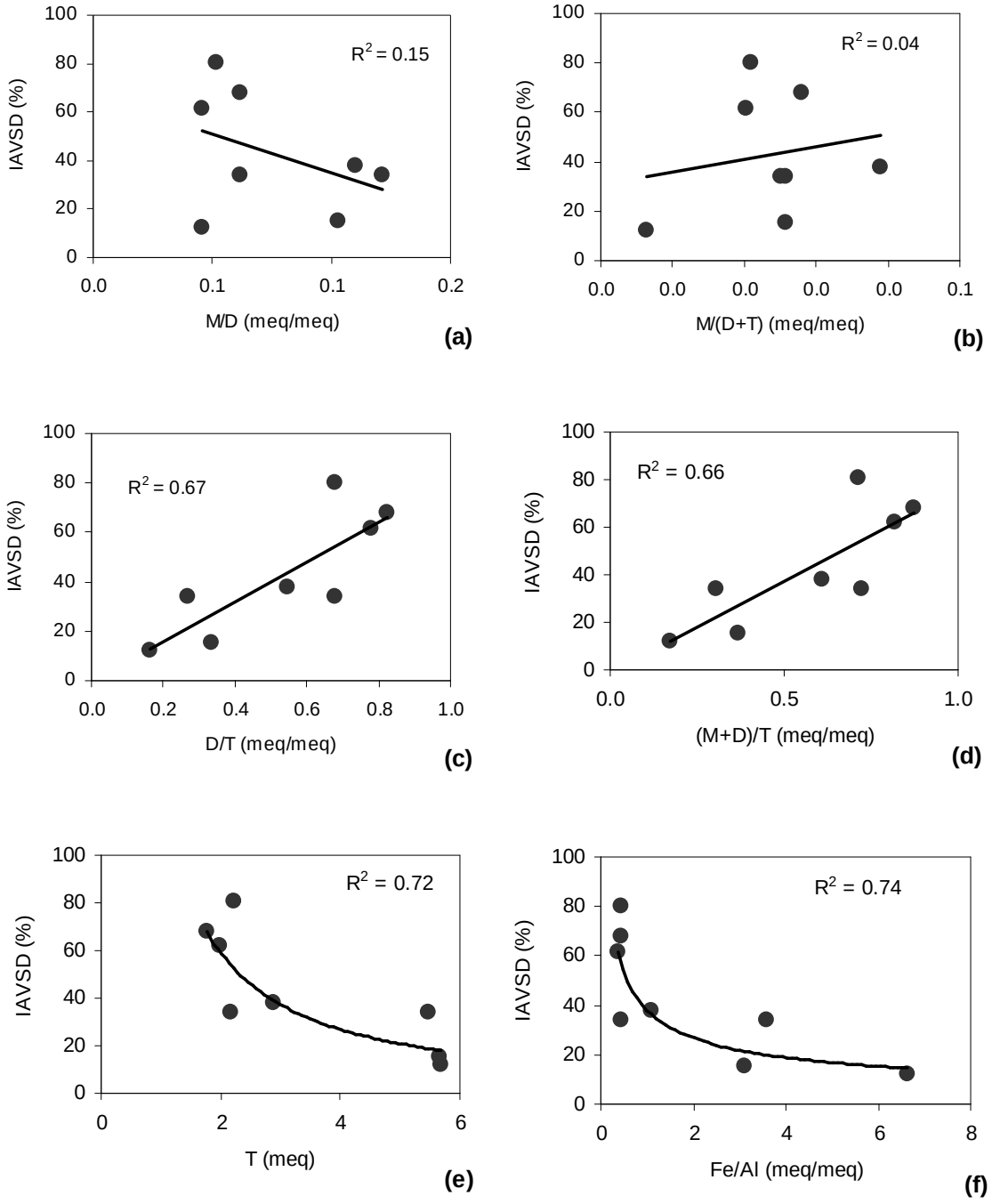


Figure 5-8: The Impact of Floc Structure on Ultrasonic Disintegration Enhanced Anaerobic Digestion: (a) Monovalent to divalent cation ratio (Higgins and Novak 1997b), (b) Monovalent to divalent plus trivalent (Park et al. 2006b) (c) divalent to trivalent ratio (d) Monovalent plus Divalent to trivalent cations "lectin-like" material verse trivalents (e) tivalent bound organic matter only (f) Iron to Aluminum, debris only

(Abbrieviations (M = Monovalent cations, D = Divalent Cations, T= Trivalent Cations, Units = meq)

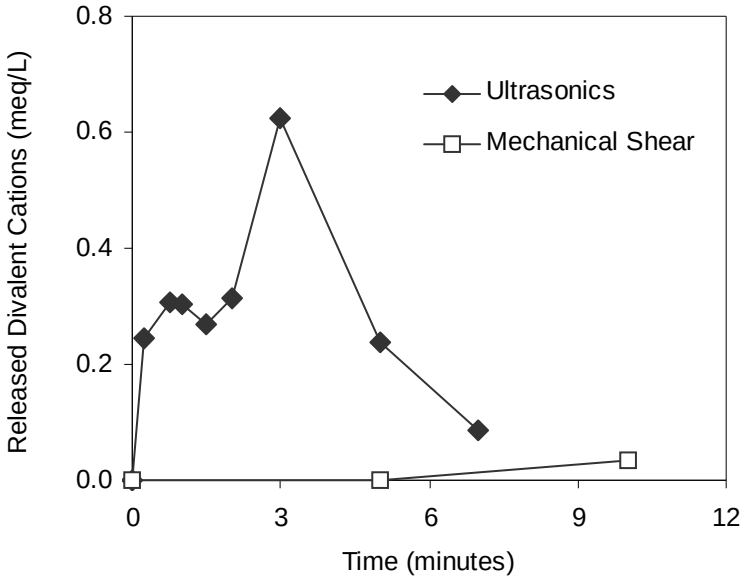


Figure 5-9: The Impact of Sonication and Mechanical Shear on Solution Divalent Cation Concentrations in WAS

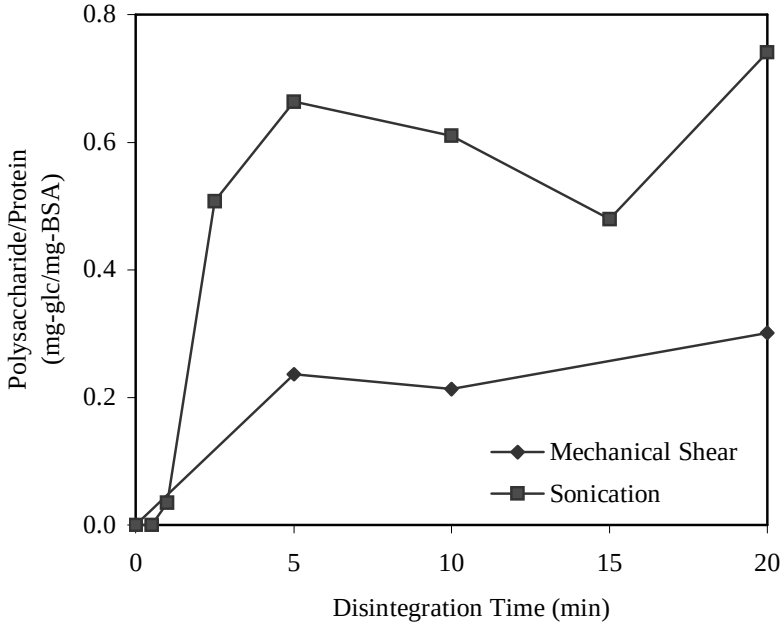


Figure 5-10: The Relative Polysaccharide and Protein Content of WAS Disintegrated by Ultrasonics and Mechanical Shear

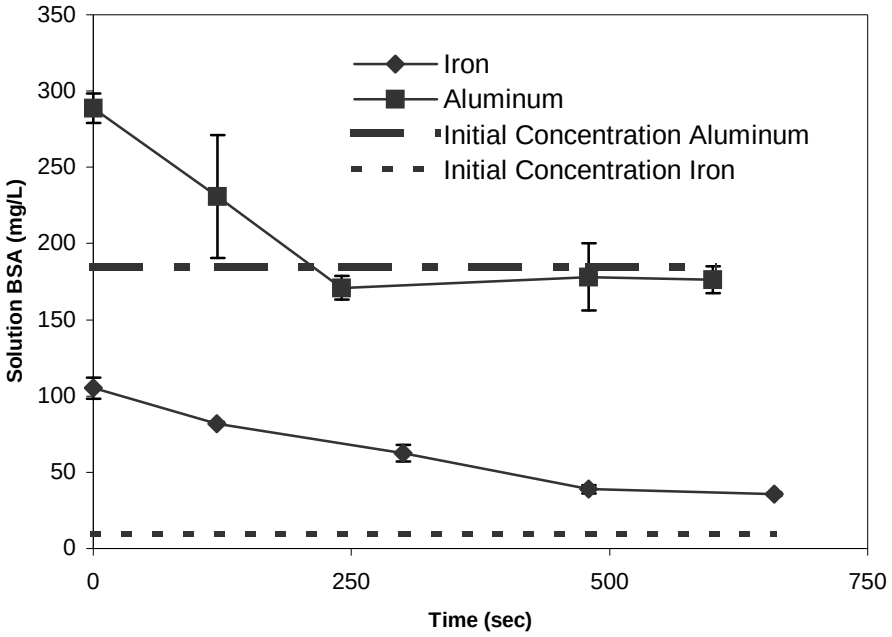


Figure 5-11: The Release of Bound Protein from Ferric and Aluminum Hydroxide Flocs Exposed to 20 kHz Ultrasonic Disintegration

VITA

Christopher D. Muller attended the Unversity of New Hampshire where he received his Bachelors of Science and Biology: Marine and Freshwater in 1996. Following graduation he worked for a short time in the aquaculture industry prior to returning to graduate school. While conducting his graduate studies at Virginia Tech, Chris received his Masters of Science in Environmental Engineering and a Ph.D. in Civil Engineering. Upon graduation Chris joined Brown and Caldwell where he is employed as a consulting engineer.

APPENDIX A: Data Associated Figures and Tables in Chapter 2

Data Associated with Figure 2 (page 76)

Figure 2: The Effect of High Intensity Shear on the Biogas Production from Batch Mesophilic Anaerobic Digesters

Cumulative Biogas

Time hours	Reactor A Control 1 ml-biogas	Reactor B Shear Digester 1 ml-biogas	Reactor C Shear Digester 2 ml-biogas	Reactor D Control 2 ml-biogas
0.0	0	0	0	0
12.3	70	112.5	109	72.5
15.8	82.5	142.5	139	92.5
19.2	96	170	164	112.5
23.1	111	212.5	199	129.5
36.2	236	363.5	359	236
47.9	284	476.5	459.5	290.5
58.9	331	545	526.5	321
65.4	361	580	566.5	343.5

Data Associated with Figure 3 (page 77)

Figure 3 Percent Volatile Solids Destruction in Traditional and Shear Enhanced Batch Mesophilic Anaerobic Digestion after a 7-Day Incubation

		Conventional		Shear Enhanced	
Data Label	**Plant Name**	**Mean VSR %**	**Std. Deviation %**	**Mean VSR %**	**Std. Deviation %**
A	Sioux City 1	5.0	0.9	8.2	0.6
A1	Sioux City 2	9.5	1.1	11.4	1.6
B	Caldwell 1	5.3	1.4	5.9	0.29
B1	Caldwell 2	8.5	1.7	9.0	0.8
B2	Caldwell 3	15.0	5.0	15.2	0.52
C	Rockland	5.2	0.5	6.3	0.33
D	Dayton Ohio	9.3	1.9	11.2	
E	Peppers Ferry	2.3	1.6	4.73	0.93

Data Label	**Plant Name**	**Net AVSD (VSR_{shear}-$VSR_{control}$)**	**Increase in AVSD %**	**p-value**
A	Sioux City 1	3.2	65	0.00004
A1	Sioux City 2	1.9	20	0.058
B	Caldwell 1	0.6	12	0.20
B1	Caldwell 2	0.5	6	0.45
B2	Caldwell 3	0.3	2	0.88
C	Rockland	1.1	22	0.001
D	Dayton Ohio	1.9	20	0.03
E	Peppers Ferry	2.4	106	0.00008

t-Test: Two-Sample Assuming Unequal Variances

Sioux City (Sample A)

	Conventional	*Shear Enhanced*
Mean	5.0	8.2
Variance	0.8	0.4
Observations	6	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-7.32	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.00004	
t Critical two-tail	2.26	

t-Test: Two-Sample Assuming Unequal Variances

Sioux City-Aluminum Ammended (Sample A2)

	Conventional	*Shear Enhanced*
Mean	9.5	11.4
Variance	1.3	2.6
Observations	7	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-2.26	
P(T<=t) one-tail	0.03	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.058	
t Critical two-tail	2.36	

t-Test: Two-Sample Assuming Unequal Variances

Caldwell Idaho (Sample B)

	Conventional	*Shear Enhanced*
Mean	5.31	6.01
Variance	1.93	0.08
Observations	8	6
Hypothesized Mean Difference	0	
df	8	
t Stat	-1.38	
P(T<=t) one-tail	0.10	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.20	
t Critical two-tail	2.31	

t-Test: Two-Sample Assuming Unequal Variances
Caldwell Idaho #2 (Sample B1)

	Conventional	*Shear Enhanced*
Mean	8.46	9.00
Variance	2.79	0.80
Observations	8	6
Hypothesized Mean Difference	0	
df	11	
t Stat	-0.78	
P(T<=t) one-tail	0.23	
t Critical one-tail	1.80	
P(T<=t) two-tail	0.45	
t Critical two-tail	2.20	

t-Test: Two-Sample Assuming Unequal Variances
Caldwell, Idaho #3 (Sample B2)

	Conventional	*Shear Enhanced*
Mean	14.96	15.23
Variance	25.26	0.28
Observations	8	6
Hypothesized Mean Difference	0	
df	7	
t Stat	-0.15	
P(T<=t) one-tail	0.44	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.88	
t Critical two-tail	2.36	

t-Test: Two-Sample Assuming Unequal Variances
Rockland, MA (Sample C)

	Conventional	*Shear Enhanced*
Mean	5.15	6.27
Variance	0.30	0.11
Observations	7	7
Hypothesized Mean Difference	0	
df	10	
t Stat	-4.61	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.81	
P(T<=t) two-tail	0.00097	
t Critical two-tail	2.2	

t-Test: Two-Sample Assuming Unequal Variances

Dayton, Ohio (Sample D)

	Conventional	*Shear Enhanced*
Mean	9.2	10.9
Variance	4.0	0.4
Observations	9	9
Hypothesized Mean Difference	0	
df	10	
t Stat	-2.48	
P(T<=t) one-tail	0.02	
t Critical one-tail	1.81	
P(T<=t) two-tail	0.03	
t Critical two-tail	2.23	

t-Test: Two-Sample Assuming Unequal Variances

Peppers Ferry Regional WWTF (Sample E)

	Conventional	*Shear Enhanced*
Mean	2.3	4.7
Variance	1.6	1.4
Observations	12	11
Hypothesized Mean Difference	0	
df	21	
t Stat	-4.88	
P(T<=t) one-tail	0.00004	
t Critical one-tail	1.72074	
P(T<=t) two-tail	0.00008	
t Critical two-tail	2.08	

Note: Statitistical analysis was conducted on the measured volatile solids destruction of the reactors. The observations value (n) is equivalent to the sum of the measurements run on a pair of duplicate digesters run on a single sample.

Data Associated with Figure 4 (page 78)

Figure 4 The Percentage of Methane and Carbon Dioxide in the Headspace of a Mesophilic Digester Under Shear and Non-Shear Conditions

Baseline Condition				Shear Enhanced Condition			
	Methane	Carbon Dioxide	Total		Methane	Carbon Dioxide	Total
Date	%	%	%	Date	%	%	%
10/23/01	59.5	34.7	94.2	12/04/01	65.9	35.8	101.7
10/25/01	51.2	29.0	80.2	12/06/01	58.2	34.6	92.8
10/30/01	23.3	12.4	35.7	12/11/01	58.2	30.5	88.7
11/01/01	36.4	18.9	55.3	01/05/02	58.8	38.8	97.6
11/06/01	61.0	29.7	90.6	01/10/02	65.8	32.2	98.0
11/08/01	56.3	28.8	85.2	01/18/02	56.5	28.1	84.5
11/13/01	59.5	30.8	90.3	01/25/02	50.6	28.0	78.7
11/15/01	55.7	30.3	86.0	02/01/02	51.4	28.6	80.0
11/20/01	59.3	31.0	90.4	02/05/02	52.8	26.2	79.0
11/29/01	41.5	20.3	61.8	02/12/02	59.3	36.7	96.0
				02/19/02	56.9	28.5	85.3
				02/26/02	56.1	34.2	90.4
				03/05/02	54.1	32.7	86.7

	Baseline		Shear Enhanced	
Compound	Mean (%)	Std. Deviation (%)	Mean (%)	Std. Deviation (%)
Methane	56	6.5	57	4.7
Carbon Dioxide	29	7.0	32	3.9

t-Test: Two-Sample Assuming Unequal Variances

Digester Gas Methane Content	Condition	
Parameter	*Control*	*Shear Enhanced*
Mean	51	56
Variance	168	20
Observations	9	10
Hypothesized Mean Difference	0	
df	10	
t Stat	-1.07	
P(T<=t) one-tail	0.16	
t Critical one-tail	1.81	
P(T<=t) two-tail	0.31	
t Critical two-tail	2.23	

t-Test: Two-Sample Assuming Unequal Variances

Digester Gas Carbon Dioxide Content	Condition	
Parameter	*Control*	*Shear Enhanced*
Mean	27.3	31.4
Variance	49.1	17.6
Observations	9	10
Hypothesized Mean Difference	0	
df	13	
t Stat	-1.53	
P(T<=t) one-tail	0.07	
t Critical one-tail	1.77	
P(T<=t) two-tail	0.15	
t Critical two-tail	2.16	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Figure 5 (page 79)

Figure 5 Total Solids Concentration in the Primary and Secondary Digesters Under Two Different Shear Regimes

		Primary Digester		Secondary Digester	
		Total Solids	Standard Deviation	Total Solids	Standard Deviation
	Date	%	%	%	%
Baseline/Conventional Condition	10/18/01	0.991	0.014	2.183	0.008
	10/23/01	0.948	0.013	2.683	0.017
	10/25/01	0.940	0.007	2.570	0.007
	10/30/01	1.110	0.001	2.665	0.235
	11/06/01	1.506	0.005	2.165	0.009
	11/08/01	1.506	0.005	2.165	0.009
	11/15/01	1.458	0.004	2.594	0.003
	11/20/01	1.308	0.007	1.990	0.008
	11/29/01	1.308	0.007	1.990	0.008
	12/04/01	1.447	0.272	2.277	0.198
	12/06/01	1.128	0.008	2.140	0.016
Shear Enhanced Condition	01/05/02	1.208	0.006	1.876	0.036
	01/10/02	1.071	0.005	1.801	0.010
	01/18/02	0.643	0.007	1.876	0.014
	01/25/02	0.834	0.005	1.922	0.003
	02/01/02	0.981	0.005	1.948	0.004
	02/05/02	0.909	0.012	2.042	0.004
	02/12/02	1.156	0.179	2.063	0.137

t-Test: Two-Sample Assuming Unequal Variances
Primary Digester Total Solids Concentration

Parameter	*Baseline*	*Shear Enhanced*
Mean	1.24	0.97
Variance	0.05	0.04
Observations	11	7
Hypothesized Mean Difference	0	
df	14	
t Stat	2.68	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.76	
P(T<=t) two-tail	0.02	
t Critical two-tail	2.14	

t-Test: Two-Sample Assuming Unequal Variances
Secondary Digester Total Solids Concentration

Parameter	*Control*	*Shear Enhanced*
Mean	2.31	1.93
Variance	0.07	0.01
Observations	11	7
Hypothesized Mean Difference	0	
df	13	
t Stat	4.32	
P(T<=t) one-tail	0.0004	
t Critical one-tail	1.77	
P(T<=t) two-tail	0.0008	
t Critical two-tail	2.16	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Figure 6 (page 80)

Figure 6 Volatile Solids Concentration in the Primary and Secondary Digesters Under Two Different Shear Regimes

		Primary Digester		Secondary Digester	
Operation	Date	Volatile Solids %	Standard Deviation %	Volatile Solids %	Standard Deviation %
Baseline/Conventional Condition	10/18/01	0.708	0.014	1.455	0.014
	10/23/01	0.672	0.007	1.782	0.007
	10/25/01	0.658	0.006	1.703	0.006
	10/30/01	0.796	0.234	1.811	0.234
	11/06/01	1.087	0.007	1.451	0.007
	11/08/01	1.087	0.007	1.451	0.007
	11/15/01	1.057	0.004	1.757	0.004
	11/20/01	0.953	0.028	1.382	0.028
	11/29/01	0.953	0.028	1.382	0.028
	12/04/01	1.140	0.198	1.606	0.198
	12/06/01	0.842	0.013	1.498	0.013
Shear Enhanced Condition	01/05/02	0.908	0.022	1.339	0.022
	01/10/02	0.793	0.008	1.278	0.008
	01/18/02	0.499	0.008	1.347	0.008
	01/25/02	0.605	0.002	1.371	0.002
	02/01/02	0.695	0.070	1.425	0.070
	02/05/02	0.639	0.001	1.459	0.001
	02/12/02	0.857	1.825	1.573	0.079

t-Test: Two-Sample Assuming Unequal Variances

Primary Digester Volatile Solids Concentration

Parameter	*Baseline*	*Shear Enhanced*
Mean	0.905	0.714
Variance	0.032	0.021
Observations	11	7
Hypothesized Mean Difference	0	
df	15	
t Stat	2.48	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.75	
P(T<=t) two-tail	0.03	
t Critical two-tail	2.13	

t-Test: Two-Sample Assuming Unequal Variances

Secondary Digester Volatile Solids Concentration

Parameter	*Control*	*Shear Enhanced*
Mean	1.57	1.40
Variance	0.03	0.01
Observations	11	7
Hypothesized Mean Difference	0	
df	16	
t Stat	2.77	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.75	
P(T<=t) two-tail	0.01	
t Critical two-tail	2.12	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Figure 7 (page 81)

Figure 7 The Effect of Mechanical Shear on Colloid COD Content (<1.5 μm-0.2 μm) of a Primary and Secondary Digester

		Primary Digester	Volatile		Secondary Digester	Volatile		KADY BLS	Volatile	
		COD 1.5μm-	Solids	COD/VS	COD	Solids	COD/VS	COD	Solids	COD/VS
	Date	0.2μm	%	mg-COD/g-VS	1.5μm-0.2μm	%	mg-COD/g-VS	1.5μm-0.2μm	%	mg-COD/g-VS
BASELINE	10/18	179.1	0.71	25.3	370.0	2.18	16.9			
	10/23	141.0	0.67	21.0	218.9	2.68	8.2			
	10/25	174.5	0.66	26.5	377.7	2.57	14.7			
	10/30	121.2	0.80	15.2	160.3	2.67	6.0			
	11/6	178.8	1.09	16.5	253.6	2.17	11.7			
	11/8	201.8	1.09	18.6	281.6	2.17	13.0			
	11/15	208.0	1.06	19.7	196.0	2.59	7.6			
	11/20	230.7	0.95	24.2	191.6	1.99	9.6			
	11/29	117.8	0.95	12.4	184.1	1.99	9.2			
	12/4	173.8	1.14	15.2	171.4	2.28	7.5	282.4	1.14	24.8
	12/6	163.5	0.84	19.4	150.3	2.14	7.0	210.1	0.84	24.9
	12/11	226.1	1.10	20.5	182.1	0.94	19.4	270.3	1.10	24.5
	1/5	273.0	0.91	30.1	121.2	1.88	6.5	374.0	0.91	41.2
Shear Enhanced	1/18	248.9	0.50	49.9	132.9	1.88	7.1	296.5	0.50	59.4
	1/25	228.1	0.60	37.7	141.0	1.92	7.3	336.2	0.60	55.6
	2/1	247.3	0.69	35.6	122.2	1.42	8.6	322.3	0.69	46.4
	2/5	212.6	0.64	33.2	132.1	1.459	9.1	241.2	0.64	37.7
	2/12	245.6	0.86	28.6	121.6	1.6	7.7			
	2/19	218.5	0.67	32.5				491.4	0.67	73.0
	2/26	262.7	0.67	39.2	108.2	0.73	14.9	613.4	0.67	91.5
	3/5	274.7	0.63	43.9	84.5	0.79	10.7	544.0	0.63	87.0

Colloidal COD	Baseline Condition		Shear Enhanced Condition	
	Mean	**St. Deviation**	**Mean**	**St. Deviation**
	mg-COD/g-VS	mg-COD/g-VS	mg-COD/g-VS	mg-COD/g-VS
Primary Digester	19.9	4.8	36.8	6.8
Secondary Digester	10.8	3.6	9.0	2.7
KADY BLS^{tm}			61.5	20.5

Data Associated with Figure 8 (page 82)

Figure 8 Mean Solution Ammonium-N Concentrations of the Digestion Systems under Normal and Enhanced Digestion Operation

Operation	Date	Primary Digester mg-NH4-N/L	Secondary Digester mg-NH4-N/L	KADY BLS^{tm} mg-NH4-N/L
Baseline Condition	10/23/01	301.4	301.4	
	10/25/01	312.7	312.7	
	10/30/01	232.2	232.2	
	11/06/01	314.6	314.6	
	11/08/01	232.8	232.8	
	11/15/01	304.8	304.8	
	11/20/01	293.2	293.2	
	~~12/04/01~~	~~222.5~~	~~222.5~~	~~230.7~~
	~~12/06/01~~	~~356.0~~	~~356.0~~	~~292.7~~
	~~12/11/01~~	~~227.0~~	~~227.0~~	~~231.8~~
	~~01/05/02~~	~~327.7~~	~~327.7~~	~~318.3~~
Shear Enhanced Condition	01/10/02	372.9	372.9	345.6
	01/18/02	353.8	353.8	264.8
	01/25/02	265.5	265.5	221.0
	01/31/02	461.6	461.6	310.2
	02/05/02	357.2	357.2	311.7
	02/12/02	307.4	307.4	208.9
	02/19/02	77.4	77.4	333.6
	02/26/02	389.9	389.9	347.8
	03/05/02	184.1	184.1	388.8

t-Test: Two-Sample Assuming Unequal Variances

Primary Digester Ammonium-N Concentrations

Parameter	*Conventional*	*Shear Condition*
Mean	225.6	301.6
Variance	3348.3	4050.1
Observations	7.0	9.0
Hypothesized Mean Difference	0.0	
df	14.0	
t Stat	-2.5	
P(T<=t) one-tail	0.013	
t Critical one-tail	1.8	
P(T<=t) two-tail	0.0257	
t Critical two-tail	2.1	

t-Test: Two-Sample Assuming Unequal Variances

Secondary Digester Ammonium-N Concentration

Parameter	*Conventional*	*Shear Condition*
Mean	284.5	330.9
Variance	1313.9	6879.8
Observations	7.0	8.0
Hypothesized Mean Difference	0.0	
df	10.0	
t Stat	-1.4	
P(T<=t) one-tail	0.1	
t Critical one-tail	1.8	
P(T<=t) two-tail	0.2	
t Critical two-tail	2.2	

t-Test: Two-Sample Assuming Unequal Variances

Primary Digester Shear Enhanced vs. KADY BLS vessel

Parameter	*Primary Digester Shear Enhanced*	*KADY BLS*
Mean	302	301
Variance	4050	3458
Observations	9	9
Hypothesized Mean Difference	0	
df	16	
t Stat	0.03	
P(T<=t) one-tail	0.49	
t Critical one-tail	1.75	
P(T<=t) two-tail	0.97	
t Critical two-tail	2.12	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Figure 9 (page 83)

Figure 9: The Change in Optimum Polymer Dose Associated with the Operation of an Internal Recycle Shear Device

	Baseline			Shear Enhanced	
	Primary Digester	Secondary Digester		Primary Digester	Secondary Digester
Date	g-Polymer/Kg-TS	g-Polymer/Kg-TS	Date	g-Polymer/Kg-TS	g-Polymer/Kg-TS
10/23/01	13.3	8.9	1/5/02	10.9	6.1
10/30/01	7.0	5.2	1/10/02	9.3	5.0
11/6/01	5.6	6.1	1/18/02	14.0	4.8
11/8/01	5.6	6.1	1/25/02	10.8	4.7
11/15/01	4.5	5.6	2/1/02	10.4	5.9
11/20/01	7.3	7.2	2/5/02		4.7
11/29/01	5.0	6.0	2/12/02	8.8	4.9
12/4/01	5.0	5.3	2/19/02	9.9	
12/6/01	6.4	6.2	2/26/02	10.7	6.2
12/11/01	6.2	6.4	3/5/02	12.2	5.3

Optimum Polymer Dose	Basline Condition		Shear Enhanced Condtion	
	Mean	Std.Deviation	Mean	Std.Deviation
Sample	g-Polymer/Kg-TS	g-Polymer/Kg-TS	g-Polymer/Kg-TS	g-Polymer/Kg-TS
Primary Digester	5.85	1.11	10.79	1.54
Secondary Digester	6.03	0.69	5.29	0.61

t-Test: Two-Sample Assuming Unequal Variances

Primary Digester Polymer Demand

Parameter	*Baseline*	*Shear Enhanced*
Mean	6.9	10.8
Variance	8.9	2.4
Observations	7	9
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.12	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.01	
t Critical two-tail	2.31	

t-Test: Two-Sample Assuming Unequal Variances
Secondary Digester

Parameter	*Baseline*	*Shear Enhanced*
Mean	6.45	5.29
Variance	1.62	0.38
Observations	7	9
Hypothesized Mean Difference	0	
df	8	
t Stat	2.22	
P(T<=t) one-tail	0.03	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.06	
t Critical two-tail	2.31	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Table 1 (page 84)

Table 1: The Reduction in Total and Fecal Coliforms during Mesophilic Digestion with and without Mechanical Shear Operation

	Feed Solids		Primary Digester		Log Reduction	
	Total Coliforms	Fecal Coliforms	Total Coliforms	Fecal Coliforms	Total Coliforms	Fecal Coliforms
Date	MPN	MPN	MPN	MPN		
10/18/01			8.00E+06	4.00E+06		
10/23/01	3.00E+08	1.00E+08	1.40E+08	1.20E+07	0.3	0.9
10/25/01	3.00E+08	5.00E+07	1.40E+08	2.00E+06	0.3	1.4
10/30/01	3.00E+09	8.00E+06	3.00E+07	2.00E+06	2.0	0.6
11/06/01	2.30E+09	2.00E+08	3.00E+07	2.00E+06	1.9	2.0
11/13/01	5.00E+09	4.00E+08	5.00E+07	2.00E+06	2.0	2.3
11/15/01	1.10E+09	2.00E+08	1.10E+07	2.00E+06	2.0	2.0
12/04/01	1.10E+09	2.00E+08	2.00E+08	8.00E+06	0.7	1.4
12/06/01	2.00E+08	2.20E+07	1.30E+07	2.00E+06	1.2	1.0
12/11/01	1.70E+09	1.10E+07	1.30E+07	8.00E+05	2.1	1.1
01/05/02	7.00E+09	2.00E+08	2.00E+08	4.00E+06	1.5	1.7
01/10/02	3.00E+09	1.10E+08	2.00E+08	2.00E+06	1.2	1.7
01/18/02	3.00E+09	3.00E+08	2.00E+08	4.00E+06	1.2	1.9
01/25/02	2.30E+09	2.00E+08	3.00E+07	2.00E+06	1.9	2.0
02/01/02	7.00E+08	8.00E+07	2.30E+07		1.5	
02/05/02	2.30E+09	4.00E+08	7.00E+08	4.00E+06	0.5	2.0
02/12/02	8.00E+09	8.00E+08	2.00E+08	1.30E+07	1.6	1.8
02/19/02	2.30E+09	2.00E+08	2.00E+08	7.00E+06	1.1	1.5
02/26/02	1.30E+09	0.00E+00	3.00E+07		1.6	
03/05/02	2.30E+09	2.00E+08	1.10E+07	8.00E+05	2.3	2.4

t-Test: Two-Sample Assuming Unequal Variances

Total Coliform Log Reductions

Parameter	*Control*	*Shear Enhanced*
Mean	1.42	1.44
Variance	0.72	0.24
Observations	6	10
Hypothesized Mean Difference	0	
df	7	
t Stat	-0.04	
P(T<=t) one-tail	0.48	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.97	
t Critical two-tail	2.36	

t-Test: Two-Sample Assuming Unequal Variances
Fecal Coliform Log Reductions

Parameter	*Control*	*Shear Enhanced*
Mean	1.5	1.9
Variance	0.5	0.1
Observations	6	8
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.14	
P(T<=t) one-tail	0.15	
t Critical one-tail	1.94	
P(T<=t) two-tail	0.30	
t Critical two-tail	2.45	

Note: Statitistical analysis was conducted on the measured biogas component concentration for during two disintinct phases of operation. The observations value (n) is equivalent to the sum of the measurements collected for that particular parameter for the duration of that specific phase of digester operation.

Data Associated with Table 2 (page 85)

Table 2: Mean Volatile Fatty Acid Composition with and without Shear Enhancement

Baseline	Acetic Acid mg/L	Propionic Acid mg/L	Butyric Acid mg/L	Valeric Acid mg/L
Feed Solids	276	163	28	N/D**
Primary Digester	16	N/D*	N/D	N/D
Secondary Digester	28.7	N/D	N/D	N/D
Shear Enhanced				
Feed Solids	364	238	161	54
Primary Digester	16	N/D*	N/D*	N/D
Secondary Digester	14	N/D*	N/D	N/D

Feed Solids				
Date	Acetic Acid mg/L	Propionic Acid mg/L	Butyric Acid mg/L	Valeric Acid mg/L
10/23/01				
10/25/01	285	218	54	N/D
10/30/01	320	204	6	N/D
11/06/01	207	98	-21	N/D
11/08/01	267	138	8	N/D
11/15/01	254	144	10	N/D
11/20/01	198	120	37	N/D
12/04/01	330	183	55	28
12/06/01	337	188	59	33
12/11/01	282	170	42	N/D
01/05/02	366	235	140	45
01/10/02	343	204	120	41
01/18/02	247	189	76	N/D
01/25/02	251	257	61	N/D
01/31/02	163	145	27	N/D
02/06/02	294	12	71	N/D
02/13/02	283	256	92	25
02/20/02	473	358	186	51
02/26/02	559	337	326	65
03/06/02	656	382	515	96

Primary Digester				
Date	Acetic mg/L	Propionic mg/L	Butyric mg/L	Valeric mg/L
10/23/01				
10/25/01	19	N/D	N/D	N/D
10/30/01	44	N/D	N/D	N/D
11/06/01	13	N/D	N/D	N/D
11/08/01	14	N/D	N/D	N/D
11/15/01	10	N/D	N/D	N/D
11/20/01	16	N/D	N/D	N/D
12/04/01	13	N/D	N/D	N/D
12/06/01	8	N/D	N/D	N/D
12/11/01	6	N/D	N/D	N/D
01/05/02	18	N/D	N/D	N/D
01/10/02	23	N/D	N/D	N/D
01/18/02	35	15	N/D	N/D
01/25/02	11	N/D	N/D	N/D
01/31/02	19	N/D	N/D	N/D
02/06/02	12	N/D	N/D	N/D
02/13/02	22	N/D	N/D	N/D
02/20/02	1	N/D	N/D	N/D
02/26/02	3	N/D	N/D	N/D
03/06/02	14	N/D	17.5083	N/D

Date	Secondary Digester Acetic Acid mg/L	Propionic Acid mg/L	Butyric Acid mg/L	Valeric Acid mg/L
10/23/01				
10/25/01	20.5	N/D	N/D	N/D
10/30/01	24.1	N/D	N/D	N/D
11/06/01	16.3	N/D	N/D	N/D
11/08/01	18.4	N/D	N/D	N/D
11/15/01	45	N/D	N/D	N/D
11/20/01	43	N/D	N/D	N/D
12/04/01	5	N/D	N/D	N/D
12/06/01	47	N/D	N/D	N/D
12/11/01	39	N/D	N/D	N/D
01/05/02	41	N/D	N/D	N/D
01/10/02	5	N/D	N/D	N/D
01/18/02	11	N/D	N/D	N/D
01/25/02	18	N/D	N/D	N/D
01/31/02	16	N/D	N/D	N/D
02/06/02	16	N/D	N/D	N/D
02/13/02	16	N/D	N/D	N/D
02/20/02	15	17.3156	N/D	N/D
02/26/02	-2	N/D	N/D	N/D
03/06/02	4	N/D	N/D	N/D

	BLS[tm]			
Date	Acetic mg/L	Proprionic mg/L	Butyric mg/L	Valeric mg/L
10/23/01				
10/25/01				
10/30/01				
11/06/01				
11/08/01				
11/15/01				
11/20/01				
12/04/01	18	N/D	N/D	N/D
12/06/01	10	N/D	N/D	N/D
12/11/01	11	N/D	N/D	N/D
01/05/02	22	N/D	N/D	N/D
01/10/02	-5	N/D	N/D	N/D
01/18/02	31	15	N/D	N/D
01/25/02	23	N/D	N/D	N/D
01/31/02	18	N/D	N/D	N/D
02/06/02	25	N/D	N/D	N/D
02/13/02	18	N/D	N/D	N/D
02/20/02	-1	N/D	N/D	N/D
02/26/02	14	N/D	N/D	N/D
03/06/02	-2	N/D	N/D	N/D

APPENDIX B: Data Associated Figures and Tables in Chapter 3

Data Associated with Table 1 (page 115)

Table 1: Summary of Sonication Application in Mesophilic Digesters

Equation Used to Calculate Ultrasonic Dose

Ultrasonic Dose = $\Sigma_{n(1->x)}$ [((P_{max}*U_t)*t_{exp})/V]

n= number of exposure events
x = number of exposures to achieve the desired dose
P_{max} = maximum power of ultrasonic unit (Dukane Horn w/ DPC-1 = 1000 W)
U_t = percent total power utilized (read from display on unit)
t_{exp} = time of exposure
V = volume of sample (2500 ml)

Procedure:

The ultrasonic power was applied as described in the materials and method. During the application of ultrasonic energy the percent total power was monitored along with the application time. During Phase 1 the ultrasonic dose was applied for at set time. After observing a reduction in power input with time the power input in time were monitored more carefully, during Phase 2. Any fluctuation in power input was recorded and the time at a higher or lower input level was recorded an the total exposure time was changed to account for the change in power to give a static dose.

Daily Ultrasonic Energy Input to Digesters

Digester				Digester			
	Pretreatment	**Recycle1**	**Recycle 2**		**Pretreatment**	**Recycle1**	**Recycle 2**
Date	*W-min/mL*	*W-min/mL*	*W-min/mL*	Date	*W-min/mL*	*W-min/mL*	*W-min/mL*
7/15/2004	2.88	1.92	0.96	8/10/2004	2.88	1.44	1.44
7/16/2004	2.88	1.92	0.96	8/11/2004	2.88	1.44	1.44
7/17/2004	2.88	1.92	1.92	8/12/2004	2.88	2.88	1.44
7/18/2004	2.88	1.92	1.92	8/13/2004	2.88	2.4	2.16
7/19/2004	2.88	1.92	0.96	8/14/2004	2.88	1.44	1.44
7/20/2004	2.88	1.92	1.92	8/15/2004	2.88	1.44	1.44
7/21/2004	2.88	1.92	0.96	8/16/2004	2.88	1.44	1.44
7/23/2004	2.88	1.92	1.92	8/17/2004	2.88	1.44	1.44
7/24/2004	2.88	2.88	2.88	8/18/2004	2.88	1.44	2.04
7/26/2004	2.88	2.88	2.88	8/19/2004	2.88	1.44	2.16
7/27/2004	2.88	2.88	1.44	8/20/2004	2.88	2.52	1.44
7/28/2004	2.88	2.88	2.88	8/21/2004	2.88	2.16	2.76
7/29/2004	2.88	2.88	2.88	8/22/2004	2.88	1.44	2.04
7/30/2004	2.88	1.44	1.44	8/23/2004	2.88	1.56	1.44
7/31/2004	2.88	2.88	2.88	8/24/2004	2.88	1.44	2.64
8/1/2004	2.88	1.44	1.44	8/25/2004	2.88	1.44	1.44
8/2/2004	2.88	2.88	2.88	8/26/2004	2.88	1.44	1.44
8/3/2004	2.88	2.88	2.88	8/27/2004	2.88	1.44	1.44
8/4/2004	2.88	1.44	1.44	8/28/2004	2.76	2.88	2.88
8/5/2004	2.88	1.44	1.44	8/29/2004	2.88	1.44	1.44
8/6/2004	2.88	2.88	1.44	8/30/2004	2.88	2.4	1.44
8/7/2004	2.88	1.44	1.44	8/31/2004	2.88	1.44	1.44
8/8/2004	2.88	2.88	1.44	9/1/2004	2.88	1.44	1.44
8/9/2004	2.88	1.44	1.44	9/2/2004	2.88	1.44	1.92

Daily Ultrasonic Energy Input to Digesters (continued)

Digester				Digester			
Date	**Pretreatment** *W-min/mL*	**Recycle1** *W-min/mL*	**Recycle 2** *W-min/mL*	*Date*	**Pretreatment** *W-min/mL*	**Recycle1** *W-min/mL*	**Recycle 2** *W-min/mL*
9/3/2004	2.76	1.44	1.44	9/27/2004	2.88	2.28	2.88
9/4/2004	2.64	1.44	1.44	9/28/2004	2.88	1.8	1.44
9/5/2004	2.88	1.44	1.44	9/29/2004	2.880048	1.44	1.44
9/6/2004	2.64	1.44	1.44	9/30/2004	2.879952	1.44	1.44
9/7/2004	2.88	1.44	1.44	10/1/2004	2.88	1.44	1.5
9/8/2004	2.64	1.44	2.28	10/2/2004	2.88	1.44	2.16
9/9/2004	2.64	1.44	1.44	10/3/2004	2.88	2.28	2.4
9/10/2004	2.16	1.44	1.44	10/4/2004	2.88	1.44	2.4
9/11/2004	2.16	1.44	1.44	10/5/2004	2.88	1.44	2.76
9/12/2004	1.92	1.44	1.44	10/6/2004	2.88	1.44	2.04
9/13/2004	2.16	1.44	1.44	10/7/2004	2.88	2.04	1.92
9/14/2004	2.88	1.44	1.44	10/8/2004	3.12	1.44	1.92
9/15/2004	1.92	1.56	1.44	10/9/2004	2.88	1.56	2.4
9/16/2004	2.88	1.44	1.44	10/10/2004	2.880048	1.44	2.04
9/17/2004	2.95152	1.44	1.44	10/11/2004	2.88	1.44	2.64
9/18/2004	2.88	1.44	1.44	10/12/2004	2.88	1.92	2.52
9/19/2004	2.88	1.44	1.44	10/13/2004	2.88	1.92	2.88
9/20/2004	2.856	1.44	2.64	10/14/2004	2.88	1.92	2.4
9/21/2004	2.88	1.56	2.88	10/15/2004	2.88	1.44	2.88
9/22/2004	2.880048	1.44	1.92	10/16/2004	2.88	0	2.88
9/23/2004	2.880048	1.44	1.44	10/17/2004	2.88	0	2.88
9/24/2004	2.800032	2.88	2.64	10/18/2004	2.880005	0	2.88
9/25/2004	2.88	1.44	1.44	10/19/2004	2.879952	0	2.88
9/26/2004	2.88	1.44	1.44	10/20/2004	2.88	0	2.88

Daily Ultrasonic Energy Input to Digesters (continued)

Digester

Date	**Pretreatment** *W-min/mL*	**Recycle1** *W-min/mL*	**Recycle 2** *W-min/mL*
10/21/2004	2.880048	0	2.880024
10/22/2004	2.88	0	2.88
10/23/2004	2.88	0	2.88
10/24/2004	2.88	0	2.88
10/25/2004	2.8799616	0	2.88
10/26/2004	2.879904	0	2.85
10/27/2004	2.88	0	2.88
10/28/2004	2.88	0	2.88
10/29/2004	2.88	0	3.1200024
10/30/2004	2.88	0	2.88
10/31/2004	2.88	0	2.85
11/1/2004	2.859996	0	2.88
11/2/2004	2.879904	0	2.88
11/3/2004	2.88	0	2.88
11/4/2004	2.88	0	2.82

Data Associated with Table 2 (page 115)

Table 2: A Comparison of Ultrasonic Dosage used to Enhance Anaerobic Digestion

All calculated data is from Table 1. All other values obtained from the literature.

Data Associated with Table 1 (page 116)
Table 3: Summary of Sonication Application in Mesophilic Digesters
Measured Biogas Composition

	Biogas Composition				
	Control		**Pretreatment**		
Date	***Methane***	***Carbon Dioxide***	***Methane***	***Carbon Dioxide***	
	%	***%***	***%***	***%***	
07/21/04	66.4	28.4	68.27	30.40	
07/24/04	70.0	29.1	72.3	30.2	
07/26/04	69.0	28.5	71.4	29.9	
07/29/04	-0.6	0.7	67.7	31.4	START UP
08/02/04	0.5	-0.1	0.47	-0.09	
08/05/04	57.6	33.0	67.3	34.3	
08/08/04	69.0	28.5	6.0	41.6	
08/11/04	69.3	35.6	70.4	35.9	
08/14/04	66.1	36.1	68.7	38.2	
08/18/04	65.0	33.0	64.7	31.9	
08/21/04	64.1	32.3			
08/24/04	67.5	31.5	67.9	32.6	
08/28/04	63.2	29.7	64.9	30.9	
08/31/04	75.1	37.7	75.0	37.4	
09/03/04	104.8	55.8	42.4	49.3	
09/07/04	78.2	29.1	73.5	31.4	
09/10/04	49.3	28.1	48.1	28.9	
09/13/04	49.7	24.3	61.2	30.2	PHASE 1
09/16/04	59.9	27.2	73.4	33.5	
09/20/04	59.1	27.4	6.1	30.8	
09/23/04	60.7	28.3	66.2	30.8	
09/26/04	65.4	28.0	49.6	21.5	
10/01/04	40.2	19.0	52.0	24.2	
10/04/04	63.5	30.6	68.3	34.0	
10/08/04	66.4	32.1	58.4	27.7	
10/18/04	79.4	50.6	59.6	33.3	
10/25/04	27.204	11.451	37.050	18.004	
11/01/04	56.626	23.717	50.935	22.623	
11/03/04	5.649	23.070	52.104	28.373	
11/09/04	63.442	33.706	77.240	41.414	
11/17/04	66.562	29.890	66.936	30.171	PHASE 2
11/20/04	50.698	26.113	52.770	27.519	
11/23/04	60.037	26.619			
11/30/04	53.274	29.524	51.306	28.808	
12/11/04	65.360	28.884	62.562	28.073	
12/17/04	102.503	39.821	80.852	32.828	

Measured Biogas Composition

Biogas Composition					
Date	**Recycle 1** ***Methane*** *%*	***Carbon Dioxide*** *%*	**Recycle 2** ***Methane*** *%*	***Carbon Dioxide*** *%*	
07/21/04	67.8	27.9	66.5	29.5	START UP
07/24/04	68.5	28.4	70.7	29.8	
07/26/04	27.3	20.8	70.6	29.5	
07/29/04	58.7	26.8	66.6	30.4	
08/02/04	0.5	-0.1	0.5	-0.1	
08/05/04	64.7	33.1	63.3	32.6	
08/08/04	57.8	39.9	58.0	40.7	
08/11/04	69.5	35.1	70.3	35.7	
08/14/04	69.7	37.8	67.1	36.8	
08/18/04	64.9	31.4	64.4	31.5	PHASE 1
08/21/04	59.3	29.7	62.6	31.2	
08/24/04	67.4	31.3	68.0	31.8	
08/28/04	63.8	29.6	63.9	29.8	
08/31/04	67.9	34.8	71.7	36.1	
09/03/04	44.9	50.8	63.9	70.7	
09/07/04	64.8	25.1	81.5	31.8	
09/10/04	52.6	30.3	47.8	28.1	
09/13/04	41.6	20.5	67.6	32.2	
09/16/04	69.9	31.2	66.1	29.8	
09/20/04	66.6	30.8	65.6	30.7	
09/23/04	65.5	29.9	50.5	22.1	
09/26/04	43.2	19.7	58.3	22.9	
10/01/04	40.0	18.0	53.7	24.1	
10/04/04	61.5	29.3	64.7	31.1	
10/08/04	59.0	28.1	67.8	32.7	
10/18/04	61.3	33.3	66.9	38.7	
10/25/04	38.708	18.209	54.145	-1.472	PHASE 2
11/01/04	60.261	24.961	70.769	29.265	
11/03/04	54.901	29.161	45.576	24.414	
11/09/04	60.898	32.710	60.910	33.884	
11/17/04	49.559	2.970	63.294	29.877	
11/20/04	56.373	30.256	55.341	29.661	
11/23/04	50.327	22.669	61.726	27.843	
11/30/04	49.861	27.508	56.993	32.171	
12/11/04	66.002	29.163	64.075	28.563	
12/17/04	76.184	31.507	78.632	31.975	

Ratio of Methane to Carbon Dioxide in Digester Headpace

Methane/Carbon Dioxide

Date	**Control**	**Pretreatment**	**Recycle 1**	**Recycle 2**	
	CH_4/CO_2	CH_4/CO_2	CH_4/CO_2	CH_4/CO_2	
07/21/04	2.34	2.25	2.4	2.3	
07/24/04	2.40	2.40	2.4	2.4	
07/26/04	2.42	2.38	1.3	2.4	
07/29/04	-0.83	2.15	2.2	2.2	START UP
08/02/04	-5.25	-5.25	-5.3	-5.3	
08/05/04	1.74	1.96	2.0	1.9	
08/08/04	2.42	0.15	1.5	1.4	
08/11/04	1.95	1.96	2.0	2.0	
08/14/04	1.83	1.80	1.8	1.8	
08/18/04	1.97	2.03	2.1	2.0	
08/21/04	1.98		2.0	2.0	
08/24/04	2.14	2.08	2.2	2.1	
08/28/04	2.13	2.10	2.2	2.1	
08/31/04	1.99	2.00	2.0	2.0	
09/03/04					
09/07/04	2.69	2.34	2.6	2.6	
09/10/04	1.76	1.66	1.7	1.7	
09/13/04	2.05	2.02	2.0	2.1	PHASE 1
09/16/04	2.20	2.19	2.2	2.2	
09/20/04	2.16		2.2	2.1	
09/23/04	2.15	2.15	2.2	2.3	
09/26/04	2.33	2.30	2.2	2.5	
10/01/04	2.11	2.15	2.2	2.2	
10/04/04	2.08	2.01	2.1	2.1	
10/08/04	2.07	2.11	2.1	2.1	
10/18/04	1.57	1.79	1.8	1.7	
10/25/04	2.38	2.06	2.1		
11/01/04	2.39	2.25	2.4	2.4	
11/03/04		1.84	1.9	1.9	
11/09/04	1.88	1.87	1.9	1.8	
11/17/04	2.23	2.22		2.1	PHASE 2
11/20/04	1.94	1.92	1.9	1.9	
11/23/04	2.26		2.2	2.2	
11/30/04	1.80	1.78	1.8	1.8	
12/11/04	2.26	2.23	2.3	2.2	
12/17/04	2.57	2.46	2.4	2.5	

Statistical Analysis of Methane and Carbon Dioxide Ratios Associated with Table 3

t-Test: Paired Two Sample for Means
Phase 1-Pretreatment

	Control	*Pretreatment*
Mean	2.09	2.07
Variance	0.07	0.03
Observations	14	14
Pearson Correlation	0.91	
Hypothesized Mean Difference	0	
df	13	
t Stat	0.66	
P(T<=t) one-tail	0.26	
t Critical one-tail	1.77	
P(T<=t) two-tail	0.52	
t Critical two-tail	2.16	

t-Test: Paired Two Sample for Means
Phase 2- Pretreatment

	Control	*Pretreatment*
Mean	2.18	2.10
Variance	0.08	0.05
Observations	8	8
Pearson Correlation	0.93	
Hypothesized Mean Difference	0	
df	7	
t Stat	2.25	
P(T<=t) one-tail	0.03	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.06	
t Critical two-tail	2.36	

t-Test: Paired Two Sample for Means
Phase 1- Recycle 1

	Control	*Recycle 1*
Mean	2.09	2.11
Variance	0.06	0.04
Observations	16	16
Pearson Correlation	0.93	
Hypothesized Mean Difference	0	
df	15	
t Stat	-0.931	
P(T<=t) one-tail	0.183	
t Critical one-tail	1.753	
P(T<=t) two-tail	0.367	
t Critical two-tail	2.131	

t-Test: Paired Two Sample for Means
Phase-2 Recycle 1

	Control	*Recycle 1*
Mean	2.19	2.12
Variance	0.08	0.06
Observations	8	8
Pearson Correlation	0.94	
Hypothesized Mean Difference	0	
df	7	
t Stat	1.87	
P(T<=t) one-tail	0.05	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.10	
t Critical two-tail	2.36	

t-Test: Paired Two Sample for Means
Phase 1- Recycle 2

	Control	*Recycle 2*
Mean	2.09	2.12
Variance	0.06	0.05
Observations	16	16
Pearson Correlation	0.94	
Hypothesized Mean Difference	0	
df	15	
t Stat	-1.78	
P(T<=t) one-tail	0.05	
t Critical one-tail	1.75	
P(T<=t) two-tail	0.09	
t Critical two-tail	2.13	

t-Test: Paired Two Sample for Means
Phase 2- Recycle 2

	Control	*Recycle 2*
Mean	2.17	2.11
Variance	0.07	0.07
Observations	8	8
Pearson Correlation	0.98	
Hypothesized Mean Difference	0	
df	7	
t Stat	3.17	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.89	
P(T<=t) two-tail	0.02	
t Critical two-tail	2.36	

Data Associated with Table 4 (page 116)

Table 4: Summary of Peak Organo-Sulfur Odors Generated under a Static Headspace from Dewatered Biosolids

Headspace VOSC Generation: SET 1

Date	Control 1 *(mg-OS/m3)/kg-VS*	Control 2 *(mg-OS/m3)/kg-VS*	Pretreatment1 *(mg-OS/m3)/kg-VS*	Pretreatment 2 *(mg-OS/m3)/kg-VS*	Recycle 1-1 *(mg-OS/m3)/kg-VS*	Recycle 1-2 *(mg-OS/m3)/kg-VS*
9/10/2004	0	0	0	0	0	0
9/11/2004	2968.4	3163.7	154.67	149.49	1492.9	1809.6
9/13/2004	417.7	360.6	234.82	247.47	277.1	288.0
9/15/2004	382.6	354.8	281.88	197.29	275.9	165.0

Headspace VOSC Generation: SET 2

Date	Control *(mg-OS/m3)/kg-VS*	Pretreatment *(mg-OS/m3)/kg-VS*	Recycle 1 *(mg-OS/m3)/kg-VS*	Recycle 2 *(mg-OS/m3)/kg-VS*
9/22/2004	0	0	0	0
9/23/2004	4239.6	1046.7	2667.3	5890.2
9/24/2004	15919.3	10112.4	11810.2	3647.1
9/25/2004	625.4	200.1	248.0	127.6
9/27/2004	529.9	476.4	360.1	299.7

Headspace VOSC Generation: SET 3

Date	Control *(mg-OS/m3)/kg-VS*	Pretreatment *(mg-OS/m3)/kg-VS*	Recycle 1 *(mg-OS/m3)/kg-VS*	Recycle 2 *(mg-OS/m3)/kg-VS*
10/9/2004	0	0	0	0
10/10/2004	2291.6	1208.2	2272.7	3990.8
10/11/2004	3927.8	3694.5	514.6	1805.0
10/12/2004	376.5	168.0	155.7	48.8

Headspace VOSC Generation: SET 3

Date	Control *(mg-OS/m3)/kg-VS*	Pretreatment *(mg-OS/m3)/kg-VS*	Recycle-1 *(mg-OS/m3)/kg-VS*	Recycle 2 *(mg-OS/m3)/kg-VS*
11/23/2004	0	0	0	0
11/24/2004	0	1	0	3464
11/25/2004	2227	1028	0	112
11/26/2004	437	171	0	105
11/29/2004	358	202	0	177

Data Associated with Figure 2 (page 117)

Figure 2: The Change in Additional Total Solids Reduction with Ultrasonic Treatment as a Pretreatment and in a Recycle Line

Equation Used to Calculate Net Solids Reduction:

Net Solids Reduction = $TSR_{treatment(t)}$-$TSR_{control(t)}$

TSR = Total Solids Reduction at time t

Total Solids Reduction (TSR) = $(TS_{feed}-TS_{sample})/TS_{feed}*100$

Statistical Significance in Total Solids Destruction was determined through analysis of TSR.

t-Test: Paired Two Sample for Means
Pretreatment-Start Up

	Control	*Pretreatment*
Mean	38.0	39.5
Variance	96.2	43.3
Observations	10	10
Pearson Correlation	0.72	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.72	
P(T<=t) one-tail	0.25	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.49	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Pretreatment Phase-1

	Control	*Pretreatment*
Mean	23.2	32.2
Variance	19.4	16.2
Observations	20	20
Pearson Correlation	0.76	
Hypothesized Mean Difference	0	
df	19	
t Stat	-13.7	
P(T<=t) one-tail	1.4E-11	
t Critical one-tail	1.73	
P(T<=t) two-tail	2.8E-11	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for Means

Pretreatment Phase 2

	Control	*Pretreatment*
Mean	23.0	27.9
Variance	33.2	30.7
Observations	9	9
Pearson Correlation	0.942	
Hypothesized Mean Difference	0	
df	8	
t Stat	-7.6775	
P(T<=t) one-tail	2.9E-05	
t Critical one-tail	1.86	
P(T<=t) two-tail	5.9E-05	
t Critical two-tail	2.31	

t-Test: Paired Two Sample for

Means

Recycle 1-Start Up

	Control	*Recycle 1*
Mean	37.98	39.38
Variance	96.17	56.29
Observations	10	10
Pearson Correlation	0.92	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.08	
P(T<=t) one-tail	0.15	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.31	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 1- Phase 1

	Control	*Recycle 1*
Mean	23.2	26.1
Variance	19.4	17.2
Observations	20	20
Pearson Correlation	0.89	
Hypothesized Mean Difference	0	
df	19	
t Stat	-6.59	
P(T<=t) one-tail	1.31E-06	
t Critical one-tail	1.73	
P(T<=t) two-tail	2.62E-06	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for

Means

Recylce 1-Phase 2

	Control	*Recycle 1*
Mean	23.0	20.6
Variance	33.2	47.3
Observations	9	9
Pearson Correlation	0.893	
Hypothesized Mean Difference	0	
df	8	
t Stat	2.251	
P(T<=t) one-tail	0.027	
t Critical one-tail	1.860	
P(T<=t) two-tail	0.054	
t Critical two-tail	2.306	

t-Test: Paired Two Sample for Means

Recycle 2- Start Up

	Control	*Recycle 2*
Mean	38.0	33.7
Variance	96.2	51.0
Observations	10	10
Pearson Correlation	0.86	
Hypothesized Mean Difference	0	
df	9	
t Stat	2.57	
P(T<=t) one-tail	0.02	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.03	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 2 Phase 1

	Control	*Recycle 2*
Mean	23.2	26.6
Variance	19.4	29.4
Observations	20	20
Pearson Correlation	0.59	
Hypothesized Mean Difference	0	
df	19	
t Stat	-3.41	
P(T<=t) one-tail	0.001	
t Critical one-tail	1.73	
P(T<=t) two-tail	0.003	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for Means

Recycle 2-Phase 2

	Control	*Recycle 2*
Mean	23.0	29.1
Variance	33.2	23.3
Observations	9	9
Pearson Correlation	0.508	
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.429	
P(T<=t) one-tail	0.004	
t Critical one-tail	1.860	
P(T<=t) two-tail	0.009	
t Critical two-tail	2.306	

Measured Total Solids Content of Sludge Samples

	Feed		Control		Pretreatment		
Date	*%*	*Std. Deviation*	*%*	*Std. Deviation*	*%*	*Std. Deviation*	
7/17/2004	2.427	0.017	1.152	0.008	1.362	0.004	
7/20/2004	2.125	0.018	1.288	0.001	1.419	0.020	
7/24/2004	2.266	0.030	1.355	0.039	1.413	0.009	
7/26/2004	2.599	0.042	1.345	0.005	1.438	0.004	START UP
7/29/2004	3.320	0.024	1.542	0.009	1.542	0.007	
8/2/2004	2.577	0.029	1.725	0.011	1.556	0.006	
8/5/2004	2.653	0.010	1.819	0.001	1.616	0.004	
8/8/2004	2.605	0.002	1.783	0.013	1.462	0.009	
8/11/2004	2.616	0.017	1.882	0.007	1.705	0.006	
8/14/2004	2.489	0.010	1.802	0.011	1.722	0.009	
8/18/2004	2.385	0.032	1.932	0.012	1.713	0.007	
8/21/2004	2.729	0.005	1.948	0.014	1.755	0.009	
8/24/2004	2.452	0.007	1.838	0.003	1.540	0.013	
8/28/2004	2.666	0.026	1.964	0.013	1.930	0.003	
8/31/2004	2.472	0.048	1.978	0.003	1.829	0.003	
9/3/2004	2.604	0.028	1.934	0.009	1.711	0.034	
9/7/2004	2.580	0.010	1.919	0.010	1.767	0.009	
9/10/2004	2.362	0.013	2.019	0.019	1.724	0.142	
9/13/2004	2.685	0.059	2.022	0.006	1.846	0.002	
9/14/2004	2.737	0.031	1.986	0.008	1.788	0.016	PHASE 1
9/16/2004	2.555	0.063	2.030	0.011	1.790	0.006	
9/20/2004	2.967	0.001	2.090	0.006	1.837	0.005	
9/23/2004	2.652	0.013	2.180	0.030	1.800	0.012	
9/25/2004	2.852	0.008	2.171	0.008	1.821	0.009	
9/26/2004	2.884	0.035	2.242	0.046	1.891	0.006	
10/1/2004	2.708	0.014	2.152	0.012	1.855	0.004	
10/4/2004	3.269	0.015	2.187	0.008	1.906	0.019	
10/8/2004	2.861	0.034	2.275	0.009	1.974	0.006	
10/9/2004	3.023	0.237	2.214	0.017	1.923	0.008	
10/18/2004	2.671	0.024	2.118	0.012	1.864	0.005	
10/25/2004	2.754	0.012	2.106	0.010	1.919	0.005	
11/1/2004	2.775	0.010	2.155	0.010	2.008	0.004	
11/3/2004	2.949	0.003	2.160	0.013	1.984	0.007	
11/9/2004	2.439	0.027	2.100	0.005	1.916	0.006	
11/17/2004	2.410	0.005	1.937	0.006	1.881	0.008	PHASE 2
11/20/2004	2.255	0.026	1.875	0.013	1.789	0.010	
11/23/2004	2.554	0.024	1.793	0.010	1.689	0.008	
11/30/2004	2.544	0.024	1.933	0.011	1.813	0.021	
12/11/2004	2.759	0.061	1.927	0.014	1.793	0.006	
12/17/2004	2.708	0.020	2.044	0.007	1.861	0.012	

Measured Total Solids Content of Sludge Samples

	Recycle 1		Recycle 2		
Date	*%*	*Std. Deviation*	*%*	*Std. Deviation*	
07/17/04	1.237	0.007	1.466	0.031	
07/20/04	1.333	0.009	1.516	0.006	
07/24/04	1.393	0.006	1.527	0.012	
07/26/04	1.391	0.007	1.533	0.014	START UP
07/29/04	1.546	0.003	1.665	0.010	
08/02/04	1.643	0.038	1.716	0.059	
08/05/04	1.722	0.007	1.810	0.006	
08/08/04	1.536	0.013	1.838	0.010	
08/11/04	1.732	0.009	1.793	0.027	
08/14/04	1.771	0.008	1.861	0.022	
08/18/04	1.831	0.007	1.908	0.009	
08/21/04	1.849	0.005	1.879	0.054	
08/24/04	1.775	0.007	1.770	0.004	
08/28/04	1.979	0.003	2.344	0.007	
08/31/04	1.985	0.011	1.916	0.006	
09/03/04	1.926	0.004	1.866	0.010	
09/07/04	1.916	0.007	1.881	0.014	
09/10/04	1.905	0.111	1.874	0.003	
09/13/04	1.977	0.008	1.896	0.008	
09/14/04	1.933	0.009	1.898	0.011	PHASE 1
09/16/04	1.946	0.009	1.894	0.005	
09/20/04	2.008	0.014	1.984	0.009	
09/23/04	1.998	0.015	1.975	0.017	
09/25/04	2.032	0.007	1.969	0.009	
09/26/04	2.064	0.022	2.026	0.010	
10/01/04	2.025	0.009	2.007	0.019	
10/04/04	2.066	0.008	2.033	0.007	
10/08/04	2.166	0.009	2.103	0.008	
10/09/04	2.113	0.010	2.055	0.010	
10/18/04	2.048	0.003	1.992	0.004	
10/25/04	2.094	0.001	1.861	0.007	
11/01/04	2.162	0.013			
11/03/04	2.177	0.010	1.840	0.003	
11/09/04	2.156	0.009	1.820	0.005	
11/17/04	2.052	0.006	1.719	0.018	PHASE 2
11/20/04	2.026	0.013	1.702	0.017	
11/23/04	1.936	0.009	1.633	0.007	
11/30/04	1.955	0.015	1.794	0.008	
12/11/04	1.935	0.012	2.099	0.017	
12/17/04	2.015	0.002	1.944	0.002	

Data Associated with Figure 3 (page 118)

Figure 3: The Net Change in Additional Voltaile Solids Reduction with Ultrasonic Treatment as a Pretreatment and in a Recycle Line

Equation Used to Calculate Net Solids Reduction:

Net Solids Reduction = $VSR_{treatment(t)}$-$VSR_{control(t)}$

VSR = Volatile Solids Reduction at time t

Volatile Solids Reduction = (VS_{feed}-VS_{sample})/VS_{feed}*100

Statistical Analysis of the Observed VSR between the Control and Experimental Digester for Each Phase of the Study.

t-Test: Paired Two Sample for Means
Pretreatment Start-up

	Control	*Pretreatment*
Mean	42.2	44.9
Variance	62.2	25.8
Observations	10	10
Pearson Correlation	0.58	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.29	
P(T<=t) one-tail	0.11	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.23	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Pretreatment Phase 1

	Control	*Pretreatment*
Mean	28.24	38.5
Variance	17.08	13.3
Observations	20	20
Pearson Correlation	0.85	
Hypothesized Mean Difference	0	
df	19	
t Stat	-21.31	
P(T<=t) one-tail	5.01473E-15	
t Critical one-tail	1.73	
P(T<=t) two-tail	1.00295E-14	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for Means

Pretreatment Phase 2

	Control	*Pretreatment*
Mean	22.9	27.8
Variance	29.6	27.3
Observations	10	10
Pearson Correlation	0.94	
Hypothesized Mean Difference	0	
df	9	
t Stat	-8.6	
P(T<=t) one-tail	6.04271E-06	
t Critical one-tail	1.83	
P(T<=t) two-tail	1.20854E-05	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 1 Start Up

	Control	*Recycle 1*
Mean	42.2	43.9
Variance	62.2	35.9
Observations	10	10
Pearson Correlation	0.89	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.39	
P(T<=t) one-tail	0.10	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.20	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 1 Phase 1

	Control	*Recycle 1*
Mean	28.24	32.10
Variance	17.08	14.55
Observations	20	20
Pearson Correlation	0.95	
Hypothesized Mean Difference	0	
df	19	
t Stat	-13.44002571	
P(T<=t) one-tail	1.86773E-11	
t Critical one-tail	1.73	
P(T<=t) two-tail	3.73545E-11	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for Means

Recycle 1 Phase 2

	Control	*Recycle 1*
Mean	22.9	20.7
Variance	29.6	42.1
Observations	10	10
Pearson Correlation	0.89	
Hypothesized Mean Difference	0	
df	9	
t Stat	2.23	
P(T<=t) one-tail	0.03	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.05	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 2- Start-up

	Control	*Recycle 2*
Mean	42.2	38.6
Variance	62.2	30.2
Observations	10	10
Pearson Correlation	0.79	
Hypothesized Mean Difference	0	
df	9	
t Stat	2.31	
P(T<=t) one-tail	0.02	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.05	
t Critical two-tail	2.26	

t-Test: Paired Two Sample for Means

Recycle 2 Phase 1

	Control	*Recycle 2*
Mean	28.24	32.57
Variance	17.08	18.76
Observations	20	20
Pearson Correlation	0.61	
Hypothesized Mean Difference	0	
df	19	
t Stat	-5.15	
P(T<=t) one-tail	2.81801E-05	
t Critical one-tail	1.73	
P(T<=t) two-tail	5.63603E-05	
t Critical two-tail	2.09	

t-Test: Paired Two Sample for Means

Recycle 2 Phase 2

	Control	*Recycle 2*
Mean	23.0	29.1
Variance	33.2	23.3
Observations	9	9
Pearson Correlation	0.51	
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.429	
P(T<=t) one-tail	0.004	
t Critical one-tail	1.860	
P(T<=t) two-tail	0.009	
t Critical two-tail	2.306	

Measured Volatile Solids Content of Sludge Samples

	Feed		Control		Pretreatment		
Date	*%*	*Std. Deviation*	*%*	*Std. Deviation*	*%*	*Std. Deviation*	
07/17/04	1.848	0.016	0.820	0.004	0.953	0.005	
07/20/04	1.638	0.013	0.925	0.003	0.994	0.013	
07/24/04	1.667	0.028	0.960	0.009	0.984	0.006	
07/26/04	1.897	0.031	0.956	0.006	1.008	0.004	START UP
07/29/04	2.385	0.019	1.078	0.009	1.057	0.002	
08/02/04	1.828	0.019	1.174	0.012	1.037	0.008	
08/05/04	1.907	0.006	1.226	0.001	1.060	0.002	
08/08/04	2.007	0.004	1.230	0.009	0.985	0.007	
08/11/04	2.050	0.021	1.309	0.002	1.147	0.003	
08/14/04	1.972	0.006	1.274	0.006	1.180	0.008	
08/18/04	1.881	0.027	1.391	0.009	1.185	0.011	
08/21/04	2.213	0.006	1.429	0.010	1.236	0.007	
08/24/04	1.957	0.009	1.334	0.002	1.070	0.008	
08/28/04	2.160	0.019	1.463	0.013	1.351	0.003	
08/31/04	1.979	0.039	1.477	0.001	1.293	0.004	
09/03/04	2.091	0.021	1.442	0.007	1.214	0.054	
09/07/04	2.038	0.011	1.416	0.008	1.263	0.009	
09/10/04	1.867	0.015	1.474	0.011	1.302	0.005	
09/13/04	2.229	0.049	1.522	0.002	1.342	0.003	
09/14/04	2.283	0.022	1.492	0.006	1.298	0.007	PHASE 1
09/16/04	2.118	0.063	1.539	0.009	1.314	0.005	
09/20/04	2.387	0.001	1.588	0.005	1.361	0.006	
09/23/04	2.127	0.012	1.638	0.024	1.331	0.012	
09/25/04	2.273	0.010	1.635	0.006	1.350	0.002	
09/26/04	2.299	0.029	1.677	0.006	1.405	0.006	
10/01/04	2.151	0.012	1.612	0.010	1.363	0.004	
10/04/04	2.316	0.011	1.614	0.006	1.374	0.017	
10/08/04	2.040	0.013	1.608	0.004	1.351	0.005	
10/09/04	2.149	0.173	1.541	0.014	1.287	0.006	
10/18/04	2.006	0.020	1.403	0.007	1.193	0.005	
10/25/04	1.900	0.011	1.388	0.007	1.231	0.005	
11/01/04	2.072	0.006	1.403	0.012	1.281	0.003	
11/03/04	2.200	0.004	1.420	0.008	1.271	0.005	
11/09/04	1.883	0.021	1.427	0.005	1.265	0.006	
11/17/04	1.855	0.012	1.360	0.004	1.275	0.007	PHASE 2
11/20/04	1.739	0.020	1.329	0.011	1.232	0.008	
11/23/04	1.926	0.015	1.279	0.002	1.160	0.008	
11/30/04	1.916	0.016	1.353	0.006	1.225	0.015	
12/11/04	1.895	0.016	1.264	0.010	1.133	0.003	
12/17/04	1.869	0.014	1.291	0.005	1.128	0.005	

Measured Volatile Solids Content of Sludge Samples

	Recycle 1		Recycle 2		
Date	*%*	*Std. Deviation*	*%*	*Std. Deviation*	
07/17/04	0.868	0.008	1.033	0.022	
07/20/04	0.948	0.008	1.073	0.005	
07/24/04	0.986	0.004	1.082	0.010	
07/26/04	0.985	0.007	1.086	0.012	
07/29/04	1.077	0.002	1.157	0.008	START UP
08/02/04	1.126	0.021	1.188	0.002	
08/05/04	1.144	0.007	1.206	0.005	
08/08/04	1.058	0.009	1.258	0.012	
08/11/04	1.191	0.012	1.220	0.030	
08/14/04	1.231	0.009	1.300	0.025	
08/18/04	1.291	0.005	1.342	0.011	
08/21/04	1.338	0.027	1.357	0.006	
08/24/04	1.257	0.005	1.242	0.002	
08/28/04	1.407	0.007	1.689	0.006	
08/31/04	1.415	0.021	1.387	0.005	
09/03/04	1.385	0.004	1.348	0.006	
09/07/04	1.385	0.006	1.368	0.012	
09/10/04	1.415	0.006	1.341	0.008	
09/13/04	1.449	0.008	1.390	0.004	
09/14/04	1.421	0.004	1.400	0.007	PHASE 1
09/16/04	1.443	0.004	1.401	0.009	
09/20/04	1.505	0.010	1.488	0.007	
09/23/04	1.491	0.010	1.469	0.016	
09/25/04	1.511	0.001	1.469	0.010	
09/26/04	1.543	0.017	1.514	0.009	
10/01/04	1.504	0.006	1.491	0.017	
10/04/04	1.516	0.006	1.495	0.004	
10/08/04	1.530	0.004	1.489	0.006	
10/09/04	1.471	0.005	1.457	0.053	
10/18/04	1.354	0.003	1.302	0.003	
10/25/04	1.382	0.003	1.212	0.005	
11/01/04	1.410	0.011			
11/03/04	1.424	0.008	1.201	0.001	
11/09/04	1.438	0.007	1.238	0.004	
11/17/04	1.404	0.004	1.205	0.013	PHASE 2
11/20/04	1.407	0.007	1.208	0.014	
11/23/04	1.337	0.010	1.146	0.005	
11/30/04	1.352	0.010	1.241	0.006	
12/11/04	1.288	0.007	1.334	0.011	
12/17/04	1.297	0.004	1.203	0.003	

Data Associated with Figure 4 (page 119)

Figure 4: The Mean Daily Biogas Yield from High Rate Anaerobic Digesters with ans without Ultrasonic Treatment

	Control		Pretreatment		Recycle 1		Recycle 2	
SRT	Mean	Std Deviation	Mean	Std Deviation	Mean	Std Deviation	Mean	Std Deviation
#	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day	m^3/kg-VS-Day
1	0.26	0.05	0.32	0.03	0.28	0.04	0.27	0.04
2	0.27	0.04	0.33	0.04	0.31	0.05	0.29	0.05
3	0.27	0.03	0.33	0.04	0.32	0.05	0.26	0.04
4	0.30	0.04	0.36	0.04	0.34	0.04	0.27	0.04
5	0.29	0.03	0.35	0.03	0.32	0.05	0.28	0.04
6	0.32	0.04	0.36	0.04	0.36	0.04	0.35	0.05
7	0.30	0.03	0.35	0.02	0.31	0.03	0.34	0.04
8	0.31	0.03	0.37	0.04	0.33	0.05	0.34	0.03
9	0.32	0.02	0.38	0.02	0.33	0.08	0.37	0.02
10	0.35	0.04	0.41	0.02	0.40	0.03	0.40	0.03
11	0.37	0.03	0.43	0.03	0.42	0.02	0.43	0.04

Statistical Analysis of Biogas Yeild Data

t-Test: Paired Two Sample for Means
Pretreament-Phase 1

	Control	*Pretreatment*
Mean	0.29	0.34
Variance	0.00	0.00
Observations	4	4
Pearson Correlation	0.97	
Hypothesized Mean Difference	0.00	
df	3	
t Stat	-14.89	
P(T<=t) one-tail	0.0003	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.0007	
t Critical two-tail	3.18	

t-Test: Paired Two Sample for Means
Pretreatment-Phase 2

	Control	*Pretreatment*
Mean	0.32	0.38
Variance	0.00	0.00
Observations	5	5
Pearson Correlation	0.99	
Hypothesized Mean Difference	0	
df	4	
t Stat	(28.02)	
P(T<=t) one-tail	0.000005	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.00001	
t Critical two-tail	2.78	

t-Test: Paired Two Sample for Means
Recycle 1-Phase 1

	Control	*Recycle 1*
Mean	0.2859337	0.32652164
Variance	0.0003895	0.00026066
Observations	4	4
Pearson Correlation	0.975182	
Hypothesized Mean Difference	0	
df	3	
	-	
t Stat	15.151304	
P(T<=t) one-tail	0.0003121	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.0006	
t Critical two-tail	3.1824493	

t-Test: Paired Two Sample for Means
Recycle 1-Phase 2

	Control	*Recycle 1*
Mean	0.30	0.31
Variance	0.00	0.00
Observations	3	3
Pearson Correlation	0.91	
Hypothesized Mean Difference	0	
df	2	
t Stat	-3.77	
P(T<=t) one-tail	0.03	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.06	
t Critical two-tail	4.30	

t-Test: Paired Two Sample for Means
Recycle 2-Phase 1

	Control	*Recycle 2*
Mean	0.286	0.283
Variance	0.000	0.001
Observations	4	4
Pearson Correlation	0.762	
Hypothesized Mean Difference	0	
df	3	
t Stat	0.23	
P(T<=t) one-tail	0.42	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.83	
t Critical two-tail	3.18	

t-Test: Paired Two Sample for Means
Recycle 2-Phase 2

	Control	*Recycle 2*
Mean	0.32	0.37
Variance	0.00	0.00
Observations	5	5
Pearson Correlation	0.98	
Hypothesized Mean Difference	0	
df	4	
t Stat	-10.8	
P(T<=t) one-tail	0.0002	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.0004	
t Critical two-tail	2.78	

Data Associated with Figure 5 (page 120)

Figure 5: The Net Increase in Ammonium-N within Digesters Treated with Ultrasonic Energy

Calculation of Ammonium-N yield

AMMONIUM-N YIELD = ($[NH_4\text{-}N_{con}]$-$[NH_4\text{-}N_{feed}]$)/($[VS_{feed}]$*(%VSD))]

$[NH_4\text{-}N_{con}]$ = ammonium concentration in control digester (mg-N/L)
$[NH_4\text{-}N_{feed}]$ = ammonium concentration in feed (mg-N/L)
$[VS_{feed}]$ = Volatile Solids Concentration in feed (mg/L)
%VSD = Volatile Solids Destruction (%) (mass reduction between feed and digester)

Ammonium-N Yield from Digestion of WAS- CONTROL DIGESTER

Control SRT		FEED VS %	Ave. VSR %	Ammonium-N Produced mg/L	Yield mg-NH4-N/mg-VSD	Mass VSd g-VS	Mass NH4-N Produced mg-NH4-N
1	7/15-731	1.89	50	318	0.5	23	11917
2	8/1-8/15	1.95	36	272	0.6	18	10183
3	8/16-8/30	2.05	32	302	0.7	16	11342
4	8/31-9-14	2.08	29	330	0.8	15	12378
5	9/15-9/29	2.24	28	290	0.7	16	10892
6	9/30-10-14	2.16	26				
7	10/15-1029	1.95	29	447	1.2	14	16780
8	10/30-11/13	2.05	31	382	0.9	16	14321
9	11/14-11/28	1.84	28	363	1.1	13	13612
10	11/29-12-13	1.91	31	337	0.8	15	12629
11	12/14-12/17	1.87	31	392	1.0	14	14717
				Phase 1 (mean)	0.7	mg-NH4-N/mg-VSD	
				Phase 1 (Std. Deviation)	0.1	mg-NH4-N/mg-VSD	
				Phase 2 (mean)	1.0	mg-NH4-N/mg-VSD	
				Phase 2 (Std. Deviation)	0.1	mg-NH4-N/mg-VSD	

Ammonium-N Yield from Digestion of WAS- PRETREATMENT DIGESTER

Pretreatment		Mean FEED VS	Ave. VSR	Ammonium-N Produced	Yield	Mass VSd	Mass NH4-N Produced
SRT		%	%	mg/L	mg-NH4-N/mg-VSD	g-VS	mg-NH4-N
1	7/15-731	1.89	47	368	0.6	22	13816
2	8/1-8/15	1.95	45	325	0.6	22	12180
3	8/16-8/30	2.05	41	367	0.7	21	13776
4	8/31-9-14	2.08	38	399	0.8	20	14945
5	9/15-9/29	2.24	40	478	0.8	22	17920
6	9/30-10-14	2.16	38				
7	10/15-1029	1.95	38	563	1.1	19	21101
8	10/30-11/13	2.05	38	403	0.8	19	15106
9	11/14-11/28	1.84	34	483	1.2	15	18120
10	11/29-12-13	1.91	38	447	0.9	18	16777
11	12/14-12/17	1.87	40	368	0.7	19	13783
				Phase 1 (mean)	0.7	mg-NH4-N/mg-VSD	
				Phase 1 (Std. Deviation)	0.1	mg-NH4-N/mg-VSD	
				Phase 2 (mean)	1.0	mg-NH4-N/mg-VSD	
				Phase 2 (Std. Deviation)	0.2	mg-NH4-N/mg-VSD	

Ammonium-N Yield from Digestion of WAS- RECYCLE -1 DIGESTER

Recycle 1		Mean FEED VS	Ave. VSR	Ammonium-N Produced	Yield	Mass VSd	Mass NH4-N Produced
SRT	Date	%	%	mg/L	mg-NH4-N/mg-VSD	g-VS	mg-NH4-N
1	7/15-731	1.89	48	317	0.5	23	11889
2	8/1-8/15	1.95	41	304	0.6	20	11385
3	8/16-8/30	2.05	36	336	0.7	18	12592
4	8/31-9-14	2.08	32	359	0.8	17	13453
5	9/15-9/29	2.24	33	345	0.7	19	12922
6	9/30-10-14	2.16	30				
7	10/15-1029	1.95	30	497	1.3	15	18625
8	10/30-11/13	2.05	31	367	0.9	16	13744
9	11/14-11/28	1.84	25	323	1.1	11	12111
10	11/29-12-13	1.91	31	454	1.2	15	17014
11	12/14-12/17	1.87	31	475	1.2	14	17799
				Phase 1 (mean)	0.7	mg-NH4-N/mg-VSD	
				Phase 1 (Std. Deviation)	0.1	mg-NH4-N/mg-VSD	
				Phase 2 (mean)	1.1	mg-NH4-N/mg-VSD	
				Phase 2 (Std. Deviation)	0.2	mg-NH4-N/mg-VSD	

Ammonium-N Yield from Digestion of WAS- RECYCLE -2 DIGESTER

Recycle 2		Mean Ave. FEED VS	VSR	Ammonium-N Produced	Yield	Mass VSd	Mass NH4-N Produced
SRT	Date	*VS %*	*%*	*mg/L*	*mg-NH4-N/mg-VSD*	*g-VS*	*mg-NH4-N*
1	7/15-731	1.89	42	354	0.7	20	13263
2	8/1-8/15	1.95	37	332	0.7	18	12444
3	8/16-8/30	2.05	31	319	0.7	16	11979
4	8/31-9-14	2.08	34	357	0.8	18	13383
5	9/15-9/29	2.24	34	340	0.7	19	12761
6	9/30-10-14	2.16	31				
7	10/15-1029	1.95	36	429	0.9	17	16070
8	10/30-11/13	2.05	41	394	0.7	21	14763
9	11/14-11/28	1.84	36	302	0.7	16	11338
10	11/29-12-13	1.91	32	177	0.4	15	6649
11	12/14-12/17	1.87	36	159	0.4	17	5979
				Phase 1 (mean)	0.7	mg-NH4-N/mg-VSD	
				Phase 1 (Std. Deviation)	0.1	mg-NH4-N/mg-VSD	
				Phase 2 (mean)	0.6	mg-NH4-N/mg-VSD	
				Phase 2 (Std. Deviation)	0.2	mg-NH4-N/mg-VSD	

Statistical Analysis of Ammonium Yeild Data

t-Test: Two-Sample Assuming Equal Variances

Pretreatment Phase 1

	Control	*Pretreatment*
Mean	0.736	0.737
Variance	0.004	0.006
Observations	3	3
Pooled Variance	0.005	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.03	
P(T<=t) one-tail	0.49	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.98	
t Critical two-tail	2.78	

t-Test: Two-Sample Assuming Equal Variances

Pretreatment Phase 2

	Control	*Pretreatment*
Mean	1.00	0.95
Variance	0.02	0.04
Observations	5	5
Pooled Variance	0.030	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.484	
P(T<=t) one-tail	0.321	
t Critical one-tail	1.860	
P(T<=t) two-tail	0.641	
t Critical two-tail	2.306	

t-Test: Two-Sample Assuming Equal Variances

Recycle 1 Phase 1

	Control	*Recycle 1*
Mean	0.74	0.73
Variance	0.00	0.00
Observations	3	3
Pooled Variance	0.004	
Hypothesized Mean Difference	0	
df	4	
t Stat	0.104	
P(T<=t) one-tail	0.461	
t Critical one-tail	2.132	
P(T<=t) two-tail	0.922	
t Critical two-tail	2.776	

t-Test: Two-Sample Assuming Equal Variances

Recylce 1 Phase 2

	Control	*Recycle 1*
Mean	1.00	1.12
Variance	0.02	0.03
Observations	5	5
Pooled Variance	0.0227801	
Hypothesized Mean Difference	0	
df	8	
t Stat	-1.24	
P(T<=t) one-tail	0.12	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.25	
t Critical two-tail	2.31	

t-Test: Two-Sample Assuming Equal Variances

Recycle 2 Phase 1

	Control	*Recycle 2*
Mean	0.736	0.719
Variance	0.004	0.003
Observations	3	3
Pooled Variance	0.003	
Hypothesized Mean Difference	0	
df	4	
t Stat	0.338	
P(T<=t) one-tail	0.376	
t Critical one-tail	2.132	
P(T<=t) two-tail	0.752	
t Critical two-tail	2.776	

t-Test: Two-Sample Assuming Equal Variances

Recycle 2 Phase 2

	Control	*Recycle 2*
Mean	1.00	0.62
Variance	0.02	0.05
Observations	5	5
Pooled Variance	0.0359806	
Hypothesized Mean Difference	0	
df	8	
t Stat	3.17	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.01	
t Critical two-tail	2.31	

Solution Ammonium-N Concentrations in Digesters

Operational Day	Date	**Control** *mg-N/L*	**Pretreatment** *mg-N/L*	**Recycle 1** *mg-N/L*	**Recycle 2** *mg-N/L*	**Feed** *mg-N/L*
2	07/17/04	496	532	440	474	157
5	07/20/04	409	459	414	416	107
9	07/24/04	453	528	475	521	121
11	07/26/04	464	502	475	491	119
14	07/29/04	451	504	464	549	179
18	08/02/04	450	421	471	464	154
24	08/08/04	377	428	391	430	90
30	08/14/04	305	442	366	418	73
35	08/19/04					97
37	08/21/04	461	535	440	421	98
40	08/24/04	381	399	447	406	85
44	08/28/04	380	482	435	446	132
47	08/31/04	382	493	481	468	110
50	09/03/04	380	511	474	460	103
54	09/07/04	441	487	450	440	117
57	09/10/04	545	441	418	465	126
60	09/13/04	447	604	514	494	87
63	09/16/04	324	529	454	396	139
67	09/20/04	459	547	470	478	93
70	09/23/04	458	728	480	518	138
95	10/18/04	532	664	610	429	129
102	10/25/04	576	675	596	641	85
109	11/01/04	377	660	523	406	69
115	11/08/04	581	339	404	575	124
124	11/17/04	554	668	384	497	177
127	11/20/04	507	634	597	444	158
137	11/30/04	439	547	662	293	142
148	12/11/04	602	715	613	429	225
154	12/17/04	599	574	681	366	207

Solution Net Ammonium-N Concentrations in Digesters

Operational Day	Date	**Control** *mg-N/L*	**Pretreatment** *mg-N/L*	**Recycle 1** *mg-N/L*	**Recycle 2** *mg-N/L*
2	07/17/04	339	375	283	317
5	07/20/04	302	352	307	309
9	07/24/04	332	408	354	401
11	07/26/04	345	383	356	372
14	07/29/04	271	324	284	370
18	08/02/04	295	267	317	309
24	08/08/04	287	339	301	341
30	08/14/04	232	369	293	346
35	08/19/04				
37	08/21/04	363	437	343	323
40	08/24/04	296	314	361	320
44	08/28/04	249	351	303	315
47	08/31/04	272	383	372	358
50	09/03/04	276	408	371	357
54	09/07/04	324	371	333	323
57	09/10/04	419	316	292	339
60	09/13/04	359	516	426	407
63	09/16/04	185	390	315	256
67	09/20/04	367	455	377	385
70	09/23/04	320	590	342	380
95	10/18/04	403	535	482	300
102	10/25/04	492	590	512	557
109	11/01/04	307	591	454	337
115	11/08/04	457	215	279	451
124	11/17/04	377	490	207	320
127	11/20/04	349	476	439	285
137	11/30/04	297	405	520	151
148	12/11/04	377	490	388	204
154	12/17/04	392	368	475	159

Data Associated with Figure 6 (page 122)

Figure 6: Comparison of Calculated and Measured Digester Ammonium-N in Phase 1 and 2 for the Recycle 2 System

Theoretical NH_4-N Yield = $\mathbf{Y_{thNH4}}$ = average NH4-N Yield of the Control Pretreatment and Recycle 1 Digesters is phase 1

Theoretical NH_4-N Produced (mg-N) = $\mathbf{Y_{thNH4}} * \mathbf{Mass\ VSD}$ (mg-VS)

Theoretical NH_4-N Concentration (mg-N/L) = ($\mathbf{Y_{thNH4}} * \mathbf{Mass\ VSD}$ (mg-VS))/$\mathbf{V_{dig}}$

$\mathbf{V_{dig}}$ = volume of digester =37.5 L

Recycle 2 SRT		**Theoretical NH_4-N Produced** *mg-N*	**Actual NH_4-N Concentration** *mg-N/L*	**Theoretical NH_4-N Concentration** *mg-N/L*	**Percent Deviation**
1	7/15-731	14700	354	392	
2	8/1-8/15	13195	332	352	
3	8/16-8/30	11846	319	316	
4	8/31-9-14	13017	357	347	
5	9/15-9/29	14187	340	378	
6	9/30-10-14				
7	10/15-1029	17854	429	476	10
8	10/30-11/13	21341	394	569	31
9	11/14-11/28	16767	302	447	32
10	11/29-12-13	15859	177	423	58
11	12/14-12/17	17080	159	455	65

Data Associated with Figure 7 (page 123)

Figure 7: The Effect of Ultrasonic Energy Input on the Optimum Dose of Anaerobically Digested Sludge as Measured by Minimum CST

Phase 1	**Polymer Demand (g-polymer/kg-TS)**			
	Control	**Pretreatment**	**Recycle 1**	**Recycle 2**
Mean	5.5	11.2	9.5	10.0
Standard Deviation	1.6	3.1	1.4	1.6
n	7	6	6	5

Statistical Analysis of Polymer Demand Associated with digesters during Phase 1 of the study.

t-Test: Paired Two Sample for Means
Pretreatment Polymer Demand

	Control	*Pretreament*	
Mean	5.8	11.4	
Variance	3.0	9.8	
Observations	6		6
Pearson Correlation	0.97		
Hypothesized Mean Difference	0		
df	5		
t Stat	-9.0951		
P(T<=t) one-tail	0.0001		
t Critical one-tail	2.0150		
P(T<=t) two-tail	0.0003		
t Critical two-tail	2.5706		

t-Test: Paired Two Sample for Means
Recycle 1 Polymer Demand

	Control	*Recycle 1*
Mean	5.8	9.6
Variance	3.0	2.1
Observations	6	6
Pearson Correlation	0.93	
Hypothesized Mean Difference	0	
df	5	
	-	
t Stat	14.75745	
P(T<=t) one-tail	0.00001	
t Critical one-tail	2.01505	
P(T<=t) two-tail	0.00003	
t Critical two-tail	2.57058	

t-Test: Paired Two Sample for Means
Recycle 2 Polymer Demand

	Control	*Recycle 2*
Mean	6.2	10.2
Variance	2.4	2.5
Observations	5	5
Pearson Correlation	0.943	
Hypothesized Mean Difference	0	
df	4	
	-	
t Stat	16.82936	
P(T<=t) one-tail	0.00004	
t Critical one-tail	2.13185	
P(T<=t) two-tail	0.00007	
t Critical two-tail	2.77645	

Data Associated with Figure 8 (page 124)

Figure 7: The Impact of Ultrasonic Energy on Total Organo-Sulfur Odor Potential from Anaerobically Digested Sludge

VOSC Concentration of Duplicate Samples of Dewatered Biosolids

		Control (a)	Control (b)	Pretreatment (a)	Pretreatment (b)	Recycle 2 (a)	Recycle 2 (b)
Date	Date	*mg-OS/m3/kg-VS*	*mg-OS/m3/kg-VS*	*mg-OS/m3/kg-VS*	*mg-OS/m3/kg-VS*	*mg-OS/m3/kg-VS*	*mg-OS/m3/kg-VS*
0	12/18/2004	0	0	0	0	0	0
1	12/19/2004	14393	16094	6189	1891	2338	1734
2	12/20/2004	90491	85398	58174	8771	23094	19527
4	12/22/2004	181023	201728	133659	462	74908	73642
6	12/24/2004	230014	238034	187995	1139	82775	85293
8	12/26/2004	275995	279937	216896	7882	93893	96248
10	12/28/2004	344803	400191	279960	1748	132447	102188
17	1/4/2005	364818	365189	325752	4680	198250	198246

Average VOSC Concentration

	Control	Pretreatment	Recycle 2
Date	mg-OS/m3/kg-VS	mg-OS/m3/kg-VS	mg-OS/m3/kg-VS
0	0	0	0
1	15244	4040	2036
2	87945	33472	21311
4	191375	67060	74275
6	234024	94567	84034
8	277966	112389	95070
10	372497	140854	117317
17	365004	165216	198248

Data Associated with Figure 9 (page 125)

Figure 9: The Impact of Ultrasonic Dose on Volatile Solids Destruction in a Mesophilic Anaerobic Digesters (SRT =15 days)

Sludge Source Study	Blacksburg & V.P.I Feasibility		
Digester Type	**Ultrasonic Dose** *W-min/mL*	**Volatile Solids Destruction** *%*	**Percent Increase in VSD**
Control	0	45.75	0.00
Pretreatment	0.576	48.75	6.56
Recycle1	0.384	46.71	2.10
Recycle 2	0.576	48.19	5.33

Sludge Source Study	Christiansburg Phase 1		
Digester Type	**Ultrasonic Dose** *W-min/mL*	**Volatile Solids Destruction** *%*	**Percent Increase in VSD**
Control	0	22.1	0.00
Pretreatment	2.8	30.4	37.62
Recycle1	1.6	25.4	15.05
Recycle 2	1.8	25.7	16.25

Sludge Source Study	Christiansburg Phase 2		
Digester Type	**Ultrasonic Dose** *W-min/mL*	**Volatile Solids Destruction** *%*	**Percent Increase in VSD**
Control	0	21.7	0.00
Pretreatment	2.88	26.8	23.39
Recycle1	0	20.9	-3.64
Recycle 2	2.88	26.4	21.38

APPENDIX C: Data Associated Figures and Tables in Chapter 4

DATA ASSOCIATED WITH FIGURE 1 (page 151)

Figure 1 was obtained from Dr. Matthew Higgins at Bucknell University, Lewisburg, PA, and all requests for the original data should be sent to him.

DATA ASSOCIATED WITH FIGURE 2 (page 152)

The values represent the mean and standard deviation of three samples.

	High Solids Centrifuge		Low Solids Centrifuge		Laboratory Centrifuge	
Incubation	**Mean**	**St.Deviation**	**Mean**	**St.Deviation**	**Mean**	**St.Deviation**
days	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*
0	0	0	0	0	0	0
2	83	2	71	4	5	1
4	253	12	130	6	5	0
6	307	24	111	21	5	1
10	274	27	95	25	8	3

DATA ASSOCIATED WITH FIGURE 3 (page 152)

Figure 3: The Impact of Shear and Polymer Interactions on VOSC Emissions from Dewatered Biosolids

The values represent the mean and standard deviation of three samples.

Peak Volatile Organic Sulfur

Polymer Dose	No Shear MeanVOSC	Std. Dev.	Shear with Post Polymer addition MeanVOSC	Std. Dev.	Shear in the Presence of Polymer MeanVOSC	Std. Dev.
g-polymer/kg-TS	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*
0	10.8		9.6		9.7	
9.0	17.7	17.3	8.8	2.3	15.3	6.7
18.0	8.0	2.5	12.1	3.2	49.6	7.4
27.0	5.9	1.0	8.3	-2.5	97	4

Peak Volatile Organic Sulfur

High Solids Centrifuge MeanVOSC	Std. Dev.	Low Solids Centrifuge MeanVOSC	Std. Dev.
ppmv/g-VS	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*
307	24.3	111	6.0

DATA ASSOCIATED WITH FIGURE 4 (page 153)

Figure 4: Headspace VOSC Profiles of High and Low Solids Centrifuges Relative to Different Laboratory Shear Regimes

	High Solids Centrifuge		**Low Solids Centrifuge**	
Incubation	Mean	St.Deviation	Mean	St.Deviation
days	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*
0	0	0	0	0
2	83	2	71	4
4	253	12	130	6
6	307	24	111	21
10	274	27	95	25

	No Shear		**Shear in the Presence of Polymer**		**Shear with Post of Polymer Addition**	
Incubation	Mean	St.Deviation	Mean	St.Deviation	Mean	St.Deviation
days	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*	*ppmv/g-VS*
0	0.0	0.0	0	0	0.0	0.0
2	4.1	0.9	96.7	3.7	6.6	1.5
4	5.5	0.9	83.3	5.5	7.0	1.6
6	4.8	1.5	53.7	5.3	4.5	0.7
10	5.5	0.9	24.6	13.9	7.5	2.8

DATA ASSOCIATED WITH FIGURE 5 (page 154)

Figure 5: The Impact of Cake Solids Concentration on Peak VOSC Emissions from Biosolids

Sludge Source	Peppers Ferry RWWTF		
Plant 1	G(t) = 27,500		
Cake Solids	**Peak VOSC**		
%	*(mg-OS/m3)/g-VS*	*(mg-OS/m3)/kg-VS*	*ppmv/kg-VS*
16.8	0.59	590	450
24.6	3.06	3064	2338
25.1	2.56	2565	1957
29.6	3.24	3239	2472
28.6	3.49	3485	2660
20.9	1.38	1384	1056
20.1	0.68	678	517
20.9	1.17	1170	893
20.8	0.52	518	396
20.5	0.81	808	617
Data Omitted from Regression			
16.6	3.37	3367	2570

Sludge Source	Los Angeles County Sanitation District			
Plant 2	G(t) =247,500			
Label	**Cake Solids**	**Peak VOSC**	**Peak VOSC**	**Peak VOSC**
	%	*ppmv*	*ppmv/g-VS*	*ppmv/kg-VS*
Centrifuge	23	1556	588	588330
64-g	27	4234	1240	1239713
37-g	29	4716	1213	1213008
15-g	32	7763	1923	1923087

DATA ASSOCIATED WITH FIGURE 6 (page 155)

Figure 6: Impact of Polymer Dose on Observed VOSC Emissions from Biosolids

G(t) = 165,000

Polymer Dose *lb/ton*	**Peak VOSC** *mg-OS/m3-kg-VS*	**Peak VOSC** *ppmv/kg-VS*
0	1426	1088
42	45022	34357
89	105348	80391
127	87205	66546
169	102334	78091

G(t) = 27,500

Polymer Dose Volume (*ml*)	**Polymer Dose** *(g-p/kg-TS)*	**Polymer Dose** *lb/ton*	**Peak VOSC** *mg-OS/m3-kg-VS*	**Peak VOSC** *ppmv/kg-VS*
15	45.7	9.1	13.6	10.4
20	61.0	12.2	25.4	19.4
25	76.2	15.2	36.1	27.6
30	91.5	18.3	28.0	21.4
35	106.7	21.3	31.6	24.1
40	122.0	24.4	34.7	26.5

DATA ASSOCIATED WITH FIGURE 7 and Table 3 (page 157 and 150)

Figure 7: The Impact of of Shearing Intensity on Peak VOSC Emissions from Biosolids

SOURCE	**Los Angeles County Sanitation District Joint Water Pollution Control Project**	**Gatlinburg TN Wastewater Treatment Plant**	**Philadelphia Water Department Biosolids Recycling Center**	**Peppers Ferry Regional Wastewater Treatment Facility**
	Sludge A	Sludge B	Sludge C	Sludge D
	Peak VOSC	**Peak VOSC**	**Peak VOSC**	**Peak VOSC**
G(t)	*ppmv*	*ppmv*	*ppmv*	*ppmv*
0	0.9	0.8	17.4	15.6
27500			66.3	
55000	72.2	0.7	103.5	
82500				128.8
165000	903.4	0.5	364.8	306.0
247500	1391.5	2.7		
R-square	0.97	0.49	0.99	0.98
Slope	0.006	0.000006	0.0021	0.0018

DATA ASSOCIATED WITH FIGURE 8 and (page 158)

Figure 8: Contribution of Cake Dryness and Shear Intensity to Peak VOSC from Dewatered Biosolids

Sludge Source	**Peppers Ferry RWWTF**	
G(t) = 0		
Cake Solids	**Peak VOSC**	**Peak VOSC**
%	*(mg-OS/m3)/kg-VS*	*(ppmv)/g-VS*
13.42	1193	1
19.27	3990	3
21.14	5594	4
24.87	33087	25

G(t) = 82,500		
Cake Solids	**Peak VOSC**	**Peak VOSC**
%	*(mg-OS/m3)/kg-VS*	*(ppmv)/g-VS*
16.8	9700	7
22.5	46315	35
27.5	192868	147
29.2	153832	117

G(t) = 165,000		
Cake Solids	**Peak VOSC**	**Peak VOSC**
%	*(mg-OS/m3)/kg-VS*	*(ppmv)/g-VS*
17.7	40700	31
22.3	114784	88
25.1	228953	175
29.2	180859	138

DATA ASSOCIATED WITH FIGURE 10 and (page 160)

Figure 10: Contribution of Cake Dryness and Shear Intensity to Peak VOSC from Dewatered Biosolids

Full-Scale Centrifuge Samples: Philadelphia Water Department Biosolids Recycling Center

	High Solids Centrifuge			Low Solids Centrifuge		
Time	**VOSC**	**VOSC**	**VOSC**	**VOSC**	**VOSC**	**VOSC**
d	*mg-OS/m3*	*mg-OS/m3-gVS*	*ppmv/g-VS*	*mg-OS/m3*	*mg-OS/m3-gVS*	*ppmv/g-VS*
0	0	0	0	0	0	0
1	64	18	14	64	20	15
2	106	29	22	174	55	42
3	172	48	36	302	95	73
4	448	124	95	295	93	71
5	494	137	104	243	77	58
6	529	146	112	255	80	61
7	511	141	108	195	62	47
8	516	143	109	226	71	54
10	541	150	114	169	53	41
13	28	8	6	7	2	2

Centrifuge Simulations at Specified Shear Intensities

	G(t) = 82,500			G(t) =165,000		
Time	**VOSC**	**VOSC**	**VOSC**	**VOSC**	**VOSC**	**VOSC**
d	*mg-OS/m3*	*mg-OS/m3-gVS*	*ppmv/g-VS*	*mg-OS/m3*	*mg-OS/m3-gVS*	*ppmv/g-VS*
0.5	2.6	0.6	0	1.6	0.4	0
1	10.8	2.7	64	3.7	0.9	1
2	28.9	7.1	174	14.3	3.5	3
3	74.5	18.4	302	34.8	8.6	7
4	135.6	33.5	295	170.9	42.2	32
6	120.1	29.7	243	471.9	116.6	89
8	78.4	19.4	255	478.1	118.1	90
10	72.9	18.0	195	677.6	167.5	128
13	8.8	2.2	226	633.9	156.7	120
15	3.7	0.9	169	154.3	38.1	29
17			7	25.7	6.3	5
24				18.9	4.7	

DATA ASSOCIATED WITH FIGURE 11 and (page 161)

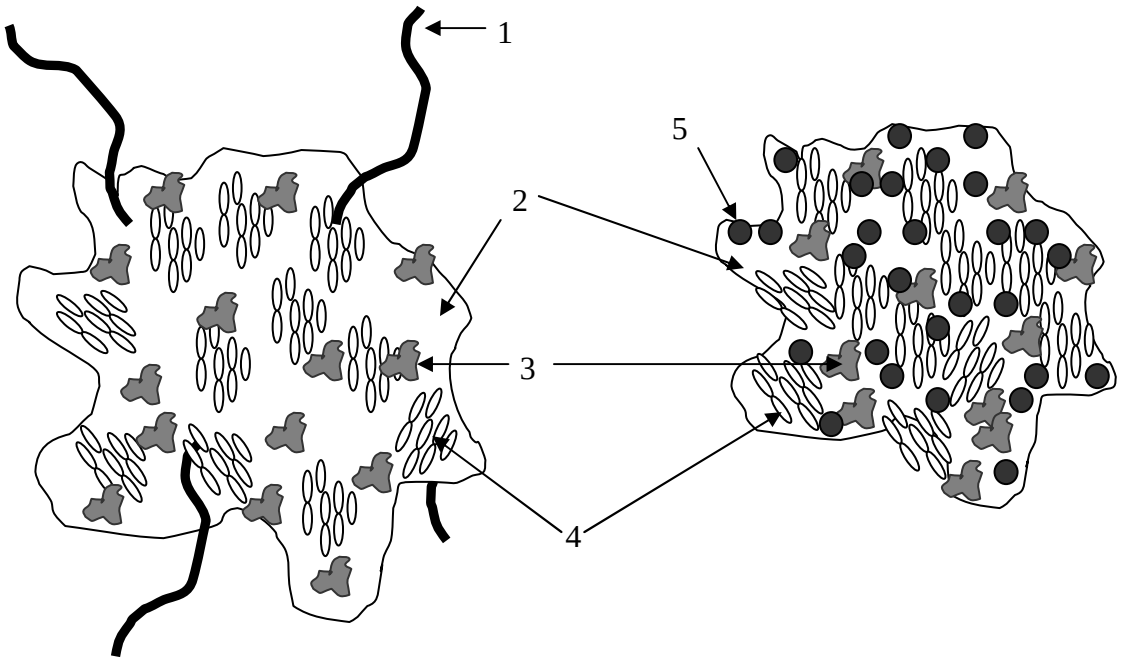
Figure 11: A Comparison of the Laboratory Centrifuge Simulation with Full-Scale Centrifuge Data for the Determination of Peak VOSC Concentrations

Lab Simulation Peak VOSC *mg-S/m3*	**Full-Scale Centrifuge Peak VOSC** *mg-S/m3*
1085	1425
1770	2244
2038	2964
678	529
0.66	6.3

APPENDIX D: Data Associated Figures and Tables in Chapter 5

DATA ASSOCIATED WITH FIGURE 1 and (page 198)

Figure 1: The Relationship between Floc Associated Cations and Floc Structure Components in Activated Sludge and Anaerobic Sludge



Aerobic Sludge Flocs (a)

Anaerobic Sludge Flocs (b)

LEGEND

1. Filamentous Bacteria
2. EPS (Monovalent and divalent cations)
3. Colloidal Organic Matter (iron and aluminum)
4. Flocculent Microorganisms (Monovalent and divalent cations
5. Ferrous Sulfide Precipitates (iron-anaerobic only)

DATA ASSOCIATED WITH FIGURE 2 and (page 198)

Figure 2: The Change in Additional Volatile Solids Destruction with Incubation Time at Mesophilic Temperatures

Day	Mill AVSD
0	0.0
3	9.7
7	11.2
12	11.1

DATA ASSOCIATED WITH FIGURE 3 and (page 198)

Figure 3: The Impact of Initial Solids Concentration on Ultrasonic Disintegration Rate
* Disintegration Rate = (PSR%/kW-hr-kg-TS); PSR = particle siz reduction

Los Angeles County Sanitation District Sample 1

Sample	**Solids Concentration** *%*	**Ultrasonic Dose** *PSR%/(kW-hr/Kg-TS)*
Stock	4.716	-0.378
75/25 TWAS	3.622	0.0055
60/40 TWAS	2.892	3.841
50/50 TWAS	2.480	53.633
40/60 TWAS	1.938	36.672
25/75 TWAS	1.272	60.442
10/90 TWAS	0.589	30.513
5/95 TWAS	0.334	16.265

DATA ASSOCIATED WITH FIGURE 4 and (page 199)

Figure 4: The Stability of Iron and Aluminum Hydroxide Associated Protein in the Presence of Sulfide

Dose Addition of Sulfide to Iron and Aluminum Hydroxide Flocculated BSA

Iron Floc Dose *uL*	**Dose** *meq*	**Sulfide/Iron** *S/Fe*	**Supernatant BSA** *mg/L*
0	0.0000	0.0000	2
5	0.0060	0.2950	115
10	0.0120	0.5899	512
15	0.0180	0.8849	993
20	0.0240	1.1798	1051

Alum Floc Dose *uL*	**Dose** *meq*	**Sulfide/Aluminum** *S/Al*	**Supernatant BSA** *mg/L*
0	0.0000	0.0000	103
4	0.0048	0.2101	49
6	0.0072	0.3151	48
8	0.0096	0.4201	87
10.3	0.0124	0.5409	101

DATA ASSOCIATED WITH FIGURE 5 and (page 199)

Can be found in Appendix A.

DATA ASSOCIATED WITH FIGURE 6 and (page 200)

Figure 6: Correlation between Proposed Floc Models or Floc Components and the Degree of Digestion Enhancement Using Mechanical Shear in an Internal Recycle: (a) Monovalent to divalent cation ratio (Higgins and Novak, 1997b), (b) Monovalent to divalent plus trivalent (Park *et al.*, 2006b) (c) Monovalent plus Divalent to trivalent cations "lectin-like" material verse trivalents (d) divalent to trivalent ratio (e) tivalent bound organic matter only (f) Iron to Aluminum, debris only

Floc Associated Metals Mass per Unit Mass Basis

		Concentration (mg-Metal/g-Solids)					
Label	**Wastewater Facility**	*Aluminum*	*Iron*	*Calcium*	*Magnesium*	*Potassium*	*Sodium*
A	Sioux City, Iowa USFilter	5.7	45.6	83.5	5.4	3.1	3.7
A1	Sioux City, IA USFilter #2	10.8	50.9	64.8	4.3	2.6	4.2
B	Caldwell, Idaho USFilter	18.1	4.4	39.0	7.5	9.8	2.7
B1	Caldwell, ID USFilter #2	13.6	6.0	35.0	7.8	6.9	1.9
B2	Caldwell, ID USFilter #3	14.9	6.8	37.5	8.7	6.4	1.9
C	Rockland, MA USFilter	36.6	102.5	12.2	1.7	1.2	1.7
D	Dayton, OH, USFilter	18.9	22.2	20.5	4.9	1.5	1.8
E	Peppers Ferry RWTP, Radford, VA						

Floc Associated Metals Equivalence per Unit Mass Basis

		Concentration (meq-Metal/g-Solids)					
Label	Wastewater Facility	*Aluminum*	*Iron*	*Calcium*	*Magnesium*	*Potassium*	*Sodium*
A	Sioux City, Iowa USFilter	0.631	2.451	4.168	0.447	0.078	0.160
A1	Sioux City, IA USFilter #2	1.196	2.736	3.236	0.356	0.067	0.181
B	Caldwell, Idaho USFilter	2.008	0.237	1.946	0.614	0.251	0.118
B1	Caldwell, ID USFilter #2	1.512	0.322	1.746	0.638	0.176	0.085
B2	Caldwell, ID USFilter #3	1.654	0.363	1.872	0.713	0.163	0.082
C	Rockland, MA USFilter	4.071	5.504	0.611	0.141	0.030	0.076
D	Dayton, OH, USFilter	2.096	1.191	1.025	0.405	0.039	0.080
E	Peppers Ferry RWTP, Radford, VA						

Floc Associated Cation Ratios

		Floc Associated Cation Ratios					
Label	**Wastewater Facility**	*M/D*	*M/(D+T)*	*(M+D)/T*	*D/T*	*T*	*Fe/Al*
A	Sioux City, Iowa USFilter	0.05	0.03	1.57	1.50	3.08	3.89
A1	Sioux City, IA USFilter #2	0.07	0.03	0.98	0.91	3.93	2.29
B	Caldwell, Idaho USFilter	0.14	0.08	1.31	1.14	2.24	0.12
B1	Caldwell, ID USFilter #2	0.11	0.06	1.44	1.30	1.83	0.21
B2	Caldwell, ID USFilter #3	0.09	0.05	1.40	1.28	2.02	0.22
C	Rockland, MA USFilter	0.14	0.01	0.09	0.08	9.57	1.35
D	Dayton, OH, USFilter	0.08	0.03	0.47	0.43	3.29	0.57
E	Peppers Ferry RWTP, Radford, VA						

Volatile Solids Destruction Data following 7-Day Batch Digestion

			Shear Enhanced Digestion Data	
Label	**Wastewater Facility**	**7-day VS Destruction** *Control (no shear) (%)*	**7-day VS Destruction** *Shear Digestion (%)*	**Increase VSR** *%*
A	Sioux City, Iowa USFilter	4.99	8.40	68.4
A1	Sioux City, IA USFilter #2	9.36	10.91	16.6
B	Caldwell, Idaho USFilter	5.31	5.94	11.9
B1	Caldwell, ID USFilter #2	8.46	9.00	6.4
B2	Caldwell, ID USFilter #3	14.96	15.39	2.9
C	Rockland, MA USFilter	5.18	6.31	21.8
D	Dayton, OH, USFilter	7.8	9.17	17.6
E	Peppers Ferry RWTP, Radford, VA	2.25	4.73	110.0

Calculations

Increase in VSR (%) = $(VSR_{experimental} - VSR_{control})/VSR_{control}*100$

VSR = volatile solids destruction

Statitical Analysis Associated with Figure 6

Mechanical Shear (M/D ratio)

Regression Statistics	
Multiple R	0.63
R Square	0.40
Adjusted R Square	0.28
Standard Error	17.71
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1059	1059.42	3.38	0.13
Residual	5	1568	313.63		
Total	6	2628			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	45.25	14.77	3.06	0.03
X Variable 1	-121.52	66.12	-1.84	0.13

Mechanical Shear (M/(D+T))

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.61
R Square	0.38
Adjusted R Square	0.25
Standard Error	18.10
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	989.63	989.63	3.02	0.14
Residual	5	1637.96	327.59		
Total	6	2627.59			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	36.37	11.15	3.26	0.02
X Variable 1	-129.63	74.58	-1.74	0.14

Mechanical Shear (D/T ratio)

Regression Statistics	
Multiple R	0.17
R Square	0.03
Adjusted R Square	-0.17
Standard Error	22.60
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	73.49	73.49	0.14	0.72
Residual	5	2554.10	510.82		
Total	6	2627.59			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	14.63	18.99	0.77	0.48
X Variable 1	6.78	17.87	0.38	0.72

Mechanical Shear ((M+D)/T ratio)

Regression Statistics	
Multiple R	0.12
R Square	0.01
Adjusted R Square	-0.18
Standard Error	22.77
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	36.16	36.16	0.07	0.80
Residual	5	2591.43	518.29		
Total	6	2627.59			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	16.51	19.25	0.86	0.43
X Variable 1	4.38	16.59	0.26	0.80

Mechanical Shear (T)

Regression Statistics	
Multiple R	0.148474199
R Square	0.022044588
Adjusted R Square	-0.173546495
Standard Error	22.670097
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	57.92415233	57.92415233	0.112707529	0.750711414
Residual	5	2569.66649	513.9332979		
Total	6	2627.590642			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	16.77764652	15.35881504	1.092378967	0.324483109
X Variable 1	1.153280538	3.435251184	0.335719421	0.750711414

Mechanical Shear (Fe/Al)

Regression Statistics	
Multiple R	0.91
R Square	0.84
Adjusted R Square	0.80
Standard Error	9.30
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2194.9	2194.897	25.363	0.004
Residual	5	432.7	86.539		
Total	6	2627.6			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	4.293	4.842	0.887	0.416
X Variable 1	13.570	2.694	5.036	0.004

DATA ASSOCIATED WITH FIGURE 7 and (page 201)

Figure 7: Additional Volatile Solids Destruction from Digested Sludge from Traditional Mesophilic Anaerobic Digestion and Internal Recycle Ultrasonic Enhanced Anaerobic Digestion

Volatile Solids Destruction Data for 7-day Batch Digestions

Sample Label		**Control**	**Sonication Enhanced**	
	Sample	*VSD(%)*	*VSD(%)*	*Percent Increase In VSD*
A	Christiansburg	7.5	12.6	67.9
A2	Christiansburg-2	6.0	9.7	61.7
B	Pepper's Ferry	5.37	9.7	80.4
B2	Pepper's Ferry-2	4.4	6.0	34.0
C	DCWASA	11.2	12.6	12.1
D	LACSD-1	13.8	18.4	33.8
D2	LACSD-2	8.3	9.6	15.2
E	San Francisco	9.7	13.4	37.7

Statistical Analysis using T-Test assuming unequal variances

t-Test: Two-Sample Assuming Unequal Variances
Peppers Ferry 2

	Conventional	*Sonication*
Mean	4.1	5.8
Variance	3.6	0.3
Observations	5.0	5.0
Hypothesized Mean Difference	0.0	
df	5.0	
t Stat	-1.8	
P(T<=t) one-tail	0.0626	
t Critical one-tail	2.0	
P(T<=t) two-tail	0.1253	
t Critical two-tail	2.6	

t-Test: Two-Sample Assuming Unequal Variances
Christiansburg-2

	Conventional	*Sonication*
Mean	6.00	9.70
Variance	0.79	0.82
Observations	6	6
Hypothesized Mean Difference	0	
df	10	
t Stat	-7.128812791	
P(T<=t) one-tail	1.59204E-05	
t Critical one-tail	1.812461505	
P(T<=t) two-tail	3.18407E-05	
t Critical two-tail	2.228139238	

t-Test: Two-Sample Assuming Unequal Variances
San Fransisco

	Conventional	*Sonciation*
Mean	9.7	13.4
Variance	3.5	6.9
Observations	6	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-2.78	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.021	
t Critical two-tail	2.26	

t-Test: Two-Sample Assuming Unequal Variances

Los Angeles Count Sanitation District-2

	Conventional	*Sonication*
Mean	6.74	9.56
Variance	1.14	3.80
Observations	5	6
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.04	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.016	
t Critical two-tail	2.31	

t-Test: Two-Sample Assuming Unequal Variances

Blue Plains WWTP

	Conventional	*Sonication*
Mean	11.2	12.6
Variance	50.9	2.9
Observations	6.0	6.0
Hypothesized Mean Difference	0.0	
df	6.0	
t Stat	-0.5	
P(T<=t) one-tail	0.332	
t Critical one-tail	1.9	
P(T<=t) two-tail	0.665	
t Critical two-tail	2.4	

t-Test: Two-Sample Assuming Unequal Variances

Peppers Ferry 1

	Conventional	*Sonication*
Mean	5.37	9.69
Variance	0.97	4.47
Observations	6	6
Hypothesized Mean Difference	0	
df	7	
t Stat	-4.54	
P(T<=t) one-tail	0.001	
t Critical one-tail	1.895	
P(T<=t) two-tail	0.003	
t Critical two-tail	2.36	

t-Test: Two-Sample Assuming Unequal Variances

Los Angeles County Sanitation District -1

	Conventional	*Sonication*
Mean	13.8	18.4
Variance	17.4	34.6
Observations	6	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.58	
P(T<=t) one-tail	0.07	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.15	
t Critical two-tail	2.26	

t-Test: Two-Sample Assuming Unequal Variances

Christiansburg-1

	Conventional	*Sonication*
Mean	7.5	12.6
Variance	4.0	1.5
Observations	6	6
Hypothesized Mean Difference	0	
df	8	
t Stat	-5.3	
P(T<=t) one-tail	0.000355988	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.000711976	
t Critical two-tail	2.306	

DATA ASSOCIATED WITH FIGURE 8 and (page 203)

Figure 8: Correlation between Proposed Floc Models or Floc Components and the Degree of Digestion Enhancement Using Ultrasonic Disintegration in an Internal Recycle: (a) Monovalent to divalent cation ratio (Higgins and Novak, 1997b), (b) Monovalent to divalent plus trivalent (Park *et al.*, 2006b) (c) Monovalent plus Divalent to trivalent cations "lectin-like" material verse trivalents (d) divalent to trivalent ratio (e) tivalent bound organic matter only (f) Iron to Aluminum, debris only

Mass Concentration of Cations in the Digested Sludge Floc

	Sodium *mg/g-Solids*	**Potassium** *mg/g-Solids*	**Magnesium** *mg/g-Solids*	**Calcium** *mg/g-Solids*	**Iron** *mg/g-Solids*	**Aluminum** *mg/g-Solids*
San Francisco	2.08	3.26	8.90	16.98	28.16	12.33
DCWASA	0.43	0.94	1.99	15.35	91.84	6.68
Christiansburg 2	0.92	1.21	5.12	22.49	10.37	12.76
Peppers Ferry 2	1.19	1.56	3.49	23.92	12.28	13.65
Christiansburg 1	1.20	1.46	5.17	20.51	9.88	11.04
Peppers Ferry 1	1.15	1.11	3.14	25.11	12.58	13.99
LACSD 2	3.65	1.36	4.58	30.31	79.59	12.40
LACSD 1	3.33	1.34	3.22	24.27	79.65	10.72

Equivalence Concentration of Cations in the Digested Sludge Floc

	Sodium *meq/g-Solids*	**Potassium** *meq/g-Solids*	**Magnesium** *meq/g-Solids*	**Calcium** *meq/g-Solids*	**Iron** *meq/g-Solids*	**Aluminum** *meq/g-Solids*
San Francisco	0.091	0.08	0.73	0.85	1.51	1.37
DCWASA	0.019	0.02	0.16	0.77	4.93	0.74
Christiansburg 2	0.040	0.03	0.42	1.12	0.56	1.42
Peppers Ferry 2	0.052	0.04	0.29	1.19	0.66	1.52
Christiansburg 1	0.052	0.04	0.43	1.02	0.53	1.23
Peppers Ferry 1	0.050	0.03	0.26	1.25	0.68	1.56
LACSD 2	0.159	0.03	0.38	1.51	4.28	1.38
LACSD 1	0.145	0.03	0.26	1.21	4.28	1.19

Floc Associated Cation Ratios

Sample Label	Sample	Fe/Al	M/(D+T)	M/D	Fe+Al	(M+D)/T	T	D/T
		meq/meq	*meq/meq*	*meq/meq*	*meq/meq*	*meq/meq*	*meq/g-TS*	*meq/meq*
A	Christiansburg	0.43	0.028	0.062	1.8	0.875	1.76	0.82
A2	Christiansburg-2	0.39	0.020	0.046	2.0	0.817	1.98	0.78
B	Pepper's Ferry	0.43	0.021	0.052	2.2	0.713	2.23	0.68
B2	Pepper's Ferry-2	0.43	0.025	0.062	2.2	0.722	2.18	0.68
C	DCWASA	6.64	0.006	0.046	5.7	0.171	5.68	0.16
D	LACSD-1	3.59	0.026	0.121	5.5	0.303	5.47	0.27
D2	LACSD-2	3.10	0.026	0.103	5.7	0.369	5.65	0.33
E	San Francisco	1.10	0.039	0.110	2.9	0.608	2.88	0.55

Volatile Solids Destruction Data for 7-day Batch Digestions

Sample Label		Control	Sonication Enhanced	
	Sample	*VSD(%)*	*VSD(%)*	*Percent Increase In VSD*
A	Christiansburg	7.5	12.6	67.9
A2	Christiansburg-2	6.0	9.7	61.7
B	Pepper's Ferry	5.37	9.7	80.4
B2	Pepper's Ferry-2	4.4	6.0	34.0
C	DCWASA	11.2	12.6	12.1
D	LACSD-1	13.8	18.4	33.8
D2	LACSD-2	8.3	9.6	15.2
E	San Francisco	9.7	13.4	37.7

Regression Analysis conducted on data associated with Figure 8. Those set in which a linear fit was not used were not included due to limitations in the software package used.

Ultrasonics (M/D ratio)

Regression Statistics	
Multiple R	0.392
R Square	0.154
Adjusted R Square	0.013
Standard Error	24.578
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	657.82	657.82	1.09	0.34
Residual	6	3624.35	604.06		
Total	7	4282.17			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	66.39	24.16	2.75	0.03	7.27
X Variable 1	-312.83	299.77	-1.04	0.34	-1046.35

Ultrasonics (M/(D+T)

Regression Statistics	
Multiple R	0.19
R Square	0.04
Adjusted R Square	-0.12
Standard Error	26.23
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	155.3	155.3	0.2	0.7
Residual	6	4126.8	687.8		
Total	7	4282.2			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	30.5	27.64	1.10	0.31
X Variable 1	518.2	1090.55	0.48	0.65

Ultrasonics (D/T)

Regression Statistics	
Multiple R	0.82
R Square	0.67
Adjusted R Square	0.61
Standard Error	15.42
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2856.05	2856.05	12.02	0.01
Residual	6	1426.12	237.69		
Total	7	4282.17			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-0.5	13.6	-0.037	0.97
X Variable 1	81.1	23.4	3.466	0.01

Ultrasonics ((M+D)/T) Ratio

Regression Statistics	
Multiple R	0.81
R Square	0.66
Adjusted R Square	0.60
Standard Error	15.68
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2806.78	2806.78	11.41	0.01
Residual	6	1475.38	245.90		
Total	7	4282.17			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	-1.35	14.21	-0.09	0.93	-36.12
X Variable 1	77.25	22.87	3.38	0.01	21.30

DATA ASSOCIATED WITH FIGURE 9 and (page 203)

Figure 9: The Impact of Sonication and Mechanical Shear on Solution Divalent Cation Concentrations in WAS

Ultrasonic Exposure	**Net Solution Divalent Cations**	**Mechanical Shear Exposure**	**Net Solution Divalent Cations**
(sec)	*meq/L*	*minutes*	*meq/L*
0	0.000	0	0.000
15	0.246	5	0.000
45	0.305	10	0.034
60	0.303	20	0.307
90	0.270	40	0.405
120	0.314	60	0.534
180	0.626	90	0.808
300	0.239		
420	0.087		

DATA ASSOCIATED WITH FIGURE 10 (page 204)

Figure 10: The Relative Polysaccharide and Protein Content of WAS Disintegrated by Ultrasonics and Mechanical Shear

Mechanical Shear of Blacksburg WAS

Time *(min)*	**Protein** *mg-BSA/L*	**Time** *(min)*	**Polysaccharide** *mg-glc/L*		**Polysaccharide/Protein**
0	29.8	0	-17.3	0	0.00
5	88.4	5	20.9	20.9	0.24
10	107.9	10	23	23	0.21
20	185.2	20	55.8	55.8	0.30
40	263.3	40	101	101	0.38
60	278.5	60	71.7	71.7	0.26
90	308.9	90	80.4	80.4	0.26
				MEAN	0.25

Ultrasonic Disintegration of Blacksburg WAS

Time *(min)*	**Polysaccharide** *mg-glc/L*	**Protein** *mg-BSA/L*	**Polysaccharide/Protein**
0	0	0	0.00
0.5	0	0	0.00
1	1	23	0.03
2.5	35	69	0.51
5	96	144	0.66
10	145	237	0.61
15	172	358	0.48
20	298	402	0.74
		MEAN	0.62

DATA ASSOCIATED WITH FIGURE 11 (page 204)

Figure 11: The Release of Bound Protein from Ferric and Aluminum Hydroxide Floc Exposed to 20 kHz Ultrasonic Disintegration

Iron Hydroxide bound Material

Iron	**Centrate BSA**	**Std. Deviation**
Time (sec)	*mg/L*	*mg/L*
Before Iron addition	1069.5	11.9
Optimum Dose	9.2	0.8
0	105.2	7.0
119.8	81.9	1.0
299.8	62.5	5.5
480.0	38.9	2.7
659.7	35.7	1.5

Aluminum Hydroxide bound Material

Aluminum	**Centrate BSA**	**Std. Deviation**
Time(sec)	*mg/L*	*mg/L*
Before Aluminum addition	1069.5	11.9
After Aluminum Addition	184.9	18.3
0	288.8	9.7
120.0	230.8	40.2
240.3	171.0	7.7
479.8	178.1	22.1
600.3	176.3	8.8