Effect of Addition of High Strength Food Wastes on Anaerobic Digestion of Sewage Sludge

Ramola Vinay Vaidya

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Environmental Engineering

Gregory D. Boardman

John T. Novak

Husen Zhang

April 29, 2015

Blacksburg, VA

Keywords: Anaerobic digestion, High-strength waste (HSW), Long chain fatty acids, Gas production, Biosolids Odors

Effect of Addition of High Strength Food Wastes on Anaerobic Digestion of Sewage Sludge

Ramola Vinay Vaidya

Abstract

Anaerobic co-digestion of municipal sludge and food wastes high in chemical oxygen demand (COD) has been an area of interest for waste water treatment facilities looking to increase methane production, and at the same time, dispose of the wastes and increase the revenue. However, addition of food wastes containing fats, oils and grease (FOG) to the conventional anaerobic digestion process can be difficult and pose challenges to utilities. Incorporating these wastes into the treatment plants can potentially inhibit the digestion process.

In this study four lab-scale, anaerobic digesters were operated under mesophilic conditions and fed municipal sludge. One of them served as the control, while the other three digesters were fed with different volumetric loadings of juice processing waste, cheese processing waste (whey), dissolved air flotation waste (DAF) from a food processor, and grease trap waste (GTW), in addition to the municipal sludge. The impact of these high strength wastes (HSWs) on digester performance was analyzed for a total period of 150 days.

Among the parameters analyzed were pH, total and soluble COD (tCOD and sCOD), Total and Total Volatile Solids (TS and TVS), Total Ammonia Nitrogen (TAN), Total Kjeldahl Nitrogen (TKN), Volatile Fatty Acids (VFA), Long Chain Fatty Acids (LCFA), and alkalinity. Biogas was collected and analyzed for methane content. The dewatering characteristics of digested sludge were also studied. Volatile organic sulfur compounds were analyzed on the dewatered sludge in order to monitor odors.

This study showed that different high strength wastes have different impacts on digester performance. HSWs have the ability to degrade along with municipal sludge and to increase biogas production. However, anaerobic digestion can be inhibited by the presence of FOG, and addition of these wastes might not always be cost effective. Careful selection of these wastes is necessary to ensure stable digester operation, while bringing about increases in gas production. Utilities need to be cautious before adding any high strength wastes to their digesters.

Acknowledgements

I would like to express my gratitude towards my advisors Dr. Gregory Boardman and Dr. John T. Novak who have helped me and guided me throughout this research. Without their mentorship and expertise in this field, this project would not have reached completion. I would also like to thank Dr. Husen Zhang for providing his inputs.

I would like to sincerely thank Robert Wimmer and Michael Hanna from Black and Veatch Corporation who gave me an opportunity to work on this project and provided the funds for this project. I would also like to thank Don Riggleman and Richard Wadkins from Opequon Water Reclamation Facility (OWRF) for providing the sludge used in this study by shipping it promptly on a weekly basis.

I am grateful to Julie Petruska and Jody Smiley for not only training me in the lab to carry out experiments but also for being patient with me.

A special thanks to all my lab mates, Peerawat Charuwat, Siddhartha Roy and Kaivalya Kulkarni for keeping the reactors running even in my absence and Ankit Pathak, for helping me set up the digesters and providing his insights on the research.

Finally, I would like to mention my friends and family for their relentless love and support throughout this time.

Table of Contents

List of Tables

Page

List of Figures

Page

Attribution

Dr. Gregory D. Boardman, *Ph.D., P.E., DEE, F.ASCE*, Professor, Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA – 24061

Dr. John T. Novak, *Ph.D., P.E.*, Professor Emeritus, Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA – 24061

Robert Wimmer, *P.E.*, Wastewater Process Specialist, Water Division, Black & Veatch Corporation, Finksburg, MD – 21048

Michael Hanna, *P.E.*, Project Manager, Black and Veatch Corporation, Finksburg, MD – 21048

Dr. Boardman served as the principal investigator of the project, while Dr. Novak served as the co-principal investigator. Most of the analyses and tests carried out were decided by Dr. Boardman and Dr. Novak. The goals and the direction of the project were decided by Robert Wimmer and Michael Hanna.

Dr. Boardman, Dr. Novak, Robert Wimmer and Michael Hanna are all equally credited as co-authors for chapter 3 in this document.

The authors would like to thank Black and Veatch Corporation and the City of Winchester for funding this study via account number 457915.

Chapter 1

Introduction

For several years most utilities have been practicing anaerobic digestion as a part of handling and disposal of biosolids. Sludge obtained after treating waste-water needs to be disposed of properly. After digesting this sludge anaerobically, it can be used for direct landfilling and land applications. With the production of biogas during this process, the plant can be made more sustainable in terms of meeting their energy requirements. Recently, utilities have been exploring the possibility of feeding different organic wastes with high amounts of degradable COD to digesters in addition to the sewage sludge. This helps in disposing of the organic wastes and has shown to increase the biogas production.

This research was conducted for the Opequon Water Reclamation Facility (OWRF), a wastewater treatment plant in Winchester, Virginia. Anaerobic digesters are being constructed at the facility, and the addition of high strength wastes is incorporated into the design. The primary and secondary sludge used for the purposes of this study was obtained from OWRF, while food wastes were collected at cheese and juice processing industries in the vicinity. Also included in the study were grease trap waste (GTW) and dissolved air flotation waste (DAF) from a food processor, with CODs greater than 50,000 mg/L, which were provided by a local grease trap waste hauling service in Winchester, Virginia.

Research Objectives

The main purpose of this study was to evaluate the effect of different types of high strength wastes on the anaerobic co-digestion process. The high strength wastes included in this project were as follows:

1. Juice waste
2. Cheese processing waste (whey)
3. Dissolved air flotation waste (DAF) from a food processor
4. Grease trap waste (GTW)

These wastes are being considered as possible options by Opequon Water Reclamation Facility (OWRF) as high strength wastes to be added to their anaerobic digesters. In the interest of checking the feasibility of adding these wastes to the digesters, four lab-scale digesters (9.75 L) were set up for this study. The digesters were fed with primary and waste activated sludge (WAS) in the ratio of 60:40. These digesters were maintained at a temperature of 37^{o}C to create mesophilic conditions. The period of study was 150 days, during which time the digesters were batch fed daily to provide a solids retention time (SRT) of 15 days.

The digester maintained as a control was fed with only sewage sludge mixture (Primary to WAS in the volumetric ratio of 60:40), while the other three digesters were fed with high strength waste in varying amounts in addition to the sludge mixture. The digester performance was analyzed by monitoring pH, total and soluble COD, total and volatile solids, total ammonia nitrogen, total Kjeldahl nitrogen, volatile fatty acids, long chain fatty acids, and alkalinity regularly. Biogas was collected daily and analyzed for methane content.

To evaluate the impact of these HSWs on the sludge dewatering, the digested sludge was dewatered and tested for solids content post dewatering. The amount of polymer required to best dewater the digested sludge was estimated using capillary suction time (CST). This dewatered sludge was further incubated and analyzed for volatile organic sulfur compounds (VOSCs) to characterize odors.

The anaerobic digestion process was inhibited by addition of GTW, resulting in digester failure. An effort was made to understand this inhibition and to find possible reasons for this failure. Long chain fatty acids were measured in the digested sludge and in the wastes themselves to study their possible inhibitory effects. To get a better perspective of the inhibition by grease trap waste, the organic loading at which the digester failed was studied and the feed was tested at different volumetric loading rates of GTW.

Finally, addition of a buffer to the digestion process was studied in order to prevent inhibition. The impact of addition of this buffer was closely monitored and conclusions were drawn on the basis of these results.

Chapter 2

Literature Review

Anaerobic Digestion

Anaerobic digestion has been utilized worldwide for about a century. It is an established process used for degrading organic matter in the form of solids and liquid wastes (Bouallagui *et al*., 2010). Anaerobic digestion is a complex biological process in which micro-organisms degrade the solid mass, thereby reducing the odors and pathogens in the biosolids with concurrent production of methane in the absence of oxygen. This process can be found in landfills, sediments, flooded soils and animal intestines (Parkin and Owen, 1986; Chynoweth *et al*., 2001). Digested sludge can then be dewatered and used for land application, depending on the pathogen content of the sludge. Thus, anaerobic digestion helps in better handling and disposal of biosolids.

Compared to aerobic treatment, anaerobic treatment is relatively inexpensive and is easy to set up on any scale. With production of methane, energy can be recovered rather than consumed, as in the case of aerobic digestion. High volumetric loading rates can be established in anaerobic systems, which helps in making smaller systems that produce lower amounts of excess sludge. This excess sludge can be stabilized and easily dewatered. Anaerobic systems can be used to recover useful products, such as ammonia and sulfur with special treatment, post-digestion (Lettinga, 1995).

A conventional anaerobic reactor consists of a single continuously stirred tank reactor (CSTR), fed once or more per day and maintained at mesophilic or thermophilic temperatures with a hydraulic retention time of 15-25 days (Grady *et al*., 2011). About 60% reduction in organic matter can be achieved with a loading rate of 1.7 kg TVS m^{-3} day^{-1}. The biogas formed has usual composition of 60% to 70% methane and 30% to 40% carbon dioxide and traces of hydrogen sulfide and water vapor (Chynoweth *et al*., 2001; Lettinga, 1995). Other designs such as two phase digesters, upflow or expanded bed reactors, and egg shaped reactors are also being used by municipal utilities (Grady *et al*., 2011).

Factors affecting anaerobic digestion

The following factors are necessary to achieve optimum design and efficient operation:

Solids Retention Time (SRT): Solids retention time (θ_c) can be defined as mass of solids contained in the reactor divided by mass of solids wasted or discharged from the system per day (Metcalf and Eddy, 2003). It is essential that a sufficient SRT be maintained to ensure complete degradation of complex organic matter to methane and carbon dioxide and to prevent washout of the bacteria (Parkin and Owen, 1986). The loading potential of the system will depend upon the amount of sludge retained in the system. Higher SRTs will result in lower loading capacity of the digester (Lettinga, 1995). Thus SRT is an important parameter when designing a digester and will eventually dictate the cost of the system. McCarty has developed a correlation between amount of methane produced per unit organic matter destroyed and SRT (McCarty, 1972). According to this correlation, the methane production increases with increase in the SRT. This emphasizes the importance of SRT in any anaerobic digestion system. Typically SRTs of 15-25 days are used; however, it is not uncommon to have an SRT of as low as 10 days (Grady *et al*., 2011). EPA regulations require a minimum of SRT of 15 days to achieve Class B biosolids (EPA, 2003).

Mixing: The main purpose of mixing is to provide sufficient contact between bacteria, bacterial enzymes and substrates. Proper mixing in a digester is essential to make sure no sludge in the reactor is deprived of substrate (Lettinga, 1995). Inefficient mixing results in inefficient use of digester volume, thereby decreasing the operational SRT. Improper mixing can lead to stratification and temperature gradients, scum formation and digester foaming (Parkin and Owen, 1986). Lack of proper mixing can reduce the effective volume of a full scale digester by as much as 70% (Monteith and Stephenson, 1981; Torpey, 1955). In this regard, egg-shaped digesters, commonly used in Europe are better than cylindrical digesters as the design ensures better mixing (Nelson and Baley, 1979).

Temperature: Anaerobic digesters are usually maintained at either mesophilic (30-38^{o}C) or thermophilic temperature (48-60^{o}C) (Parkin and Owen, 1986). Studies have shown that thermophilic digestion leads to better organic removal, higher methane production and improved sludge dewatering characteristics as compared to mesophilic digestion (Parkin and Owen, 1986; Gavala *et al*., 2003). However, most municipal digesters are operated at mesophilic temperature

due to greater stability of operation and lower energy requirements (Gavala *et al*., 2003). Digesters tend to be sensitive to temperature variations. Thus a stable operating temperature is essential for the digestion process (Grady *et al*., 2011).

pH: The pH range for stable anaerobic digestion is between values 7 to 7.6. (Clark and Speece, 1970). Since methanogens are most sensitive to pH changes, system changes such as those in organic loading, temperature or presence of toxic materials can lead to a sudden drop in pH unless the system contains sufficient buffering capacity. Maintaining adequate alkalinity (2,500 – 3,500 mg/L) can help to keep the pH stable (Parkin and Owen, 1986). Thus pH can be used as indicator of digester health.

Absence of toxic materials: Inhibition of the digestion process due to toxic agents can lead to digester failure (Swanwick *et al.,* 1969). The nature and concentration of a substance will determine its toxic nature. Many substances stimulate the digestion process at low concentrations, but can be inhibitory at higher concentrations. Total ammonia nitrogen is stimulatory at concentrations of 300-1,000mg/L, while at concentrations above 3,500mg/L it can be strongly inhibitory (Parkin and Owen, 1986). Heavy metals in soluble form can be inhibitory at concentrations of 0.1 – 10mg/L (Kugelman and Chin, 1971). Table 2.1 gives a list of inorganic compounds that are considered to be inhibitory to anaerobic digestion. Toxicity can be minimized by ensuring adequate mixing, suitable environment and sufficient SRT (Parkin and Owen, 1986).

Table 2.1. Concentrations of Inorganics reported to be inhibitory to Anaerobic Digestion (Parkin Gene F., Owen William F., Fundamentals of Anaerobic Digestion of Wastewater Sludge. Journal of Environmental Engineering, 1986. 112(5): p. 867-920, Used with permission from ASCE, 2015). All concentrations are in mg/L

Substance	Moderately Inhibitory	Strongly Inhibitory
Na^+	3,500-5,500	8,000
K^+	2,500-4,500	12,000
Ca^{2+}	2,500-4,500	8,000
Mg^{2+}	1,000-1,500	3,000
NH_3-N	1,500-3,000	3,000
S^{2-}	200	200
Cu^{2+}	–	0.5 (soluble) 50-70 (total)
Cr (VI)	–	3.0 (soluble) 200-260 (total)
Cr (III)	–	180-420 (soluble)
Ni^{2+}	–	2.0 (soluble) 30 (total)
Zn^{2+}	–	1.0 (soluble)

Anaerobic Co-digestion

In addition to the conventional anaerobic digestion, wastewater treatment plants are seeking alternatives to increase the methane production. Co-digestion is the process of digesting municipal sewage sludge with other organic wastes to get a higher methane recovery, while also disposing of the organic wastes. The advantages of co-digestion technology is that it increases gas production during anaerobic digestion and provides cost savings to the plant, since multiple waste streams can be processed in a single facility. Moreover, recovery of methane helps in reducing greenhouse gas emissions to the atmosphere (Mondragon *et al*., 2006).

A variety of solid and liquid organic wastes have been tested and co-digested with municipal sewage sludge in the literature. Municipal sewage sludge, organic fraction of municipal solid waste (OFMSW) and cattle manure are the main wastes used. These are usually co-digested with industrial organic wastes, wood wastes and farm wastes (Mondragon et al., 2006). All these organic wastes have biodegradable chemical oxygen demand. In past years a number of treatment options have been considered for the disposal of these wastes. These options include land application, landfilling, composting, chemical hydrolysis of cellulose for production of ethanol, biodiesel production, manufacturing of soaps, lubricants and anaerobic co-digestion (Callaghan et al., 1999; Long et al., 2012).

Digestion of different wastes

Several food wastes can be found in the literature. Co-digestion with food and yard wastes have resulted in increases in methane production and the volumetric production of biogas by 1.7 times as compared to usual anaerobic digestion (Brown and Li, 2013). Food wastes such as onion juice have resulted in a TVS destruction of 72 to 91% while wastes from potatoes have shown 70% TVS reduction and 64% COD removal in a two stage digestion process (Romano and Zhang, 2008; Zhu et al., 2008). Digestion of food wastes collected from the city of San Francisco, California, resulted in an average methane content of 73% and average TVS destruction of 81% in 1 Liter, batch-fed reactors (Zhang *et al*., 2007). In Portugal, different types of coffee wastes were added to granular sludge collected from a reactor treating brewery effluent. Four of the five wastes tested showed high reduction of TS (50-73%) and TVS (75-80%) and a methane yield of 0.24–0.28 m^3 CH_4/kg $TVS_{initial}$ were achieved (Neves *et al*., 2006).

Studies related to organic fraction of municipal solid wastes (OFMSW) have shown positive results. It was found that effluent pH was higher, COD reductions were greater and VFA concentrations were lower when co-digesting industrial sludge with OFMSW in anaerobic landfill reactors than while digesting the industrial sludge alone (Agdag and Sponza, 2005). High solids digestion of OFMSW and pre thickened sewage sludge resulted in gas production rates of 5.5L biogas/L active volume /day at a retention time of 17 days and an organic loading of 11.2 g TVS/ L active volume/ day (Stroot *et al*., 2001).

Co-digestion of cow manure and food processing waste containing a mixture of cheese whey, animal blood, used cooking oil, fried potato residue was inhibited at 35^{o}C due to possible ammonia inhibition from the food processing waste mixture. However, co-digestion at thermophilic temperature (55^{o}C) was shown to be more efficient than digestion of manure alone (Yamashiro *et al*., 2013). In a two phase, pilot-scale study involving co-digestion of OFMSW, cow manure and cotton gin waste, a synergistic effect was observed, improving biodegradation and resulting in higher methane yield, as compared to the digestion of manure alone. Also, a TS reduction of 52-78% was observed as compared to 16% TS reduction while digesting cow manure alone (Maccias-Corral *et al*., 2008). Similar results were obtained while co-digesting cheese whey and cow manure (Bertin *et al*., 2013). A few other experiments related to addition of wastewater from olive mills and glycerol to boost the biogas production were carried out successfully (Fountoulakis *et al*., 2010; Athanasoulia *et al*., 2012).

Recently, a number of utilities have been adding fats, oils and grease (FOG) from the wastes of restaurants, kitchens and cafeterias. It is illegal to directly release FOG into sewers. Once it is released in the sewers, this FOG can accumulate on pipe walls and form deposits. This results in reduction in conveyance capacity and pipe overflows, resulting in increased expenses for maintenance and repair (He *et al*., 2011; Long *et al*., 2012). For segregating FOG from rest of the waste stream, municipalities install 'grease traps' or 'grease interceptors', which are gravity separation devices that retain the grease and food solids by providing sufficient time for flotation and sedimentation of influent wastes. Grease traps are around 50 gallons while grease interceptors are about 1000-2000 gallons in size. Regular removal of grease from these devices can prevent sewers from clogging (Long *et al*., 2012).

In Greece, a 55% increase in biogas was obtained after addition of grease from sewage sludge at an organic loading rate of 3.5 kg TVS/m^3/day. The grease constituted about 60% of the total TVS feed loading (Noutsopoulos *et al*., 2013). In another study, GTW from a meat processing plant resulted in an increase in methane production at a loading of 46% of feed TVS. However, at higher loadings (55 and 71% of feed TVS), the methane production declined due to LCFA inhibition (Luostarinen *et al*., 2009). Similarly a 65% increase in methane production occurred after adding

GTW at a 4% loading (v/v). Process inhibition and decrease in biogas production occurred at higher GTW loadings (Zhu *et al*., 2011). Anaerobic co-digestion of restaurant grease waste and municipal wastewater sludge led to an increase in biogas production by up to 65% and 120%, at a feed loading of 150% (5.57 g COD/L day) and 190% COD (3.84 g COD/L day) relative to the control receiving only municipal wastewater sludge (mixture containing primary sludge and thickened waste activated sludge in the ratio of 4:1), at steady state operation. However, GTW loading at 300% COD (8 g COD/L day) relative to the control, in the feed led to a decline in pH, alkalinity, biogas production, and methane. The study was carried out at mesophilic temperature (37^{o}C) at a 20 day SRT. Pyrosequencing showed that the number of aceticlastic methanogens, those responsible for methane production, decreased at higher loadings (8 g COD/L day). (Razaviarani and Buchanan, 2014). The highest increase in methane seen was 317% in North Carolina, after co-digestion of grease interceptor waste and thickened waste activated sludge at a 20% (v/v) grease loading. At loadings higher than 20%, the process was inhibited (Wang *et al*., 2013).

Problems associated with addition of FOG

It is difficult to characterize FOG, as grease from different sources can differ making it difficult to predict how it will behave in a digester study. This could be due to different degradability and toxicity at different degrees of saturation of FOG. The organic loading rate for FOG can vary greatly. At higher loadings of GTW, the methane production can decline, resulting in a drop in pH and biogas production, and increases in VFA and LCFA concentrations (Cuetos *et al*., 2008; Razaviarani and Buchanan, 2014; Luostarinen *et al*., 2009; Zhu *et al*., 2011). Other studies have shown that grease from slaughter house waste resulted in foaming in the digester despite mechanical stirring. Lipids have a tendency to form foam and floating aggregates that can cause stratification within the digester (Salminen *et al*., 2001; Cuetos *et al*., 2008).

In some studies, full scale experiments were unable to obtain the results seen in pilot-scale experiments (Bailey, 2007; Muller *et al*., 2010). Full-scale experiments may differ from laboratory scale experiments and further studies need to be carried out to determine the organic loading rates

of GTW that a full scale treatment plant can successfully incorporate into their digestion process (Long *et al*., 2012).

Table 2.2. Summary of FOG added

Source of FOG	Maximum Loading Rate	Reactor Configuration	Biogas /Methane Production	References
Domestic waste water (obtained by surface skimming from primary settling tanks)	3.5 kg TVS/m^3/day	Single stage 3 L CSTR batch fed once a day 15 day HRT Mesophilic temperature	700 m^3 biogas/ton TVS $_{added}$	Noutsopoulos *et al*., 2013
Meat processing plant	3.46 kg TVS/m^3/day	5 L CSTR batch fed once a day, five days a week 16 day HRT Mesophilic temperature	463 m^3 CH_4/ton TVS $_{added}$	Luostarinen *et al*., 2009
Local restaurants and food processing facilities	-	100ml batch fed CSTR Mesophilic temperature	1034 m^3 CH_4/ton TVS $_{GTW}$	Zhu *et al*., 2011
Thickened WAS plus restaurant food waste	20% (v/v) of TVS	6 L CSTR batch fed every other day 20 day SRT Mesophilic temperature	0.752 m^3 CH_4/ kg TVS $_{added}$	Wang *et al*., 2013
Restaurant garbage oil	Substrate to inoculum ratio of 0.46	190 ml CSTR Mesophilic temperature	418 mL CH_4/g total TVS	Li *et al.*, 2011
Local waste collection company	5.57 g COD/ L day	8 L CSTR batch fed once a day 20 day SRT Mesophilic temperature	0.5 L CH_4/ g TVS $_{added}$	Razaviarani and Buchanan, 2014

Inhibition of Anaerobic Digestion and Co-digestion

A substance is said to be inhibitory when it causes a detrimental effect on the bacterial growth and population (Chen *et al*., 2008). Inhibition results in a decline in methane production and accumulation of organic acids (Kroeker *et al*., 1979). A variety of substances in the waste stream can be toxic, depending on their concentration and the origin of waste water. These include LCFAs, ammonia, sulfides, light metal ions, heavy metals and other organics (Parkin and Owen, 1986; Chen *et al*., 2008).

LCFAs

Sewage sludge contains lipids consisting of neutral fats and LCFAs. These neutral fats can hydrolyze to LCFAs and glycerol. The degradation of LCFAs takes place via β oxidation pathway that leads to the formation of acetate and H_2, which are further converted to methane (Hanaki *et al.,* 1981; Long *et al*., 2012). The LCFA degradation reaction is as follows:

$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_n - 2COOH + CH_3COOH + H_2 \quad (1)$$

(Kim *et al*., 2004). Unsaturated fatty acids degrade to saturated fatty acids and further by β oxidation (Novak and Carlson., 1970).

During the acid phase of anaerobic digestion, Heukelekian and Mueller (1958), found that LCFA degradation was incomplete and methane was not produced. Oleic and stearic acids are major constituents of vegetable oils. Lalman and Bagley (2001), showed that oleic acid degraded anaerobically at 21^{o}C, while stearic acid degraded slowly in unacclimated cultures. Also, oleic acid inhibited methane production at a concentration 30 mg/L, while stearic acid did not inhibit methane production at this concentration (Lalman and Bagley, 2001). In another study, it was seen that oleate and stearate were both inhibitory at concentrations of 0.1-0.2 g/L and 0.5 g/L respectively, in a thermophilic (55^{o}C) environment, with the toxic effect of oleate being slightly higher than stearate. This toxicity effect was irreversible (Angelidaki and Ahring, 1992).

Hanaki *et al*. (1981), showed that addition of soluble calcium salts (8.7 mmol/L $CaCl_2$) reduced the inhibitory effects of LCFAs (upto 1000 mg/L of oleate), but this addition was ineffective after

the anaerobic culture was exposed to LCFAs for more than 24 hours. Cavaleiro *et al*. (2008), suggested application of pulses of substrate into the biomass, leading to accumulation of LCFA, and degrading them later. This seemed to increase the tolerance of acetotrophic methanogens towards LCFAs. LCFAs can inhibit the digestion process and get accumulated in the digester (Cavaleiro *et al*., 2008). Toxic effects of oleic, caprylic, capric, lauric and myristic acids were studied by Koster and Cramer (1987). Table 2.3 shows the inhibitory concentrations of single long chain fatty acids to aceticlastic methanogens at 30^{o}C. It was seen that the toxic effect of LCFAs increases with increasing double bonds. Lauric acid was found to be most inhibitory, while caprylic was slightly inhibitory. Oleic was almost as inhibitory as lauric. A synergistic effect was seen when presence of lauric acid enhanced the toxic effect of capric and myristic acids (Koster and Cramer, 1987).

Table 2.3. Inhibitory concentrations of single long chain fatty acids to aceticlastic methanogens at 30^{o}C. All concentrations are in mM. (Koster I. W., Cramer A., Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. Applied and Environmental Microbiology, 1987. 53: p. 403-409, Used under fair use, 2015)

Fatty acid	Inhibitory concentrations
caprylic acid	6.75
capric acid	2.6
lauric acid	1.6
myristic acid	2.6
oleic acid	2.4

Several mechanisms for LCFA toxicity have been proposed in the literature. The most commonly found are surfactant effects, sludge flotation and washout, digester foaming, and substrate and product transport limitation (Long *et al*., 2012; Henderson, 1973). According to Pereira *et al*. (2003) multiple inhibition mechanisms might be occurring simultaneously during LCFA inhibition, making it difficult to ascertain that one particular mechanism is responsible. It has been reported that LCFAs at low concentrations are inhibitory to gram positive bacteria, but not gram negative bacteria. Since methanogens have a cell wall that resembles that of gram positive bacteria, they can be prone to LCFA inhibition (Koster and Cramer, 1987; Kabara *et al*., 1977). LCFAs get

adsorbed onto the cell walls, preventing the transport of substrate in the sludge and methane out of the sludge. Addition of LCFAs to biomass leads to sludge flotation and washout (Rinzema *et al*., 1989; Pereira *et al*., 2003). From these mechanisms it can be seen that inhibition of anaerobic digestion and digester failure can be expected during addition of FOG.

Ammonia inhibition

During digestion, organics containing nitrogen produce ammonia nitrogen and bicarbonate alkalinity (Parkin and Owen, 1986). According to McCarty (1964), ammonia nitrogen concentrations between 50 and 200 mg/L are beneficial since it is an essential nutrient. However, ammonia can be toxic to digestion. Free ammonia is considered to be inhibitory since it is membrane permeable (Kroeker *et al*., 1979). Since pH and temperature can drastically change the concentration of ammonia nitrogen, thermophilic digesters are more prone to ammonia inhibition. It was seen that TAN concentrations greater than 4 g/L caused severe inhibition (methane production declined by 39%) in a thermophilic experiment with a 7 day HRT (Sung and Liu, 2003). In another thermophilic experiment, ammonia nitrogen concentrations of 1.1 g N/L or more in swine and cattle manure caused inhibition and lower biogas yield (Hansen *et al*., 1997). In mesophilic digesters, inhibitory concentration of TAN were around 1700-1800 mg/L for an unacclimated inoculum, whereas after acclimation, the inhibitory concentration was in the area of 5000 mg/L (Yenigun and Demirel, 2013). However, the ammonia toxicity was reversible and can be removed by lowering of pH and to reduce the free ammonia concentration (Kroeker *et al*., 1979).

Sulfides

Sulfides are formed by reduction of sulfates by sulfate reducing bacteria (SRB). Competition for organic and inorganic substrates by SRB can result in inhibition of anaerobic digestion, resulting in decrease in methane production. Sulfide itself can be due to toxic to certain bacteria (Chen *et al*., 2008). Gas production decreased by about 30% after addition of 200 mg/L of sulfide to anaerobic digesters (Rudolfs and Amberg, 1952). Sulfide removal techniques such as stripping, coagulation by precipitation of FeS, oxidation, and precipitation can prevent sulfide toxicity (Chen *et al*., 2008).

Metal Ions

Toxicity of salts is mainly determined by cations such as sodium, potassium, calcium and magnesium (McCarty and McKinney, 1961). These cations stimulate microbial growth at moderate concentrations, but at higher concentration can lead to inhibition of anaerobic digestion. Concentrations causing inhibition to unacclimated systems were 0.025M for Na^+, 0.1M for K^+ and Ca^{2+}, and 0.05M for Mg^{2+} (Kugelman and Chin, 1971). It was also found that acclimation was possible and addition of antagonistic cations can help reduce toxicity. Soluble heavy metals such as copper, nickel, zinc and chromium VI are reported to be highly toxic even at low concentrations (Kugelman and Chin, 1971).

Organic compounds

Organic compounds that are poorly soluble in water or adsorb to sludge surfaces can accumulate in digesters. This can cause bacterial cell membranes to swell and leak, leading to cell lysis (Chen *et al*., 2008). Included among these organic compounds are alkyl benzenes, phenols, alcohols, halogenated benzenes, nitrophenols, amines, amides, nitriles and carboxylic acids. Chlorophenols disrupt the proton gradient across membranes and affect the energy transduction of cells. Among the chlorophenols, pentachlorophenol (PCP) was found to be most toxic, causing inhibition to the acidogens and methanogens at a concentration of approximately 0.5 to 10 mg/L (Chen *et al.*, 2008). Table 2.4 shows inhibitory concentrations of certain organic compounds.

Table 2.4. Concentrations of Organics reported to be inhibitory to Anaerobic Digestion (Parkin Gene F., Owen William F., Fundamentals of Anaerobic Digestion of Wastewater Sludge. Journal of Environmental Engineering, 1986. 112(5): p. 867-920, Used with permission from ASCE, 2015). All concentrations are in mg/L

Organic Compound	Inhibitory concentration
Formaldehyde	50 - 200
Chlorobenzene	0.5
Ethyl Benzene	200 - 1000
Ethylene Dichloride	5
Kerosene	500
Linear alkyl benzene sulfonates (detergent)	1% of dry solids

Sludge Dewatering and Odors

The final step, post-digestion is sludge conditioning and treatment to make it viable for land filling or land application. For this purpose, separating the liquid phase from the biological solids and further dewatering of these biosolids are important steps (Higgins and Novak, 1997). Several studies have shown that both aerobic and anaerobic digestion lead to poor dewatering characteristics (Novak *et al*., 1977; Bruss *et al*., 1993). Sludge settling and dewatering depend on bioflocculation. A large portion of biopolymer gets incorporated into the activated sludge floc matrix. The portion that remains unattached are called biocolloids and are responsible for polymer conditioning demand and deterioration of sludge properties (Novak *et al*., 2003). The extent of bioflocculation depends on many factors such as temperature, cations, turbulence, dissolved oxygen concentration and substrate loading at the plant (Ahmed, 2007).

Many studies have been carried out on the effect of cation content on floc formation and sludge dewatering. A monovalent to divalent cation ratio of more than 2 seemed to deteriorate the sludge dewatering characteristics (Higgins and Novak, 1997; Ahmed, 2007). Higgins and Novak (1997), showed that a minimum concentration of 0.72-2 meq/L of calcium and magnesium each was necessary for good settling and dewatering. According to the authors, sludge dewatering properties for wastewaters with imbalanced ratios of monovalent to divalent cations can be improved by addition of appropriate cations to the feed (Higgins and Novak, 1997).

Bruss *et al*. (1993) showed that reduction of iron during storage of activated sludge under anaerobic conditions can lead to poor dewatering. Further, Neilsen and Keiding (1998) found that addition of sulfide to activated sludge led to disintegration of floc due to reduction of Fe(III) to FeS. This disintegration in floc further affects the sludge dewatering capability. It was seen that addition of sulfide (H_2S) to activated sludge led to the production of amorphous iron sulfide and elemental sulfur. For one mole of sulfide added to the sludge, 0.68 moles of FeS and 0.37 moles of S^o was produced. The disintegration of floc was quantified by increase in the turbidity after addition of sulfide. The turbidity increased from 0.1 NTU for no sulfide addition to 0.5 NTU for addition of 4.8 mM of sulfide. The effect on dewatering was seen by increase in specific resistance to filtration. This increase was found to be almost linear with increase in turbidity.

Biosolids odor is one of the main limiting factors in the land applications of digested sludge. Volatile sulfur compounds (VSCs) are among the major, odor-causing compounds in anaerobically digested biosolids (Higgins *et al*., 2006). The VSCs of significance are methanethiol (MT), hydrogen sulfide (H_2S), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS). Higgins and Forbes showed that the concentrations of the VSCs produced during cake storage continue to increase, until they reach a peak and then reduce (Forbes *et al*., 2003; Higgins *et al*., 2002). The production of VSCs takes place in three steps. Initially sulfur-containing amino acids and cysteine are biodegraded to H_2S, while methionine gets biodegraded to MT. Further H_2S gets methylated to form MT, while MT is methylated to form DMS. Finally, MT is oxidized to DMDS (Higgins *et al*., 2006).

The odors generated vary according to sludge treatment and handling processes (Novak *et al*., 2006). According to Muller *et al*. (2004), there is a positive correlation between cake solid concentration and VSCs. They also proposed that applying shear force by dewatering equipment, such as centrifuges and belt presses, makes the floc-bound protein more bioavailable in post dewatered solids. In the same study, the authors showed that addition of polymer and application of shear force at the same time helped to reduce biosolids odors. In a study carried out by Novak *et al*. (2006), it was concluded that presence of iron in wastewater determined the production of H_2S. H_2S reacts with iron and precipitates as FeS. In absence of iron, H_2S odors might be prevalent for a long period. Thus, addition of iron in wastewater treatment plants can help to reduce odors generated from H_2S. Another observation from this study was that the odors can be dissipated completely by storing dewatered cakes for 20-30 days at 22-25^{o}C. This sludge can then be land applied.

References

1. Ağdağ, O.N. and D.T. Sponza, Co-digestion of industrial sludge with municipal solid wastes in anaerobic simulated landfilling reactors. Process Biochemistry, 2005. 40(5): p. 1871-1879.

2. Alvarez J. M., M.S., Llabres P., Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technology, 2000. 74: p. 3-16.

3. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., van Lier, J. B., Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Science and Technology, 2009. 59(5): p. 927-934.

4. Angelidaki I., A.B.K., Effect of free long chain fatty acids on thermophilic anaerobic digestion. Applied Microbiology and Biotechnology, 1992. 37: p. 808-812.

5. Appels, L., et al., Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 2008. 34(6): p. 755-781.

6. Athanasoulia, E., P. Melidis, and A. Aivasidis, Anaerobic waste activated sludge co-digestion with olive mill wastewater. Water Sci Technol, 2012. 65(12): p. 2251-7.

7. Athanasoulia, E., P. Melidis, and A. Aivasidis, Co-digestion of sewage sludge and crude glycerol from biodiesel production. Renewable Energy, 2014. 62: p. 73-78.

8. Aymerich, E., M. Esteban-Gutierrez, and L. Sancho, Analysis of the stability of high-solids anaerobic digestion of agro-industrial waste and sewage sludge. Bioresour Technol, 2013. 144: p. 107-14.

9. Bailey, R.S. Anaerobic digestion of restaurant grease wastewater to improve methane gas production and electrical power generation potential. in 80th Annual Technical Exhibition and Conference of the Water Environment Federation. 2007. San Diego, CA.

10. Bertin, L., et al., Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. Bioresour Technol, 2013. 128: p. 779-83.

11. Bjornsson L., M.M., Mattiasson B., Evaluation of parameters for monitoring an anaerobic co-digestion process. Applied Microbiology Biotechnology, 2000. 54: p. 844-849.

12. Bolzonella, D., et al., Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochemistry, 2005. 40(3-4): p. 1453-1460.

13. Bond T., B.C.J., Foxon K.M. and Buckley C.A., A critical review of experimental and predicted methane generation from anaerobic codigestion. water Sci Technology, 2012. 65(1): p. 183-189.

14. Bouallagui, H., L. Marouani, and M. Hamdi, Performances comparison between laboratory and full-scale anaerobic digesters treating a mixture of primary and waste activated sludge. Resources, Conservation and Recycling, 2010. 55(1): p. 29-33.

15. Brown, D. and Y. Li, Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresour Technol, 2013. 127: p. 275-80.

16. Bruss J. H., C.J.R., Rasmussen H., Anaerobic storage of activated sludge: effects on conditioning and dewatering performance. Water Sci Technol, 1993. 28: p. 350-357.

17. Buendia, I.M., et al., Feasibility of anaerobic co-digestion as a treatment option of meat industry wastes. Bioresour Technol, 2009. 100(6): p. 1903-9.

18. Callaghan F. J., W.D.A.J., Thayanithy K., Forster C. F., Co-digestion of waste organic solids: batch studies. Bioresource Technology, 1999. 67: p. 117-122.

19. Calli, B., et al., Effects of high free ammonia concentrations on the performances of anaerobic bioreactors. Process Biochemistry, 2005. 40(3-4): p. 1285-1292.

20. Cavaleiro, A.J., M.A. Pereira, and M. Alves, Enhancement of methane production from long chain fatty acid based effluents. Bioresour Technol, 2008. 99(10): p. 4086-95.

21. Chen, Y., J.J. Cheng, and K.S. Creamer, Inhibition of anaerobic digestion process: a review. Bioresour Technol, 2008. 99(10): p. 4044-64.

22. Chynoweth David P., O.J.M., Legrand Robert, Renewable methane from anaerobic digestion of biomass. Renewable Energy, 2001. 22: p. 1-8.

23. Clark, R.H., and Speece, R. E., The pH Tolerance of Anaerobic Digestion, in 5th International Conference on Water Pollution Research. 1970: San Francisco, California.

24. Cuetos, M.J., et al., Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). Biochemical Engineering Journal, 2008. 40(1): p. 99-106.

25. Dai, X., et al., Biodegradation of polyacrylamide by anaerobic digestion under mesophilic condition and its performance in actual dewatered sludge system. Bioresour Technol, 2014. 153: p. 55-61.

26. Esposito, G., Frunzo, L., Liotta, F., Panico, A., Pirozzi, F., Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates. The Open Environmental Engineering Journal, 2012. 5: p. 1-8.

27. F., A., Effects of cation addition on the flocculation behaviour of activated sludge at applied constant shear force, in Department of Civil and Environmental Engineering Water Environment Technology. 2007, Chalmers University of Technology: Göteborg, Sweden. p. 86.

28. Forbes, R.H.H., L.; Adams, G.; Witherspoon, J.; Murthy, S. N.; Card, and R.G. T.; Hargraves, D.; Higgins, M. J. Impacts of In-Plant Operational Parameters on Biosolids Odor Quality: Preliminary Results of WERF Phase 2 Study. in Water Environment Federation and American

Water Works Association Annual Biosolids and Residuals Conference. 2003. Baltimore, Maryland.

29. Fountoulakis, M.S., I. Petousi, and T. Manios, Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Manag, 2010. 30(10): p. 1849-53.

30. G., L., Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek, 1995. 67: p. 3-28.

31. Gavala, H.N., et al., Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Research, 2003. 37(19): p. 4561-4572.

32. Glassman, H.N., Surface active agents and their application in bacteriology. Bacteriology Reviews, 1948. 12(2): p. 105-148.

33. Grady L.C.P., D.G.T., Love N.G., Filipe C.D.M., Biological Wastewater Treatment. Third ed. 2011, New York: CRC press, Taylor and Francis Group.

34. Hansen, K.H., Angelidaki, I., Ahring, B.K., Anaerobic digestion of swine manure: inhibition by ammonia. Water Research, 1997. 32: p. 5-12.

35. He, X., Iasmin, M., Dean, L., Lappi, S., Ducoste, J.J., de los Reyes III, and F.L., Evidence for how fat, oil, and grease (FOG) deposits form in sewer lines. Envionmental Science and Technology, 2011. 45(10): p. 4385-4391.

36. Henderson, C., The effects of fatty acids on pure cultures of rumen bacteria. Agric. Sci. Camb., 1973. 81: p. 107-112.

37. Heukelekian, H., Mueller, P., Sew. Ind. Wastes 1958. 30: p. 1108.

38. Higgins, M.a.N., J., Characterization of Exocellular Protein and Its Role in Bioflocculation. Environmental Engineering, 1997. 123(5): p. 479-485.

39. Higgins, M.J., et al., Cycling of Volatile Organic Sulfur Compounds in Anaerobically Digested Biosolids and its Implications for Odors. Water Environment Research, 2006. 78(3): p. 243-252.

40. Higgins, M.J.M., S. N.; Striebig, B.; Hepner, S.; Yamani, S.; Yarosz, D. P.; Toffey, W. Factors Affecting Odor Production in Philadelphia Water Department Biosolids. in Proceedings of Water Environment Federation Odors and Toxic Air Emissions. 2002. Albuquerque, New Mexico.

41. Holm-Nielsen, J.B., T. Al Seadi, and P. Oleskowicz-Popiel, The future of anaerobic digestion and biogas utilization. Bioresour Technol, 2009. 100(22): p. 5478-84.

42. Hosseini Koupaie, E., et al., Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: process and cost-benefit analysis. Bioresour Technol, 2014. 152: p. 66-73.

43. K., H., Mechanism of Inhibition Caused by Long-Chain Fatty Acids in Anaerobic Digestion Process. Biotechnology and Bioengineering, 1981. 23(7): p. 1591-1610.

44. Kaare Hvid Hansen, I.A.a.B.K.A., Anaerobic Digestion of Swine Manure: Inhibition by Ammonia. Water Research, 1998. 32(1): p. 5-12.

45. KabaraJ. J., V.R., Jie L. K., Antimicrobial Lipids: Natural and Synthetic Fatty Acids and Monoglycerides. Lipids, 1977. 12(9): p. 753-759.

46. Kim, S.H., Han, S.K., Shin, H.S., Kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation and beta-oxidation. Environ. Sci. Health A: Toxic/Hazard. Subst. Environ. Eng., 2004. 39(4): p. 1025-1036.

47. Kobayashi, T., et al., Characterization of start-up performance and archaeal community shifts during anaerobic self-degradation of waste-activated sludge. Bioresour Technol, 2009. 100(21): p. 4981-8.

48. Koster I. W., K.A., Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. Applied and Environmental Microbiology, 1987. 53: p. 403-409.

49. Kroeker, E.J., Schulte, D. D., Lapp, H. M., Methane Production from Animal Wastes II—Process Stability. ASAE, 1976: p. 76-208.

50. Kroeker, E.J., et al., Anaerobic Treatment Process Stability. Water Pollution Control Federation, 1979. 51: p. 718.

51. Kugelman, I.J., and Chin, K. K., Toxicity, Synergism, and Antagonism in Anaerobic Waste Treatment Processes, in Anaerobic Biological Treatment Processes. 1971, American Chemical Society.

52. Lalman J. A., B.D.M., Anaerobic Degradation and Methanogenic Inhibitory effects of oleic and stearic acids. Water Research, 2001. 35: p. 2975-2983.

53. Lansing, S., et al., Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. Bioresour Technol, 2010. 101(12): p. 4362-70.

54. Le Hyaric, R., et al., Pilot-scale anaerobic digestion of screenings from wastewater treatment plants. Bioresour Technol, 2010. 101(23): p. 9006-11.

55. Leiva, M.B., E.H. Koupaie, and C. Eskicioglu, Anaerobic co-digestion of wine/fruit-juice production waste with landfill leachate diluted municipal sludge cake under semi-continuous flow operation. Waste Manag, 2014. 34(10): p. 1860-70.

56. Li, C., P. Champagne, and B.C. Anderson, Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions. Bioresour Technol, 2011. 102(20): p. 9471-80.

57. Long, J.H., et al., Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. Process Safety and Environmental Protection, 2012. 90(3): p. 231-245.

58. Lou, X.F., J. Nair, and G. Ho, Field performance of small scale anaerobic digesters treating food waste. Energy for Sustainable Development, 2012. 16(4): p. 509-514.

59. Luostarinen, S., S. Luste, and M. Sillanpaa, Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. Bioresour Technol, 2009. 100(1): p. 79-85.

60. Lv, W., W. Zhang, and Z. Yu, Evaluation of system performances and microbial communities of two temperature-phased anaerobic digestion systems treating dairy manure. Bioresour Technol, 2013. 143: p. 431-8.

61. Macias-Corral, M., et al., Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. Bioresour Technol, 2008. 99(17): p. 8288-93.

62. Martin, M.A., et al., Semi-continuous anaerobic co-digestion of orange peel waste and residual glycerol derived from biodiesel manufacturing. Waste Manag, 2013. 33(7): p. 1633-9.

63. McCarty, P.L., and McKinney, R. E., Volatile Acid Toxicity in Anaerobic Digestion. Water Pollution Control Federation, 1961. 33: p. 223.

64. McCarty, P.L., Anaerobic Waste Treatment Fundamentals: I. Chemistry and Microbiology; II. Environmental Requirements and Control; III. Toxic Materials and Their Control; IV. Process Design. Public Works, 1964: p. 9-12.

65. McCarty, P.L. Stoichiometry of Biological Reactions. in International Conference Toward a Unified Concept of Biological Waste Treatment Design. 1972. Atlanta.

66. Mikkelsen L.H., K.K., Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. Water Research, 2002. 36: p. 2451-2462.

67. Mondragón F. A., S.P., Cox H. J., Ahring B. K. and Iranpour R., Anaerobic Codigestion of Municipal, Farm, and Industrial Organic Wastes: A Survey of Recent Literature. Water Environment Research, 2006. 78(6): p. 607-636.

68. Monroy Oscar, F.G., Meraz Monika, Montoya Leticia and Macarie Herve, Anaerobic Digestion for Wastewater treatment in Mexico: State of the Technology. Water Research, 2000. 34: p. 1803-1816.

69. Monteith, H.D., and Stephenson, J. P., Mixing Efficiencies in Full-Scale Anaerobic Digesters by Tracer Methods. Journal of the Water Pollution Control Federation, 1981. 53(1): p. 78-84.

70. Muller, C., Lam, P., Lin, E., Chapman, T., Devin-Clark, D., and J. Belknap-Williamson, Krugel, S. Co-digestion at Annacis Island WWTP: metro Vancouver's path to renewable energy and greenhouse gas emission reductions. in Water Environment Federation. 2010. New orleans, LA.

71. Muller, C.D.V., N.; Higgins, M. J.; Novak, J. T. The Role of Shear in the Generation of Nuisance Odors from Dewatered Biosolids. in Proceedings of the 77th Annual Water Environment Federation Technical Exposition and Conference. 2004. New Orleans, LA.

72. Nallathambi, G.V., Anaerobic Digestion of Biomass For methane Production: A Review. Biomass and Bioenergy, 1997. 13: p. 83-114.

73. Nelson, E.D., and Baley, J. F., The Egg is Shape of Things to Come. Water and Sewage Works, 1979: p. 30-32.

74. Neves, L., R. Oliveira, and M.M. Alves, Anaerobic co-digestion of coffee waste and sewage sludge. Waste Manag, 2006. 26(2): p. 176-81.

75. Nielsen P. H., K.K., Disintegration of Activated Sludge flocs in the presence of Sulfide. Water Research, 1998. 32(2): p. 313-320.

76. Nishio, N. and Y. Nakashimada, Recent development of anaerobic digestion processes for energy recovery from wastes. J Biosci Bioeng, 2007. 103(2): p. 105-12.

77. Noutsopoulos, C., et al., Anaerobic co-digestion of grease sludge and sewage sludge: the effect of organic loading and grease sludge content. Bioresour Technol, 2013. 131: p. 452-9.

78. Novak J. T., B.H., Zurow A., Factors influencing activated sludge properties. Environmental Engineering, 1977. 103: p. 815-828.

79. Novak John T., P.C., Higgins Matthew J., Chen Y., Morton R., Gary D., Forbes R., Erdal Z. , WERF Odor Study Phase III: Impacts of the MicroSludge Process on Odor Causing Compounds in Residuals and Biosolids management Conference. 2007.

80. Novak, J.T., Carlson, D.A., The kinetics of anaerobic long chain fatty acid degradation. Water Pollution Control Federation, 1970. 42(11): p. 1932-1943.

81.Novak, J.T., et al., Generation Pattern of Sulfur Containing Gases from Anaerobically Digested Sludge Cakes. Water Environment Research, 2006. 78(8): p. 821-827.

82. Novak, J.T., M.E. Sadler, and S.N. Murthy, Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. Water Research, 2003. 37(13): p. 3136-3144.

83. Owen, W.F., Stuckey, D. C., Healy, J. B., Young, L. Y., McCarty, P. L., Bioassay for monitoring Biochemical Methane Potential and Anaerobic Toxicity. Water Research, 1979. 13: p. 485-492.

84. Pahl, O., et al., Anaerobic co-digestion of mechanically biologically treated municipal waste with primary sewage sludge - a feasibility study. Bioresour Technol, 2008. 99(9): p. 3354-64.

85. Parkin Gene F., O.W.F., Fundamentals of Anaerobic Digestion of Wastewater Sludge. Journal of Environmental Engineering, 1986. 112(5): p. 867-920.

86. Pereira, M.A., Cavaleiro, A.J., Mota, M., Alves, M.M., Accumulation of long chain fatty acids onto anaerobic sludge under steady state and shock loading conditions: effect on acetogenic and methanogenic activity. Water Sci Technol, 2003. 48(6): p. 33-40.

87. Rajagopal, R., D.I. Masse, and G. Singh, A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour Technol, 2013. 143: p. 632-41.

88. Rajeshwari K.V., B.M., Kansal A., Lata Kusum, and K. V.V.N., State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renewable and Sustainable Energy Reviews, 2000. 4: p. 135-156.

89. Raskin, L., Stroot, P. G., McMahon, K.D., Mackie R.I., Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions - I. Digester Performance. Water Research, 2001. 35(7): p. 1804 - 1816.

90. Razaviarani, V. and I.D. Buchanan, Reactor performance and microbial community dynamics during anaerobic co-digestion of municipal wastewater sludge with restaurant grease waste at steady state and overloading stages. Bioresour Technol, 2014. 172: p. 232-40.

91. Razaviarani, V., et al., Pilot-scale anaerobic co-digestion of municipal wastewater sludge with restaurant grease trap waste. J Environ Manage, 2013. 123: p. 26-33.

92. Rinzema A., A.A., Lettinga G., The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. Chem. Technol. Biotechnol., 1989. 46: p. 257-266.

93. Rinzema A., B.M., Knippenberg K. and Lettinga G., Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion. Water Environment Research, 1994. 66: p. 40-49.

94. Romano, R.T. and R. Zhang, Co-digestion of onion juice and wastewater sludge using an anaerobic mixed biofilm reactor. Bioresour Technol, 2008. 99(3): p. 631-7.

95. Rudolfs, W.C., and Amberg, H. R, White Water Treatment II. Effect of Sulfides on Digestion. Sewage and Industrial Wastes, 1952. 24: p. 1278.

96. Salminen E., R.J., Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. Bioresour Technology, 2002. 83: p. 13-26.

97. Sheng, K., et al., Effect of ammonia and nitrate on biogas production from food waste via anaerobic digestion. Biosystems Engineering, 2013. 116(2): p. 205-212.

98. Shilton, A., Powell, N., Broughton, A., Pratt, C., Pratt, S., Pepper, C., Enhanced biogas production using cow manure to stabilize co-digestion of whey and primary sludge. Enviromental Technology, 2013. 34(17): p. 2491-2496.

99. Silvestre, G., et al., Thermophilic anaerobic co-digestion of sewage sludge with grease waste: Effect of long chain fatty acids in the methane yield and its dewatering properties. Applied Energy, 2014. 117: p. 87-94.

100. Sung, S. and T. Liu, Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere, 2003. 53(1): p. 43-52.

101. Swanwick, J.D., Shurgen, D. G., and Jackson, S., A Survey of the Performance of Sewage Sludge Digesters in Great Britain. Water Pollution Control, 1969. 68(6): p. 186-279.

102. Tchobanoglous, G., Burton, F. L., Stensel, D. H., Metcalf and Eddy: Wastewater Engineering, Treatment and Reuse. 4 ed. 2003: Tata McGraw Hill.

103. Torpey, W.N., Loading to Failure of a Pilot High-Rate Digester. Sewage and Industrial Wastes, 1955. 27(2): p. 121-148.

104. Wang, L., T.N. Aziz, and F.L. de los Reyes, 3rd, Determining the limits of anaerobic co-digestion of thickened waste activated sludge with grease interceptor waste. Water Res, 2013. 47(11): p. 3835-44.

105. Ward, A.J., et al., Optimisation of the anaerobic digestion of agricultural resources. Bioresour Technol, 2008. 99(17): p. 7928-40.

106. Yamashiro, T., et al., Anaerobic co-digestion of dairy cow manure and high concentrated food processing waste. Journal of Material Cycles and Waste Management, 2013. 15(4): p. 539-547.

107. Yenigün, O. and B. Demirel, Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry, 2013. 48(5-6): p. 901-911.

108. Zhang, R., et al., Characterization of food waste as feedstock for anaerobic digestion. Bioresour Technol, 2007. 98(4): p. 929-35.

109. Zhen, G., et al., Enhanced dewatering characteristics of waste activated sludge with Fenton pretreatment: effectiveness and statistical optimization. Frontiers of Environmental Science & Engineering, 2013. 8(2): p. 267-276.

110. Zhu, H., et al., Biohydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges. International Journal of Hydrogen Energy, 2008. 33(14): p. 3651-3659.

111. Zhu, H., et al., Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. Bioresour Technol, 2008. 99(11): p. 5078-84.

112. Zhu, Z., M.K. Hsueh, and Q. He, Enhancing biomethanation of municipal waste sludge with grease trap waste as a co-substrate. Renewable Energy, 2011. 36(6): p. 1802-1807.

Chapter 3

Manuscript 1

Title:

Evaluation of the Impacts of the nature of High Strength Food Wastes on Anaerobic Co-digestion with Sewage Sludge

R. Vaidya[a]*, G.D. Boardman[a], J.T. Novak[a], R. Wimmer[b], M. Hanna[b]

a: Virginia Polytechnic Institute & State University, Blacksburg, VA, United States

b: Black & Veatch, Baltimore, MD, United States

*Corresponding author

Abstract

Anaerobic co-digestion has been practiced by water treatment plants for the past several years to increase the methane production. During anaerobic co-digestion, addition of a variety of organic wastes can have beneficial impacts on digester performance. The feasibility of addition of fats, oils and grease (FOG) and other high strength wastes (HSWs) in addition to municipal sewage sludge were studied and their impact on digester performance was assessed with the help of several process parameters.

Four lab-scale, continuously mixed digesters were set up with a working volume of 9.75 L, 15 day SRT and temperature of 37ºC. Wastes from different food processing industries such as cheese (whey), juice, grease trap waste (GTW) and dissolved air flotation waste (DAF) from a food processor, in addition to municipal sewage sludge, were fed to the digesters in varying amounts. Biogas was collected regularly and analyzed for total volume and methane content. The polymer addition necessary for sludge dewatering was calculated through the results obtained with the capillary suction time (CST) analysis and biosolids odors measurements were made on the dewatered sludge.

The results showed that the addition of HSWs led to higher methane production at lower (6% whey, 18% GTW and 76% sludge mix on a volumetric basis) organic loadings. However, at higher organic loadings, the GTW appeared to be toxic to the methanogens, leading to digester failure. During this period of failure, there was a drop in the digester pH and biogas production, and an accumulation of volatile fatty acids within the digester. The reason for digester failure was attributed to some kind of toxicity present in the GTW.

Keywords: Anaerobic co-digestion, High-strength waste (HSW), Long chain fatty acids, Digester failure, Sludge dewatering, Biosolids odors

Introduction

Anaerobic digestion was first discovered by Volta, in 1776 when he found that the organic matter degraded by microorganisms gets converted to biogas. Anaerobic digestion has been utilized by mainly wastewater treatment plants for processing and disposal of biosolids, post treatment of waste water. The main purpose of this digestion procedure is to degrade the degradable organic solids contents of the wastewater in the absence of oxygen to produce biogas, thus reducing the odors and contributing to the overall energy requirements of the plant. This digested sludge can then be used for land application and landfilling. To increase the methane content and to dispose of wastes containing high levels of degradable organic matter, anaerobic co-digestion (addition of non-sewage sludge organics) has been widely utilized. This includes addition of high strength wastes (COD greater than 50,000 mg/L) to municipal sewage sludge and digesting the wastes together.

Examples of anaerobic co-digestion of wastes can be found in the literature. The main wastes include food wastes from the dairy and agricultural industry, restaurants and cafeterias, poultry and slaughter house waste, and an organic fraction of municipal solid waste (OFMSW) (Salminen and Rintala, 2002; Alvarez and Llabres, 2000; Brown and Li, 2013; Yamashiro *et al*., 2013). These wastes have greater concentrations of degradable organic matter, as compared to sewage sludge, and therefore produce more methane. These wastes have been successfully tested at lab-scale, as well as full-scale and at different temperatures.

Food wastes, such as potato wastes and onion juice, when used in anaerobic co-digestion, resulted in stable digester performance and greater methane yield. A 70% reduction in TVS and 64% reduction in COD was obtained in a lab-scale, two-stage digestion process using mashed potatoes as feedstock (Zhu *et al*., 2008). A methane yield of 0.37 L/g TVS was obtained after using mixtures of onion juices with wastewater sludge (Romano and Zhang, 2008). Food wastes collected from the city of San Francisco, California, produced a methane yield of 435 mL/g TVS after anaerobic co-digestion under thermophilic conditions (Zhang *et al*., 2007). These studies show the feasibility of using food wastes as possible substrates during anaerobic co-digestion.

Studies involving co-digestion of cheese whey waste have shown that this waste tends to work with dairy manure. Anaerobic co-digestion of dairy manure and food processing wastes containing

a mixture of potato waste, cheese whey, animal blood and used cooking oil resulted in a two fold increase in methane production per digester volume as compared to digestion of cow manure alone. However, this digestion was possible only at thermophilic (55^{o}C) temperature. The process was inhibited at mesophilic (35^{o}C) temperature. The authors claim that the digested effluent can be directly used as organic fertilizer (Yamashiro *et al*., 2013). In another study, a two stage batch process at mesophilic temperature, with an equal ratio of cow manure and cheese whey in the feed, led to a methane yield of 258 mL methane/g TVS. This was more than twice the methane yield obtained in the one-stage process (Bertin *et al*., 2013). A 151% increase in biogas production was obtained after co-digestion of cheese whey with primary sludge at an organic loading rate (OLR) of 3.2 kg COD/m^3 day, as compared to primary sludge alone. Further addition of chemical alkalinity boosted the biogas production by 208% at an OLR of 6.4 kg COD/m^3 day. Replacing this chemical addition with cow manure resulted in an increase in biogas production by 268% and an OLR of 5.2 – 6.9 kg COD/m^3 day (Shilton *et al*., 2013). The digesters were stable in these studies despite addition of cheese whey to anaerobic digestion.

Similarly, co-digestion studies with fruit juice and wine processing wastes have also been studied. In a lab-scale, co-digestion experiment receiving four different waste streams, thickened waste activate sludge (TWAS), screen cake from fruit juice/winery waste water, landfill leachate and municipal sludge cake, resulted in stable operational conditions due to additional buffering capacity and readily biodegradable compounds, as compared to digestion of TWAS alone. The thickened WAS increased the dewatering capacity of digested sludge. A cost-benefit analysis indicated a total reduction in capital and operational costs by 22% through co-digestion of the four streams together (Leiva *et al*., 2014). Similar results were obtained in another study using fruit juice, industrial waste, and municipal sludge under mesophilic conditions (Hosseini *et al*., 2014).

Recently, studies have been carried out to include glycerol from biodiesel production in anaerobic digestion to boost biogas production. Co-digestion of glycerol and orange peel waste led to a methane production of 330 ml methane/g TVS at an organic loading rate of 1.91 kg TVS/m^3 day. However, at organic loadings greater than 2.10 g TVS/L the process was inhibited, leading to decrease in the pH and accumulation of volatile fatty acids (Martin *et al*., 2013). In another experiment, addition of 1% (v/v) glycerol to the feed containing sewage led to a methane production of 2,353 mL methane/day as compared to 1,106 mL CH_4/day with sewage sludge alone.

At glycerol feed higher than 1% (v/v), the digestion process was inhibited (Fountoulakis *et al*., 2010).

Fat, oils and grease (FOG) have been sought by many utilities to increase the biogas production. FOG disposal can be an issue to many wastewater utilities and can be toxic to biological treatment steps in a wastewater treatment facility. Direct disposal of this FOG in sewers can clog pipelines, resulting in increase in the overall cost on the maintenance of the plant. To prevent this, grease traps and grease interceptors are installed in sewers to separate the grease and food solids from the wastewater (Long *et al*., 2012). Grease trap waste (GTW) from various sources such as restaurants, cafeterias and kitchens has been used for co-digestion, since it has high levels of degradable organic solids.

Noutsopoulos *et al*., (2013), showed that addition of grease sludge from primary settling tanks in wastewater treatment plants, up to 60% of feed TVS, led to an increase in biogas yield by 55%. The OLR of grease sludge for stable operation was found to be 2.4 kg TVS/m^3 day. Similar results were observed after addition of GTW from restaurants and from meat processing plants. A methane potential of 145 L methane/L GTW was observed at a feed loading of 5.5 g TVS/L, while 918 m^3/t TVS added at a feed of 3.46kg TVS/m^3 day for these wastes. However, at higher organic loadings the process was inhibited, resulting in a decrease in methane production (Zhu *et al*., 2011; Loustarinen *et al*., 2009). The addition of GTW from restaurant has been shown to inhibit the digestion process. Though mesophilic co-digestion resulted in stable digester operation (120% increase in methane yield), when the feed loading was increased to 300% COD relative to municipal wastewater sludge, the methane production and alkalinity declined and the pH dropped below 6.5, while the VFA concentration increased. Pyrosequencing on the digested effluent revealed that there was decline in the number of methanogens after addition of GTW (Razaviarani and Buchanan, 2014).

The highest increase in the methane production from GTW has been shown by Wang *et al*., 2013. A 317% increase in methane yield was obtained after addition of 20% (v/v) of grease interceptor waste. Higher loadings resulted in digester failure due to long chain fatty acid (LCFA) inhibition. These studies show that although addition of FOG leads to an increase in the biogas production, it has the potential to inhibit anaerobic digestion, resulting in digester failure and unstable operation. Thus, it is important to understand the organic loading rate at which stable digester operation is

possible. It is necessary to characterize the FOG before addition to the digesters to check for the possibility of toxicity.

The aim of this project was to evaluate the feasibility of adding four different wastes, namely, juice processing waste, cheese whey, GTW and DAF from a food processor along with primary sludge and WAS (the ratio of primary sludge to WAS was 60:40). The OLR at which methane production increased was observed at every stage of operation. The loading rate at which digester inhibition took place was studied and the impact of FOG on digester performance was evaluated. Further, the dewatering capacity of the digested sludge and the polymer addition required were tested using capillary suction time (CST). This digested sludge was then tested for biosolids odors to evaluate the effect of HSWs on biosolids disposal.

Digester Setup and Operation

Four continuously mixed, lab-scale, anaerobic digesters, as shown in Fig. 3.1 and 3.2 were set up at Virginia Tech in a constant temperature room maintained at 37ºC. The digesters were made of high density polyethylene and supplied by the Hobby Beverage Equipment Company (Temecula, California). These lab-scale digesters were cylindrical in shape with a conical bottom for efficient mixing and grit removal. To monitor temperature, a stainless steel thermometer was inserted in the digesters, which was also supplied by Hobby Company. The volume of the digesters was 25 L, but the liquid volume was maintained at 9.75 L.

The gas collected in the headspace was recirculated to a valve at the bottom of the digesters by means of a variable speed peristaltic pump (Cole Parmer, Vernon Hills, Illinois) in order to ensure complete mixing in the digesters. The degree of mixing was controlled by changing the pump speed. The pumps were set at 50% their maximum value, corresponding to 0.8 L/minute (600 rpm). Masterflex® Tygon tubing (Cole Parmer, LFL- 17) was used in the connection of the headspace to the conical bottom.

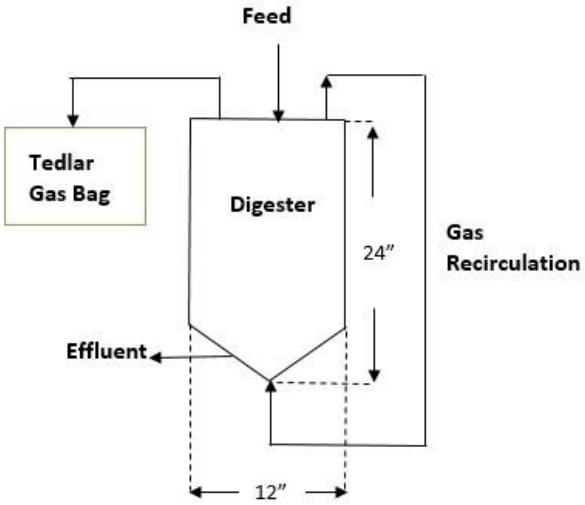


Fig. 3.1. Schematic diagram showing digester setup

Fig. 3.2. Photograph of the lab-scale setup employed

The digesters were sealed to maintain anaerobic conditions. The biogas generated was collected from each digester in 25 L, TEDLAR® gas sampling bags for analysis.

Raw municipal sewage sludge (sludge mix) added to the digesters was a mix of primary and waste activated sludge (WAS) at a ratio of 60:40. The sludge was obtained from Opequon Water Reclamation Facility (OWRF), a wastewater treatment plant in Winchester, Virginia. Both primary sludge and WAS were shipped from Winchester every week in ice-packed coolers. The other

Table 3.1: High Strength Waste Characteristics

High Strength Waste	Total COD (mg/L)	TS (mg/L)	TVS (mg/L)
GTW	50,000	40,000	37,000
Whey	87,000	78,000	56,000
DAF	600,000	300,000	285,000
Juice waste	76,000	44,000	43,000
Sludge Mix	~35,000	~30,000	~23,000

wastes used in the study were juice and cheese processing waste (whey) obtained from industries in Winchester, and grease trap waste (GTW) and dissolved air flotation waste (DAF) obtained from a grease trap waste hauling service in Winchester, Virginia. The characteristics of these wastes are summarized in table 3.1.

Initially, the digesters were seeded with 8 L of effluent from mesophilic, anaerobic digesters at the wastewater treatment plant in Christiansburg, Virginia. Once the digesters were stabilized, they were fed with the sludge mix shipped from Winchester. The characteristics of the sludge from the Christiansburg plant were similar to that obtained from Winchester; thus, the feed to the digesters was similar. The digesters were operated at a SRT of 15 days. All the digesters were fed with sludge mix only until stable conditions were observed.

Feeding scheme

All four digesters were batch fed once a day with a feed volume of 650 mL. The same volume of effluent was wasted every day to maintain a constant volume inside the digester. Analytical tests were performed on the wasted effluent. All tests, except for sludge dewatering and biosolids odors, were carried out once every week. These tests were performed after every change in the feed to the digesters. Digester 1 was maintained as the control and was fed only with sludge mix (60% primary and 40% WAS). Digesters 2, 3 and 4 were fed with sludge mix and high strength wastes in varying amounts.

The study was carried out in three different phases characterized by the volume of HSWs added to the feed. The aim was to increase the organic loading rate in steps in each phase to allow for acclimation of the microbes and a better evaluation of negative effects. The loadings at which digesters failed was noted, and after failure, digesters were re-inoculated with effluent from the control to stabilize them for further experiments. Day 0 marks the beginning of HSW addition to the digesters. The digesters were operated for a period of 150 days. Table 3.2 summarizes the feeding scheme employed in the three different stages. The food waste percentage in the table was calculated on the basis of volume (v/v).

In the first phase, (84 days), the HSWs included only whey, GTW and DAF. Juice waste was not fed to the digesters. In the second phase, (134 days), the feed to the digesters was increased gradually to assess the capability of the reactors to digest these wastes. In the third phase, (150 days), the first digester (which had served at the control) was also fed with HSW. Digesters 2 and 4 were fed with $Mg(OH)_2$ to provide additional alkalinity so that the impact of maintaining a higher pH could be evaluated. Finally, Digester 3 was fed the highest possible loading intended to be fed at the full-scale digesters under construction at OWRF. An important consideration while assessing digester failure was that the feed to the digesters was stopped as soon as it was certain that the digester was in failure. Failure was not allowed to reach a steady state. The digesters were re-inoculated with effluent from the control during failure in phase II and were shut down after failure in phase III.

Table 3.2. Feeding regime

Phase	Days	Digester 1	Digester 2	Digester 3	Digester 4
I	0	100% Sludge mix	6% whey 9% GTW 85% Sludge mix	8% DAF 92% Sludge mix	6% whey 18% GTW 76% Sludge mix
II	85	100% Sludge mix	8% whey 16% GTW 8% DAF 68% Sludge mix	6% whey 12% DAF 10% juice waste 72% Sludge mix	8% whey 22% GTW 10% juice waste 60% Sludge mix
III	135	16% GTW 84% Sludge mix	16% GTW 84% Sludge mix 1.1 g $Mg(OH)_2$	8% whey 16% DAF 21% juice waste 55% Sludge mix	22% GTW 78% Sludge mix 0.26 g $Mg(OH)_2$

Analytical Methods

The following parameters were monitored regularly to evaluate digester performance (Table 3.3). Most of these tests were conducted according to Standard Methods for the Examination of Water and Wastewater (APHA 2012). The rest of the methods have been described in brief.

Table 3.3. Analytical Methods

Parameter	Standard Method	Frequency
pH	4500 H^+	Alternate day
Total and Total Volatile Solids (TS and TVS)	2540 E. Fixed and volatile solids ignited at 550^oC	Twice a week
Total and Soluble COD (tCOD and sCOD)	5220C, closed reflux titrimetric method	Once a week
Total Ammonia Nitrogen (TAN)	4500, distillation and titrimetry	Once a week

Total Kjeldahl Nitrogen (TKN)	4500-N_{org} Semi Micro Kjeldahl method	Once a week
Alkalinity	2320 Potentiometric titration	Twice a week
Volatile Fatty Acids (VFAs)	-	Once in two weeks
Long Chain Fatty Acids (LCFAs)	-	Once a month
Gas Production and Composition	-	daily
Capillary Suction Time (CST) and Sludge Dewatering	-	Once a month
Biosolids Odors	-	Once a month
Metals Analyses	3125-B	Once a month
Biomethane Potential (BMP) Analyses	-	Once at the end of study

Volatile Fatty Acids (VFA)

For measurement of VFAs samples were diluted and filtered through a 0.45 micron syringe filter and were frozen to prevent any methanogenic activity. These samples were then thawed before analysis and acidified with 85% phosphoric acid. VFA were measured using a gas chromatograph (Shimadzu GC 14A) with flame ionization detector. Nukol™ fused silica column (15 m x 0.53 mm, 0.5 μm film thickness) was used for species separation. For data analysis, a computer integrator (Shimadzu, Model CR501 Chromatopak) was used. Helium at a flow rate of 17 ml/min was used as the carrier gas. VFAs were measured in mg/L and the acids monitored were acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, n-valeric acid, isocaproic acid, hexanoic acid and heptanoic acid. These were expressed as Total VFAs in meq/L.

Long chain Fatty Acids (LCFA)

LCFAs were analyzed by using hexane extraction method based on Standard methods 5520D. LCFAs were prepared for GC analysis by conversion to Fatty Acid Methyl Esters (FAMEs). Ten milliliters of sample was taken in a test tube and two drops of sulfuric acid were added to lower

the pH. The solvent was initially extracted by adding extraction solvent mixture containing hexane and MTBE. The test tube was centrifuged to allow the hexane and aqueous layer to separate. The hexane layer was then extracted and this procedure was repeated three times to extract as much fat from the sample as possible. The solvent was then evaporated under a stream of nitrogen leaving behind the oil residue for quantification.

A transesterification solution mixture containing methanol, hydrochloric acid and chloroform in the ratio of 10:1:1 was added to the oil residue and heated at 90^{o}C for 30 minutes to replace the carboxylic acid with methane group. After cooling down the mixture, LCFAs were extracted using an extraction solution mixture containing hexane and chloroform in the ratio of 4:1. LCFAs were then analyzed using gas chromatograph with FID as described for VFA measurement. LCFAs from C 12:0 to C 18:2 were quantified and expressed in mg/L.

Gas Production and Composition

Biogas production was important to the study and thus was monitored daily. Biogas was collected in 25 L gas bags (TEDLAR®) connected to each digester. The gas bags were emptied on a daily basis using a pump at a fixed flowrate. The gas produced was then calculated using the time required to completely empty these bags.

Biogas was characterized using a gas chromatograph (Shimadzu, 14A) with a Thermal Conductivity Detector (TCD). The column (Restek) was packed with Haysep Q media (4 m length, 6.35 mm ID) and the carrier gas was Helium. An inlet temperature of 110°C was employed. Percentages of methane and carbon dioxide were recorded. Methane and carbon dioxide standards of 25%, 50%, 75% and 100% were used for calibration. These calibrations were made at least twice a week.

Capillary Suction Time (CST) and Sludge Dewatering

The effluent obtained post-digestion was used to prepare dewatered sludge cakes based on the method described by Mueller *et al*. (2004). For sludge conditioning, polymer (FLOPAM 3551-Branched Polyacrylamide polymer with charge density 55%) was used. Polymer concentration of 2g/L was prepared by mixing it with tap water with a blender at 300 rpm for 55 min and allowing it to sit for another 30 min. A mixture of 50mL of effluent sludge and polymer was sheared in a blender (Oster 12 speed, 6811-C) at 450 Watts for 10 sec to simulate shearing in a centrifuge. The

polymer dosage required for minimum CST was determined with the help of Triton CST apparatus type P304M with Whatman 17-CHR chromatography paper. The polymer dosage that resulted in a capillary suction time of 20 sec was considered as the optimum polymer dosage.

For sludge dewatering, a mixture of 350 mL of effluent sludge and the corresponding polymer dosage required for minimum capillary suction time was sheared in the blender as described above, and then centrifuged (Beckman-Coulter Avanti-JE) at 10,000 rpm (17,700 G) for 30 min. The centrate was discarded and the pellet was dewatered using a hydraulic piston press over a Whatman 41 filter paper at 38 psi for 15 min to achieve a higher solids concentration in the dewatered cake. Percent solids in the dewatered cake were estimated by measuring the TS values post-centrifugation and post-dewatering with the piston press.

Biosolids odors

The biosolids odors were analyzed based on the method described by Higgins *et al*. (2004). In order to analyze the odors from digested sludge, the centrifuged and dewatered sludge cake were tested for volatile organic sulfur compounds. Five grams of dewatered sludge from the four digesters were incubated in 40 mL EPA vials sealed with screw caps and Teflon™-lined rubber septa at room temperature (25^{o}C). The sealed vials were used to make sure that the biosolids were not exposed to air, thus simulating the environment in a biosolids pile. Headspace gas of 100 µL from the incubated vials was injected each day into a gas chromatograph (Model No. GC 6890, MSD 5970, Hewlett-Packard) with a cryo-trapping system. The cryo-trap formed by liquid nitrogen was employed to accumulate gas samples and to generate narrow chromatographic peaks. The column used for injection was a Supelco column (Model No. 20751-01A, 30 m long and 0.25 mm ID) with helium as the carrier gas at a flowrate of 2 mL/min. The sulfur compounds analyzed were: hydrogen sulfide (H2S), methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS). Each was expressed in terms of mg/L of sulfur.

Biomethane potential (BMP) Analyses

The rate of biogas production was used as an estimate for biomethane potential of GTW and DAF, separately. For this purpose 125 mL serum bottles, sealed with pierceable septa were used. The blank was filled with 100 mL of digested sludge. To analyze biogas production for DAF, 100 mL of sample containing 8, 12, 16, 20 and 25% DAF and remaining sludge on a volumetric basis was

used. The amount of GTW added was 5, 9, 12, 16 and 22%. Diluted GTW (1:10) was added to two separate bottles at a composition of 16 and 22%. Bottles containing control, DAF, GTW and diluted GTW (1:10) were prepared and kept in a temperature controlled room at 35^{o}C. Anaerobically digested sludge was obtained from a wastewater treatment plant in the neighborhood. After adding the samples, the bottles were flushed with high purity nitrogen gas to remove all the oxygen present in the bottle. The bottles were then sealed and the gas production was then determined periodically with the help of a liquid filled manometer. The displacement solution was saturated with sodium sulfate and the pH was adjusted to less than 3.0 to prevent dissolution of carbon dioxide during measurement. The pressure developed in the bottles due to biogas production was then released by a hypodermic needle connected to the manometer, which caused a downward movement in the manometer liquid level, corresponding to the volume of biogas produced. Measurements were taken twice a day initially, for the first seven days and then once a day for the next five days until the biogas production plateaued.

Results and Discussion

Variation in pH during the study

A digester pH of 7 or more is considered to be optimum for methanogenic activity (Grady *et al*., 2011). Digester pH above 6.8 was acceptable for the digester to be considered stable. The pH values of all digesters were monitored every alternate day. Fig. 3.3 shows the digester influent and effluent pH. The arrows on the plots correspond to the beginning of the second and third phases. It can be seen that during phase I, all four digesters were stable and the pH values within the digesters for all four digesters were consistently in the range of 6.9 to 7.1. The influent pH during this phase varied from 4.7 to 5.4.

Although the influent pH was consistent during days 85 to 135, during phase II, there was a rapid drop in the effluent pH from 7.0 to 6.5 in digesters 2 and 4, which received GTW. Digester 2 failed immediately after feeding began in phase II, which can be seen by a drop in pH. The pH continued to drop below 6.2, after which time the digester feeding was stopped. The digester was re-inoculated using the effluent from digester 1 (control) until the pH stabilized around pH 6.9. The initial drop in pH observed in digester 4 was due to the addition of fryer oil, a waste that was accidently shipped in place of GTW. Once digester 4 was stabilized using effluent from the control, the feeding scheme from phase II was started. Though the pH remained stable for a couple of days, the failure in digester 4 was soon evident from a gradual decline in the pH. As soon as the pH dropped below 6, the digester feeding was stopped. The digester was re-inoculated using inoculum from the control to re-attain a stable operation. Digesters 1 and 3 were stable during phase II, which can be seen from the pH values that were around 6.9-7.0.

The failures during phase II were attributed to the addition of GTW. To prevent this failure, $Mg(OH)_2$ was added in phase III to act as a buffering agent. This can be seen by an increase in the influent pH of digesters 2 and 4. The effluent pH of digester 1, which was no longer the control, dropped immediately since it did not receive any buffer. The effluent pH of digester 3 was stable around 6.9-7.0, even though the loading rate was increased. The pH of digester 4 dropped below 6 even after receiving the buffer. To increase the pH, the $Mg(OH)_2$ dosage was increased from 0.26 g/day to 0.40 g/day. This resulted in a rise in pH to 6.8. However, this did not prove to be a long term solution and the digester continued to undergo failure with the pH dropping below 6.

Digester 2 was fed with 1.1 g $Mg(OH)_2$ /day, but eventually failed. However, the pH dropped only to values close to 6.7, due to the $Mg(OH)_2$ addition.

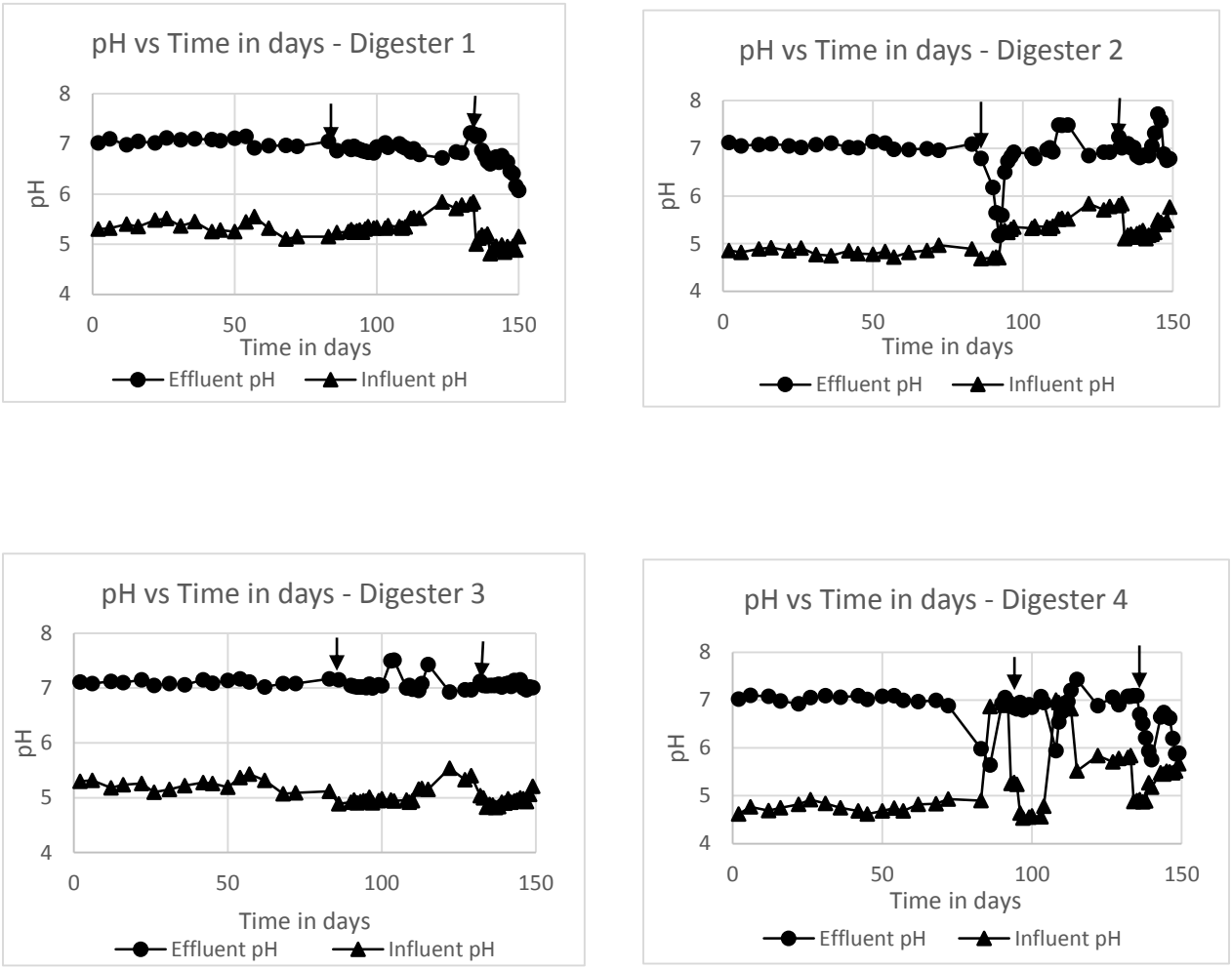


Fig. 3.3. pH vs Time

Total Volatile Solids (TVS) Destruction

VS reductions are usually in the range of 40-60 % for a mixture of primary and WAS, while TS reductions are in the range of 45-50% (Metcalf and Eddy, 1979). Fig. 3.4 shows the average %TVS destruction for all three phases. The influent TVS values fluctuated since the nature of the sludge varied considerably. The influent TVS values varied between 5,000 – 15,000 mg/L for all digesters, except for digester 2 that received both GTW and DAF. The influent TVS values for

digester 2 were slightly higher, in the range of 5,000 – 20,000 mg/L. The average %TVS destruction values differed for different digesters and phases. Digester 1, the control, had the same TVS destruction of 40% in phases 1 and 2. This value for digester 1 is lower than the other digesters, because it did not receive any food waste.

The TVS destruction values for digester 3 were around 50% during all three phases, showing stable operation throughout the study. The TVS destruction for digester 2 increased from 40 – 60% with increasing food waste. This shows that even though the influent TVS was higher than all other digesters due to food waste addition, and the digester underwent failure in phase 2 and 3, a good reduction in TVS was achieved until failure occurred. A maximum TVS destruction of 65% was achieved by digester 4 in phase 3, which received an influent TVS of about 15,000 mg/L.

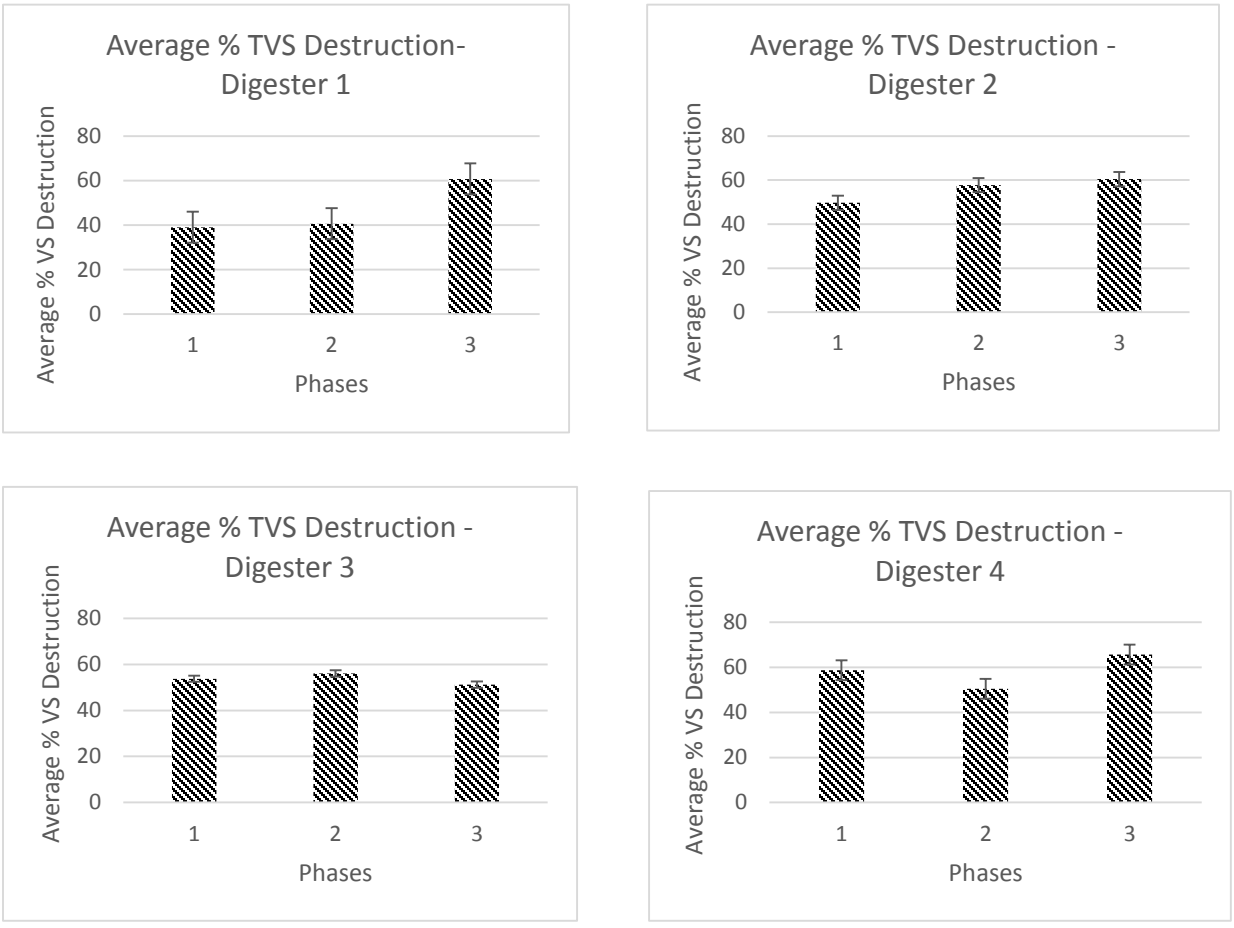
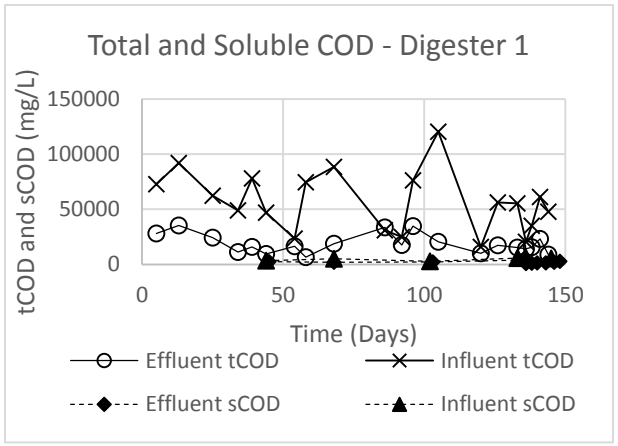


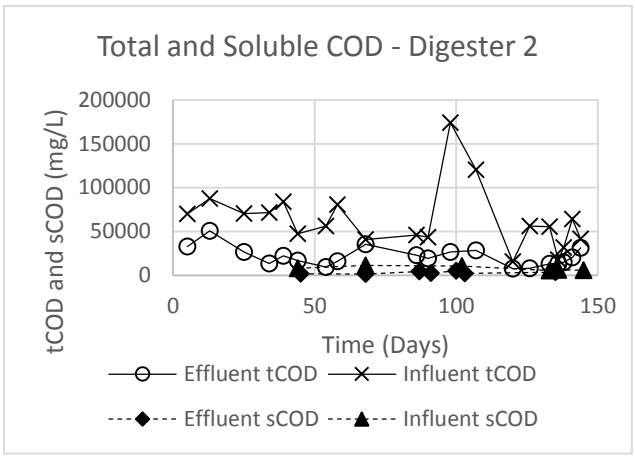
Fig. 3.4. Average % TVS destruction for different phases

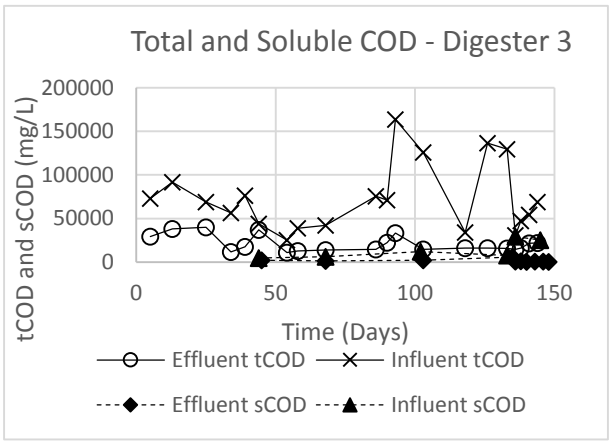
Total and Soluble COD (tCOD and sCOD)

Total COD was measured twice a week, while soluble COD was measured only when there was a change in the feeding scheme. Due to the varying nature of the sludge, the tCOD values fluctuated throughout the experiment, as seen in the Table 3.4. Fig. 3.5, comparing tCOD and sCOD shows that failure in digesters 1, 2 and 4 can be seen by increase in the effluent tCOD values by approximately a factor of two during phase III. The soluble COD values for the effluent are less than 5,000 mg/L, with digester 3 having the lowest effluent sCOD despite receiving the highest sCOD.

COD reductions are in the same range as that of TVS reductions (40-60%) (Parkin and Owen, 1986). The average tCOD destruction was consistently around 60-65% for digester 1. The average tCOD destroyed for digester 2 was 10% higher than the control for phase II even though it was not stable. The average tCOD destroyed in digester 2 was around 70% during phase II, while in phase III, this value dropped to 40% due to failure. The highest average tCOD destruction of 76% was achieved by digester 3 during phase III. Digester 4, however, showed tCOD destructions as low as 8% during the time that the digesters were fed in phase III. This shows the rapid failure of digester 4, after adding GTW.







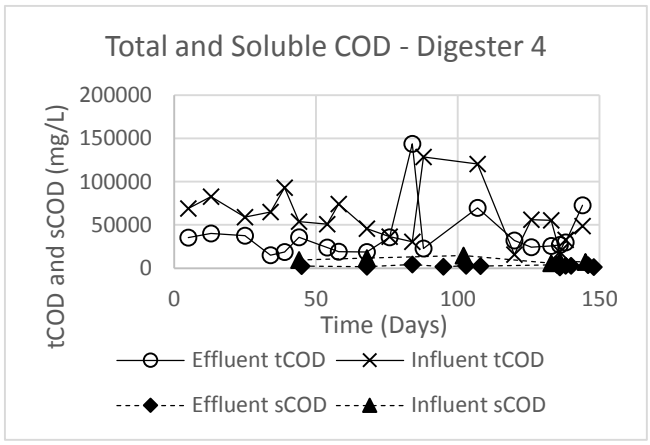


Fig. 3.5. tCOD and sCOD plots

Table 3.4. Average tCOD and sCOD values

Phase	Digester	Average Influent tCOD mg/L	Average Effluent tCOD mg/L	Average Influent sCOD mg/L	Average Effluent sCOD mg/L
I	1	65,000	18,000	4,000	2,000
	2	68,000	25,000	10,000	2,000
	3	58,000	24,000	5,500	2,000
	4	60,000	39,000	10,500	3,000
II	1	55,000	21,000	4,000	3,000
	2	73,000	18,000	8,000	4,000
	3	105,000	19,000	10,000	4,000
	4	75,000	35,000	10,000	3,000
III	1	41,000	15,000	6,000	2,000
	2	39,000	20,000	6,000	1,500
	3	50,000	19,000	27,000	700
	4	45,500	27,500	7,500	2,000

Total Ammonia Nitrogen and Total Kjeldahl Nitrogen (TAN and TKN)

TAN values above 1,000 mg/L can be inhibitory to mesophilic digestion (Parkin and Owen, 1986). Since the ammonia nitrogen was below 1,000 mg/L during all the phases, the failure of the digesters cannot be attributed to ammonia toxicity. During digester failure, the TAN values declined from approximately 400 mg/L to 300 mg/L. This could be due to loss in the buffering capacity. An overall decrease in the TAN values from 400 mg/L to 300 mg/L can be seen for the control, too, which shows that the sludge mixture itself had a lower nitrogen content.

The influent TAN values ranged from 400 – 600 mg/L for all digesters during phase I. However, the range of influent TAN values dropped to 200 – 400 mg/L in phases II and III. This might explain the drop in effluent TAN values during latter phases and shows the drop in buffering capacity of the sludge itself. The influent and effluent TKN values ranged from 500 mg/L to 2500 mg/L. From Fig. 3.6 it can be seen that TAN constitutes nearly half of the TKN content.

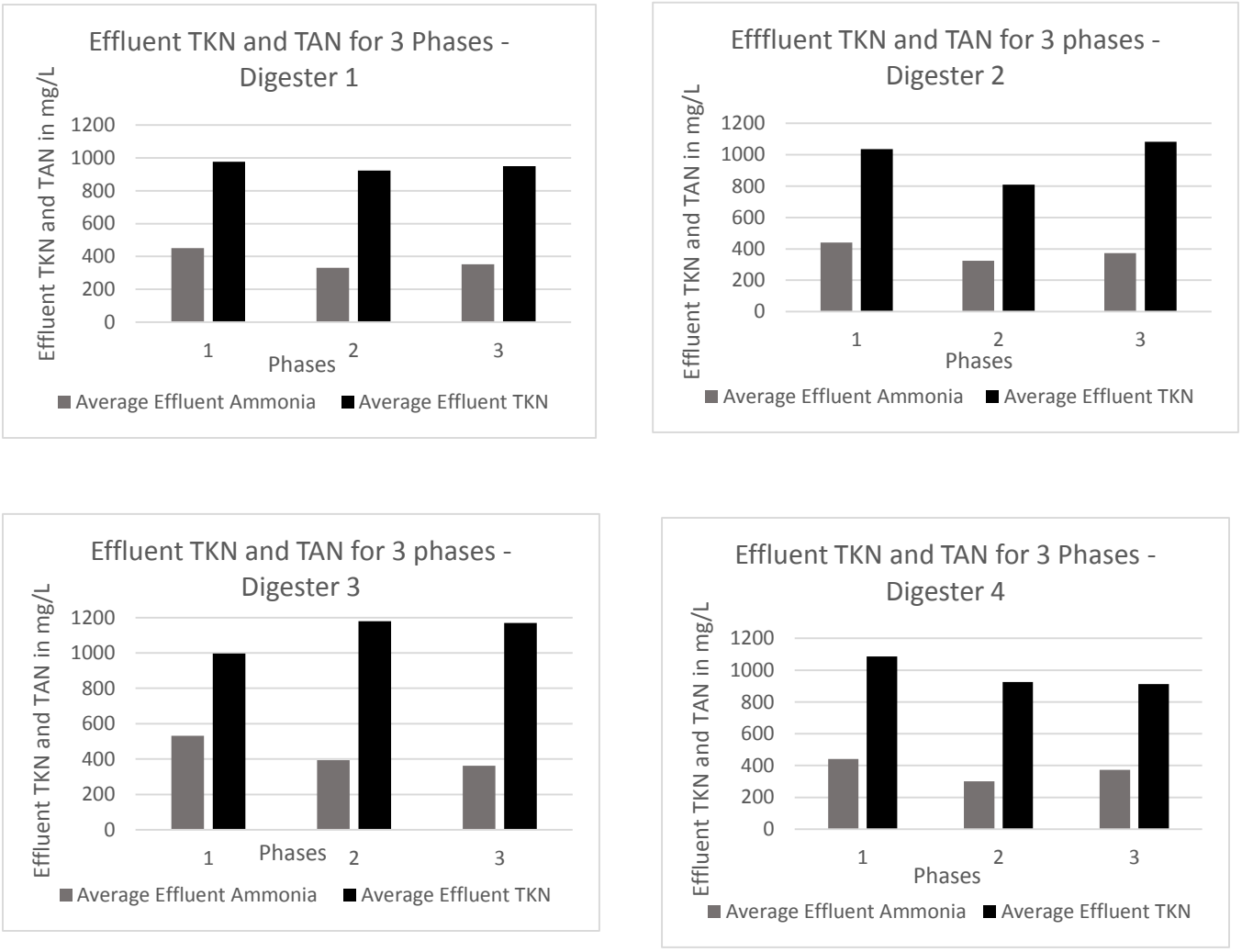


Fig. 3.6. Average TAN and TKN values during all the phases

Volatile Fatty Acids (VFAs) and Alkalinity

The arrows in Fig. 3.7 indicate the points of addition for a new feed regime (phases II and III). There was an overall decrease in the alkalinity values in all digesters. This is in agreement with the effluent TAN values, showing that the buffering capacity of the sludge decreased over time. Fig. 3.7 shows that during digester failures, the alkalinity values and VFA concentrations in the digester, tended to approach each other. Digester 1, being the control, did not exhibit an accumulation of VFAs in phases I and II. However, the failure during phase III can be seen by the rapid drop in alkalinity from 50 to nearly 20 meq/L and increase in the VFA values to 20 meq/L. In digester 2, even during digester failure in phase III, the alkalinity was around 50 meq/L, which

was attributed to the addition of magnesium hydroxide. There was accumulation of VFAs up to 20 meq/L in digester 2 during the failures seen in phases II and III. Digester 3, being stable throughout all phases, did not show an accumulation of VFAs; while in digester 4, the alkalinity and VFA values approached (about 35 meq/L) each other during digester failure. The addition of $Mg(OH)_2$ during phase III, did not help to maintain the alkalinity values in digester 4, resulting in immediate failure.

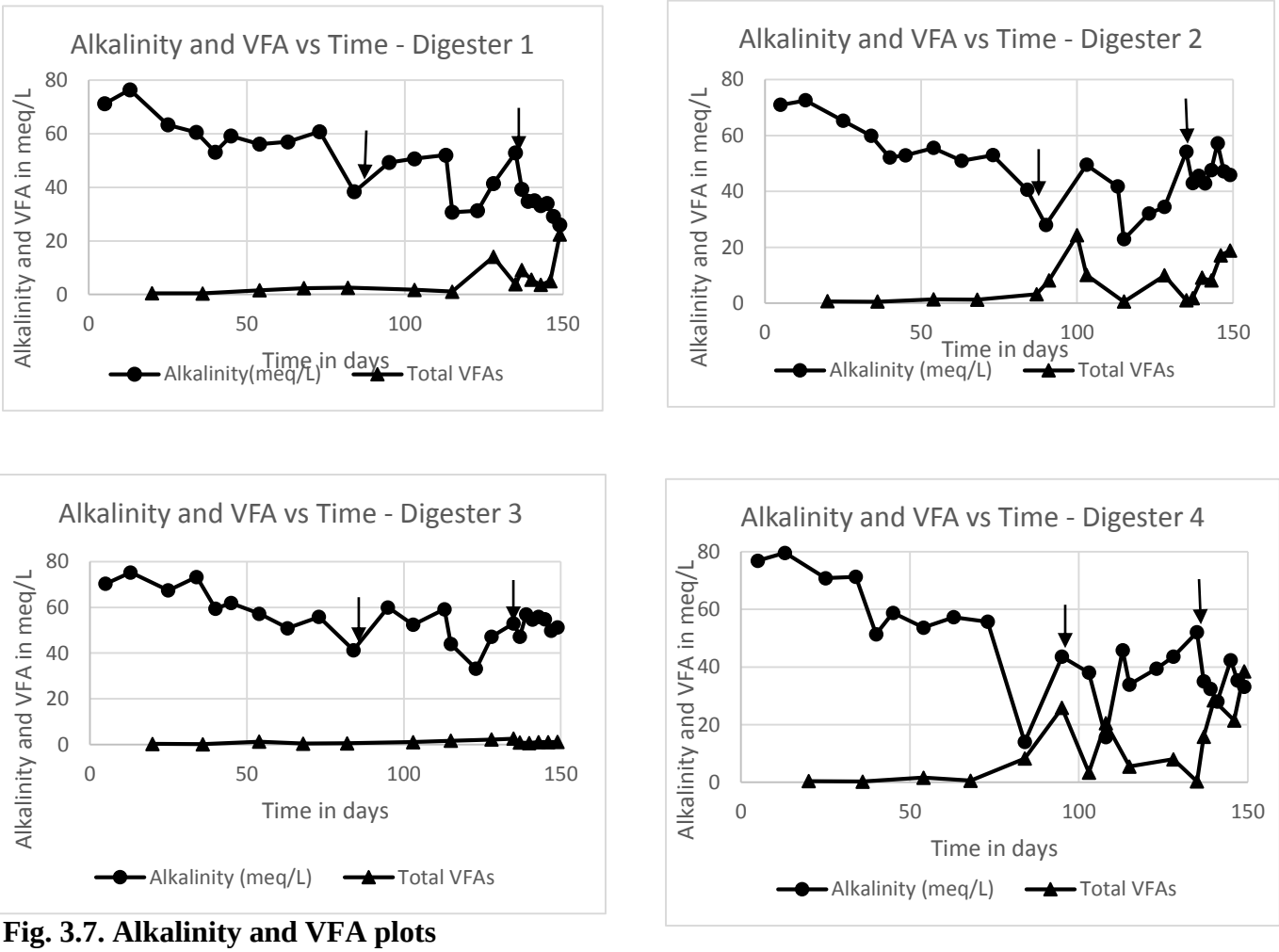


Fig. 3.7. Alkalinity and VFA plots

Gas Production

The ideal gas production under standard conditions is about 0.35 L CH_4/ g COD used up (Grady *et al*., 2011). Fig. 3.8 shows the gas production on the basis of COD fed per day. The addition of HSWs resulted in greater gas production (~0.3-0.4 L gas/g COD-day or 12-13 L/day) than the control (~0.2 L gas/g COD-day or 8.6 L/day) during phase I. This shows that addition of HSWs have the capacity to increase overall gas production and can be co-digested with sewage sludge at the feed loadings described in phase I. However, the addition of GTW effected increase (up to 0.5, 1 and 1.3 L gas/g COD-day or 7.8, 9.4 and 7.2 L/day) in gas production prior to failure in digesters 1, 2 and 4, as seen in phase III (days 140-145). During failure the gas production declined and finally stopped. Digester 3 exhibited elevated levels of gas production throughout the period of increased HSW loading. Gas productions of about 1.0 L gas/g COD-day or 33 L/day were seen in digester 3 without failure. This value shows that there is a 400% increase in the gas production in digester 3 as compared to the control.

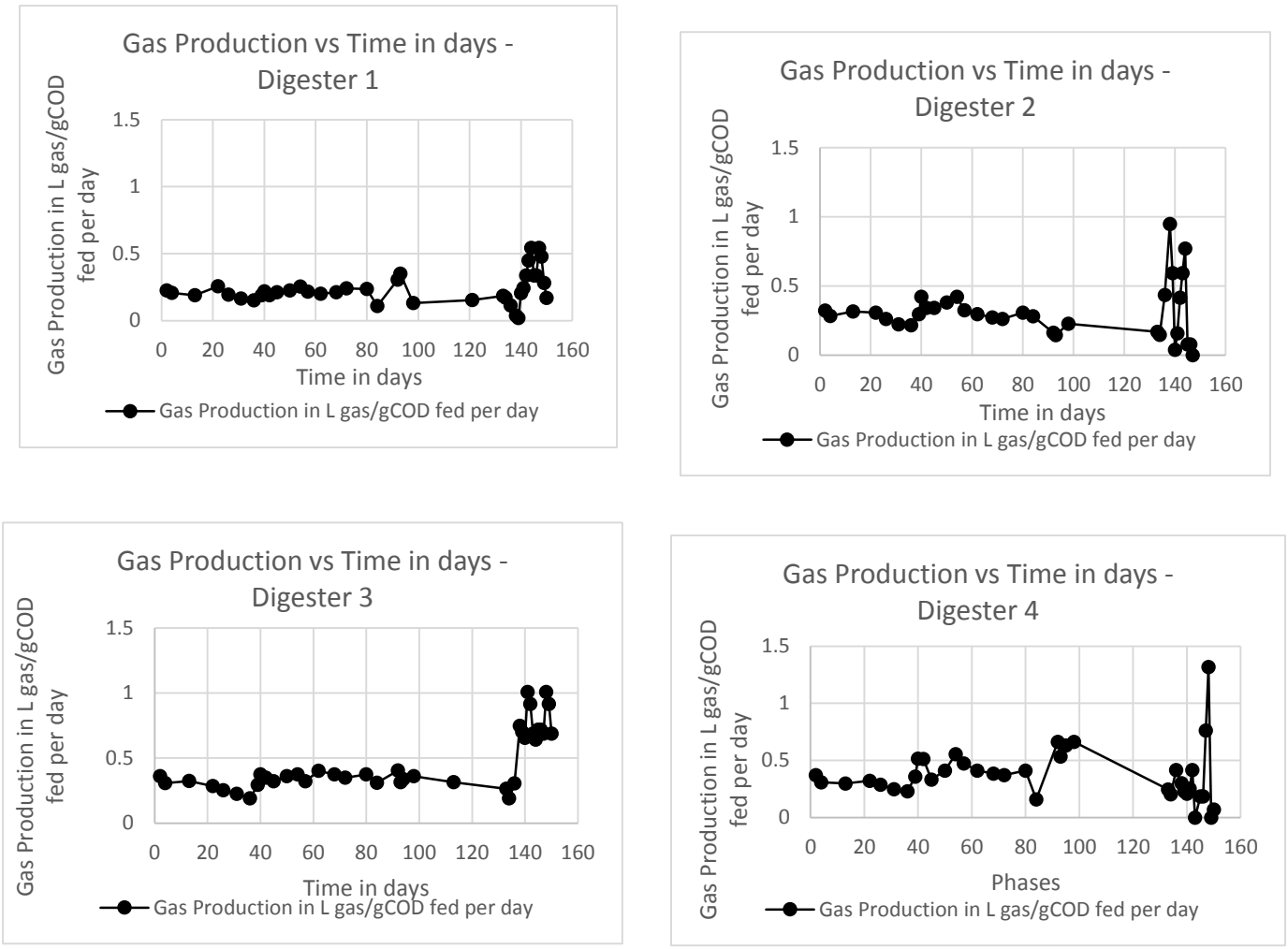


Fig. 3.8. Gas production plots

Gas Composition

The composition of the gas produced was measured once a week. The methane and carbon dioxide content of the gas produced was measured using their respective standards at concentrations of 5%, 50%, 75% and 100%. Fig. 3.9 shows the gas composition for all four digesters. The methane content of digester 3 was consistently around 60 - 70% during all the phases. The gas for digester 1 (control) had a methane content around 40 - 60%, even during failure in phase III.

Although the addition of GTW and DAF resulted in failure of digester 2, they helped to keep the methane content more or less in the area of 60%, slightly higher than the control. The highest methane content of 80% was observed in digester 4 prior to failure in phase III. The methane

content, however, dropped to nearly zero and 20% in digester 4, during the failures in phases II and III, respectively. This shows that even though GTW had the ability to increase the gas production and methane content during anaerobic digestion, it can result in rapid failure of the digesters.

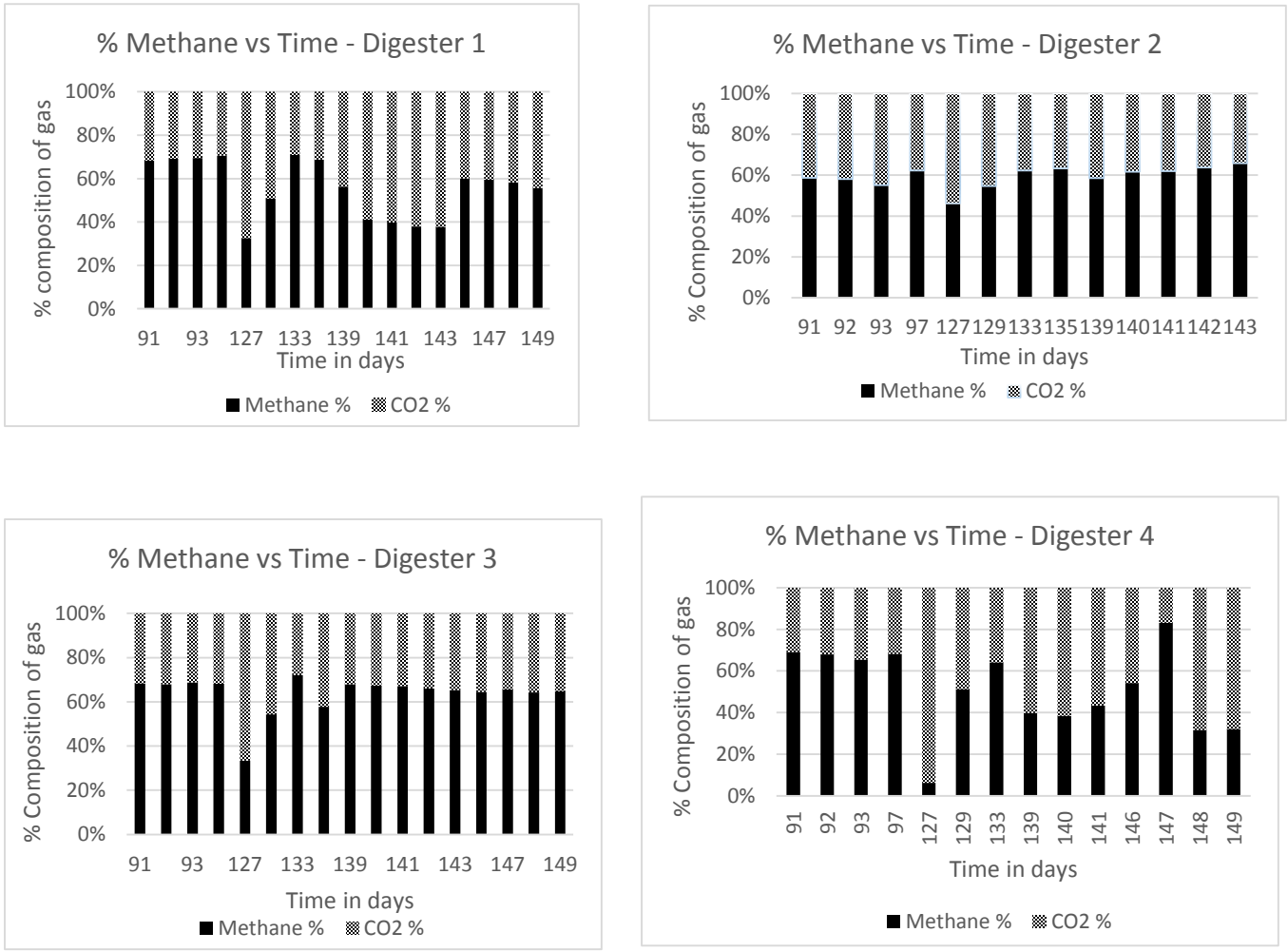


Fig. 3.9. Gas composition

Capillary Suction Time

Capillary suction time vs. polymer dosage during phase I can be seen in Fig. 3.10. The polymer dosage at the lowest CST and at 20 seconds can be seen in Table 3.5 during all the phases. With a usual optimum polymer dosage of 5-15 lb/ton dry solids used in wastewater treatment plants, Table

3.5 shows that the polymer dosage at 20 sec was slightly higher and in the range of 10-25 lb/ton dry solids. It can be seen that the polymer dosage for dewatering effluent from digester 3 is the highest during phase I. The dosage required for digesters 2 and 4 is comparable to digester 1. This shows that during phase I, when all digesters were running under stable operation, digester 3, receiving DAF and no GTW, needed the highest polymer dosage, which was 9.30 g polymer/kg dry solids at the lowest CST. During phase II, digester 2 underwent failure immediately. Thus, the CST values were obtained only for digesters 1, 3, and 4. Though the polymer dose at the lowest CST is comparable for all the digesters, the polymer dose at 20 seconds CST shows that the control, in fact, needed a higher polymer dosage of 10.59 g polymer/kg dry solids as compared to 9.59 and 6.44 g polymer/kg dry solids for digesters 3 and 4, respectively. The addition of HSWs did not impact dewatering and might even help in lowering the required polymer dosage. Though the polymer dosage for the lowest CST was slightly higher during phase III, (17.84 g polymer/kg dry solids for digester 2) as compared to other phases, the polymer dosage at 20 seconds CST (9.40 g polymer/kg dry solids for digester 2) is still in the same range as that for phases I and II. CST and dewatering tests could not be carried out for digesters 1 and 4 in phase III due to their immediate failure.

Table 3.6 shows the percentage cake solids in the dewatered effluent, after adding the polymer dose at the lowest CST. Cake solids of 15 - 30% were obtained post-centrifugation and about 20 - 40% cake solids were obtained post-use of the hydraulic piston press. Digester 4 received the highest volumetric loading of HSWs, but was still able to produce a sludge that dewatered to 36% with the hydraulic press in phase II, as compared to 31% achieved for sludge from digester 1. This shows that addition of HSWs can increase the solids content of the dewatered sludge.

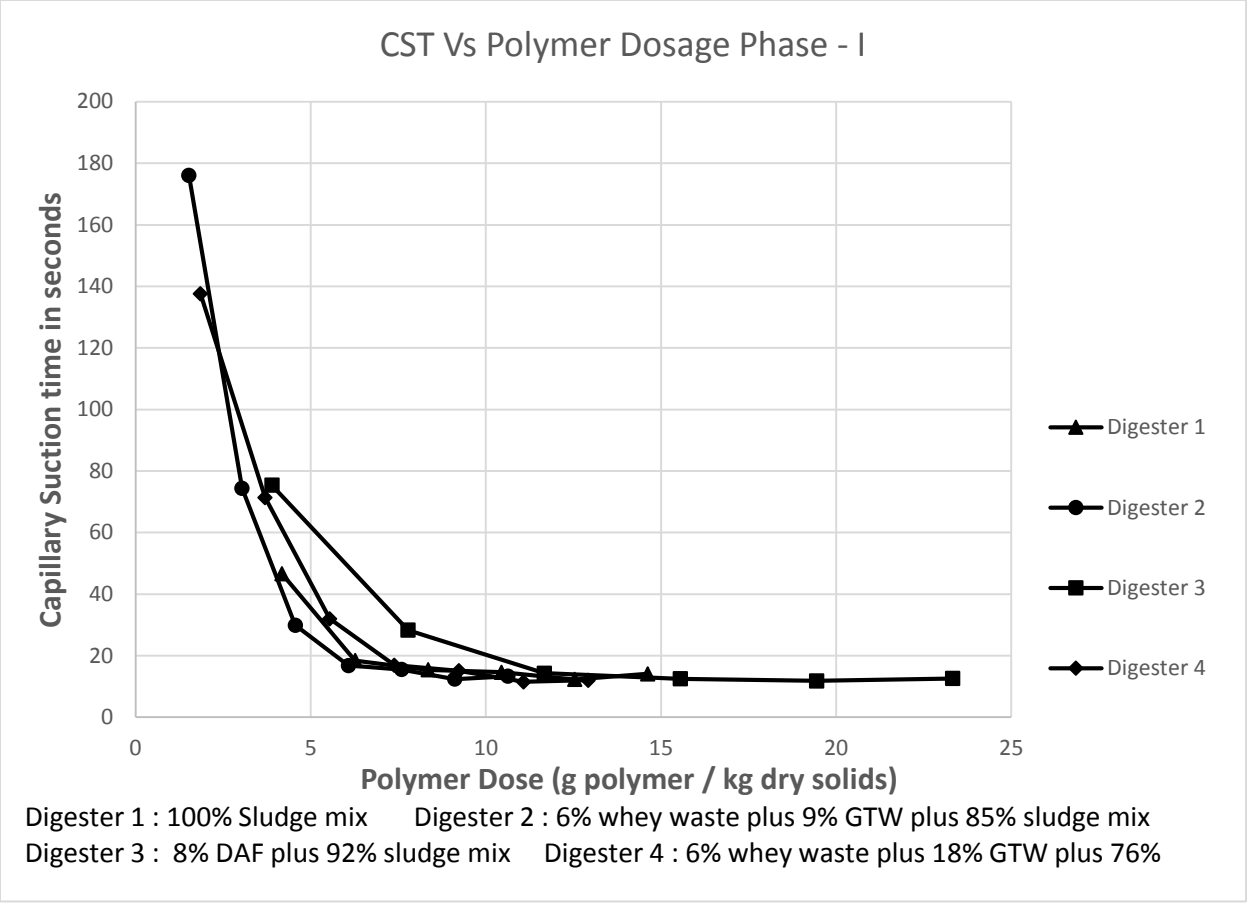


Fig. 3.10. CST vs Polymer dosage for phase I

Table 3.5. Optimum polymer dose for different phases

Phase	Digester	Polymer dose at 20 sec CST, g polymer/kg dry solids	Polymer dose for lowest CST, g polymer/kg dry solids	Polymer dose at 20 sec CST, lb polymer/ton dry solids
I	1	6.03	12.53	13.29
	2	5.51	9.11	12.15
	3	9.30	19.4	20.50
	4	6.89	11.07	15.19
II	1	10.59	16.93	23.35
	3	9.59	15.48	21.14
	4	6.44	10.04	14.20
III	2	9.40	17.84	20.72
	3	8.76	16.35	19.31

Table 3.6. % Cake Solids

Digester		Phase I	Phase II	Phase III
1	Post Centrifuge	15	17	_
	Post Hydraulic Piston Press	31	31	_
2	Post Centrifuge	16	_	20
	Post Hydraulic Piston Press	28	_	25
3	Post Centrifuge	16	21	18
	Post Hydraulic Piston Press	31	34	28
4	Post Centrifuge	23	21	_
	Post Hydraulic Piston Press	32	36	_

Volatile Sulfur Compounds

The sludge dewatered with the hydraulic piston press was incubated and used in measurements of the VSCs. Fig. 3.11 and 3.12 show the buildup and decline of hydrogen sulfide (H_2S), methanethiol (MT) and dimethyl sulfide (DMS) in the dewatered cake solids over time. Fig. 3.11, for phase I, shows that digester 1 had low amounts of MT and DMS peaking at 100 and 5 mg/L as S, respectively. MT is converted to H_2S, peaking at 950 mg/L as S, which shows there were elevated levels of sulfur in digester 1, but there was no inhibition of methanogens since MT did not accumulate in the vials. Digester 2 had high MT and DMS concentrations with MT peaking at 250 mg/L as S and the highest DMS concentrations of 40 mg/L as S. This indicates there could be some amount of inhibition of the methanogens. Methanogens convert MT to methane and sulfide. If methanogens are inhibited, MT can persist in the headspace vials (Higgins *et al*., 2006). Digester 3 had the lowest MT, DMS and H_2S levels, indicating no inhibition. Digester 4 had the highest MT concentrations of 350 mg/L. This shows the high possibility of inhibition. Although, during phase I all the digesters were stable, there could be some inhibition caused by the GTW in digester 2 and 4 as seen from the MT and DMS values.

The VSC results for phase II were unclear and therefore have not been presented. Fig. 3.12, phase III, shows VSCs for digesters 2 and 3. Digesters 1 and 4 underwent failure immediately and could not be tested for CST and sludge dewatering. Fig. 3.12, for phase III, shows that digester 3 was not being inhibited; however, there was some amount of sulfur present, which can be seen from the H_2S values peaking to 110 mg/L. The inhibition in digester 2 is evident from the MT concentration peaking up to 250 mg/L. These results show that even though the sludge from the digesters fed with HSWs dewatered well, the HSW wastes affected the odors of the dewatered cake solids.

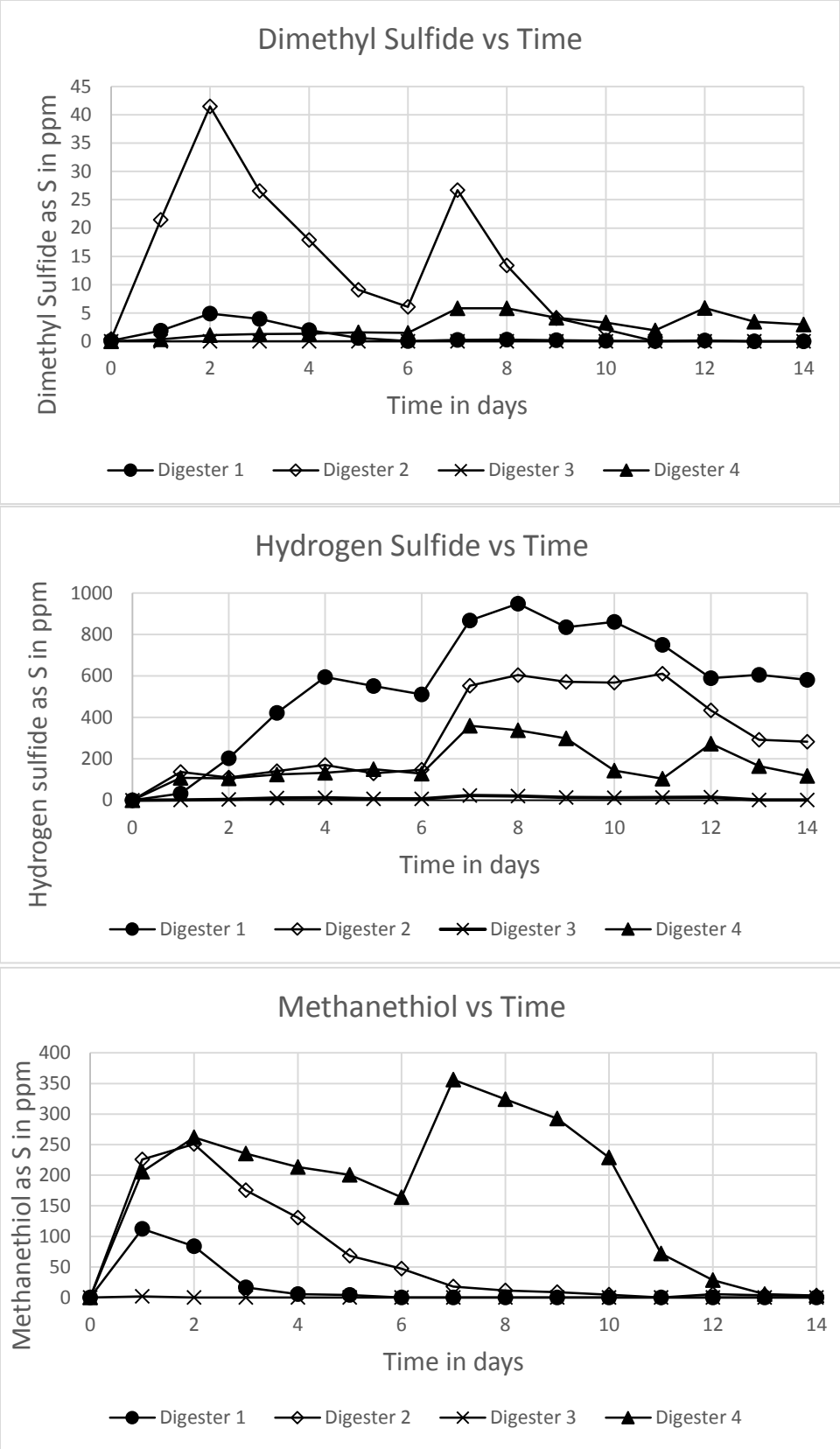
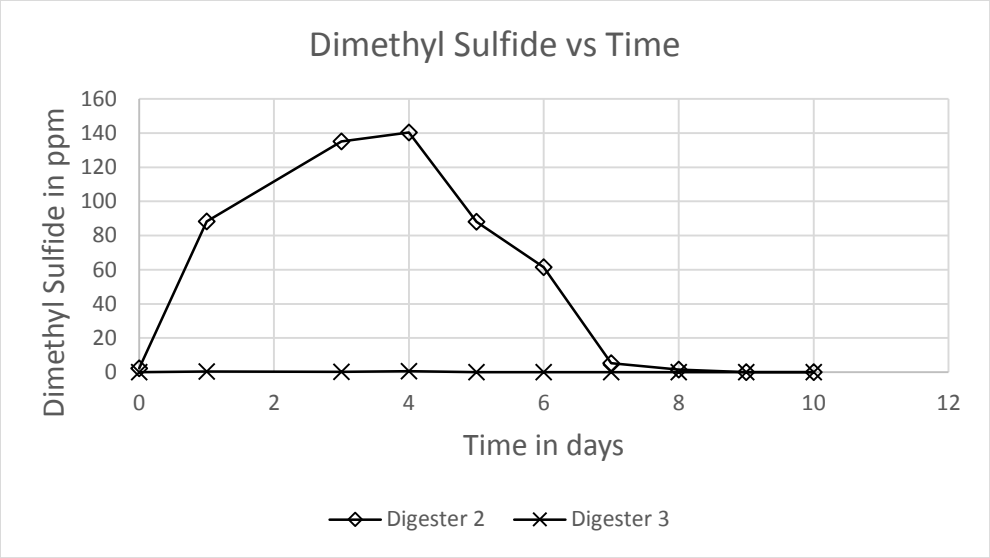
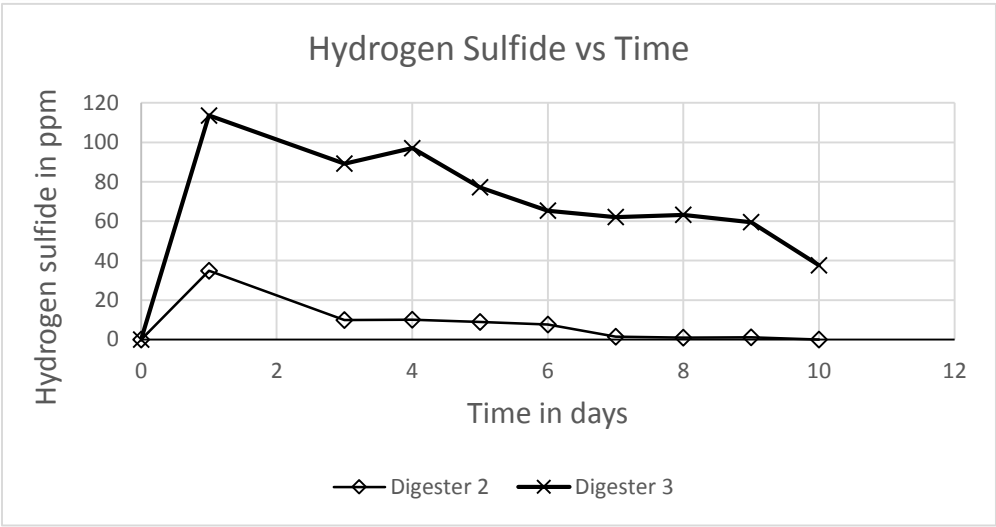


Fig. 3.11. VSCs for phase I





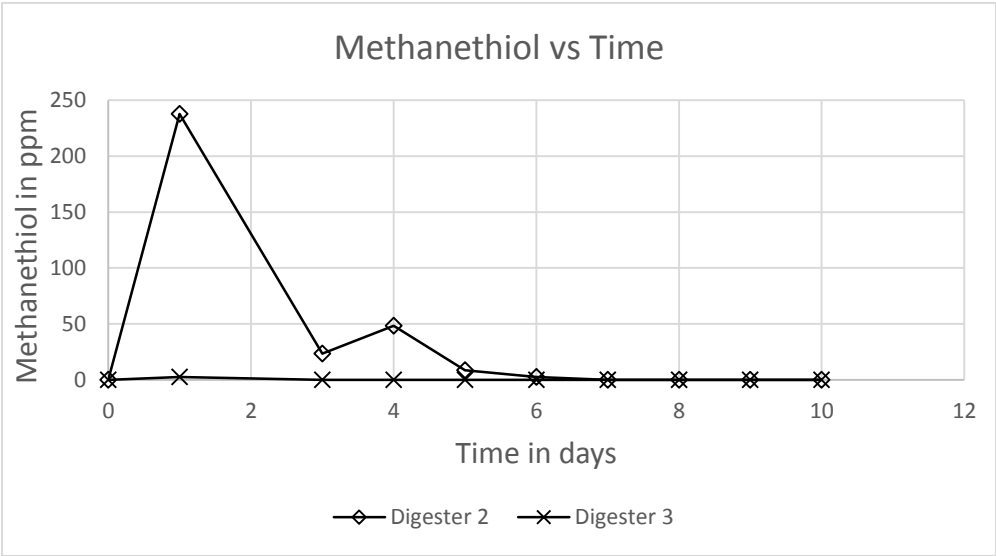


Fig. 3.12. VSCs for phase III

Metal Analyses

The effluents from the digesters were analyzed for metals to look at potential toxicity due to the presence of metal ions. Table 3.7 provides the concentrations of metals that were present in significant amounts (>1 mg/L) in the digested effluents and HSWs. The highly inhibitory concentrations of Na, K, Ca and Mg are reported to be 8,000, 12,000, 8,000 and 3,000 mg/L, respectively (Parkin and Owen, 1986). Table 3.7 shows that the effluent contains metal concentrations lower than the inhibitory concentrations. The metal concentrations in HSWs seem to be low, except for whey. The Na and K concentrations in whey are around 5,000 and 2500 mg/L, respectively. These values can be moderately inhibitory to anaerobic digestion according to Parkin and Owen, (1986).

Iron in digesters reacts with H_2S and precipitates as FeS (Novak *et al*., 2006). The amount of Fe in the digesters was ~1 mg/L. This low amount of Fe might explain the high amounts of H_2S (950 mg/L in digester 1, and 600 mg/L in digester 2) seen during phase I (Fig 3.11).

Table 3.7. Metal Analyses. All concentrations are in mg/L.

	Elements						
Sample	Na	Mg	Al	P	K	Ca	Fe
Digester 1	112.1	12.5	0.6	35.0	78.3	20.9	1.1
Digester 2	361.8	14.3	0.7	39.6	217.0	16.9	0.9
Digester 3	178.3	15.7	0.4	32.4	90.3	19.8	0.7
Digester 4	376.9	18.8	0.3	47.7	216.3	32.7	0.7
GTW	272.8	28.3	1.7	52.9	87.6	414.1	38.9
DAF	700.1	31.0	0.1	116.4	171.2	215.4	46.7
Juice waste	113.6	12.8	0.3	7.7	79.6	54.8	1.6
Whey	4,840.0	120.2	0.1	846.9	2,520.0	1,081.0	1.1

LCFAs

Table 3.8 shows the LCFAs present in digester effluent and HSWs. The effluents from digesters 2 and 4 (which underwent failure in phase II) were also analyzed for LCFAs. Since no consistent trend is seen in LCFA data, no failure or inhibition can be conclusively attributed to LCFA toxicity. The inhibitory concentrations for C14:0 (myristic acid) and C18:1 (oleic acid) are reported to be 593 mg/L and 677 mg/L respectively (Koster and Cramer, 1987). Table 3.8 shows that digesters 1

and 3 were not inhibited by LCFA since the concentrations were low (>100 mg/L), while C16:0 (palmitic acid) and C18:0 (stearic acid) were present in digesters 2 and 4 at about 1,000 and 500 mg/L, respectively. This shows that LCFAs were being accumulated in digesters 2 and 4 prior to failure. Palmitic and stearic acid concentrations increased up to 1,500 and 1,000 mg/L, respectively, during digester 2 and 4 failure. Although whey contained 300 mg/L of stearic acid, the LCFA concentrations present in juice waste and whey were negligible (<20 mg/L). DAF contained about 1,300 mg/L of palmitic acid and C18:1V (methyl vaccenate), while stearic acid and C18:1O (methyl oleate) were present at concentrations more than 500 mg/L. GTW contained lesser LCFAs (except for C18:1) as compared to DAF, 400 mg/L of palmitic acid and 500 mg/L of stearic acid. However, DAF and GTW both contained considerable amounts of LCFAs. The effluent from digesters 2 and 4, which both received GTW show that the LCFAs were not being degraded, whereas digester 3 (received DAF) had LCFAs less than 20 mg/L. Although the LCFAs present in GTW were less than those present in DAF, they did not seem to be degraded during digestion. Thus, GTW, appeared to exhibit some toxicity that was not due to LCFAs.

Table 3.8. LCFAs in digester effluent and HSWs. C18:1O = Methyl Oleate, C18:1V = Methyl Vaccenate. All concentrations are in mg/L.

Sample	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1O	C18:1V	C18:2
Juice waste	n.d.	n.d.	5.2	n.d.	n.d.	n.d.	n.d.	n.d.
Whey	4.6	n.d.	18.4	n.d.	298.0	5.8	n.d.	n.d.
DAF	63.5	13.2	1367.6	9.1	679.5	533.9	1227.6	447.5
GTW	22.9	n.d.	339.6	27.7	152.0	574.6	24.3	261.6
Digester1	1.4	n.d.	57.4	n.d.	52.0	20.6	n.d.	10.9
Digester2	91.8	14.6	1092.2	12.8	725.0	17.2	4.3	13.8
Digester3	n.d.	n.d.	18.9	n.d.	11.1	n.d.	n.d.	n.d.
Digester4	82.9	10.4	1065.0	14.8	449.9	160.4	12.9	25.9
Digester 2 failure	119.4	17.2	1137.4	5.1	737.1	199.5	6.3	44.6
Digester 4 failure	151.4	22.5	1626.4	6.6	893.1	213.6	24.0	76.5

n.d. = not detected.

BMP Analyses

Fig. 3.13 shows cumulative biogas production for the control, DAF, GTW and diluted GTW (1 to 10 dilution) reactors. The control consisted of 100% digested sludge, whereas the HSWs were present on a volumetric basis, as specified in the legend for Fig 3.13. It can be seen that all the HSWs produced more biogas than the control. The highest biogas production of 67 mL was seen for 8% DAF which corresponds to the DAF concentrations used during phase I. However, the biogas production seemed to be inhibited for 12%, 16%, 20% and 25% DAF since the production was less than 67 mL. The 12% and 16% DAF corresponded to DAF concentration during phase II and III. On the other hand, the 5%, 9% and 12% GTW was not inhibited, since the biogas productions were 39, 50 and 65 mL, respectively. The biogas production values for 16% and 22% GTW were 41 and 22 mL, respectively, and thus seemed to be inhibited. These volumetric loadings are similar to those used for GTW during phase II and phase III of the study, during which failure was observed. The diluted GTW did not produce much biogas (~15 mL) and the amount is lower than that produced for the corresponding, not diluted GTW concentrations. The maximum cumulative biogas production and maximum rate of biogas increase can be seen in Table 3.9.

Table 3.9. Maximum cumulative biogas production

Sample	Maximum cumulative biogas production (mL)	Maximum rate of biogas increase for each reactor
Control	12	1.24
8% DAF	67	8.76
12% DAF	48	6.03
16% DAF	16	1.85
20% DAF	14	1.51
25% DAF	12	1.32
1.6% GTW	14	1.51
2.2% GTW	15	1.66
5% GTW	39	1.69
9% GTW	50	2.14
12% GTW	65	4.37
16% GTW	41	4.06
22% GTW	22	3.70

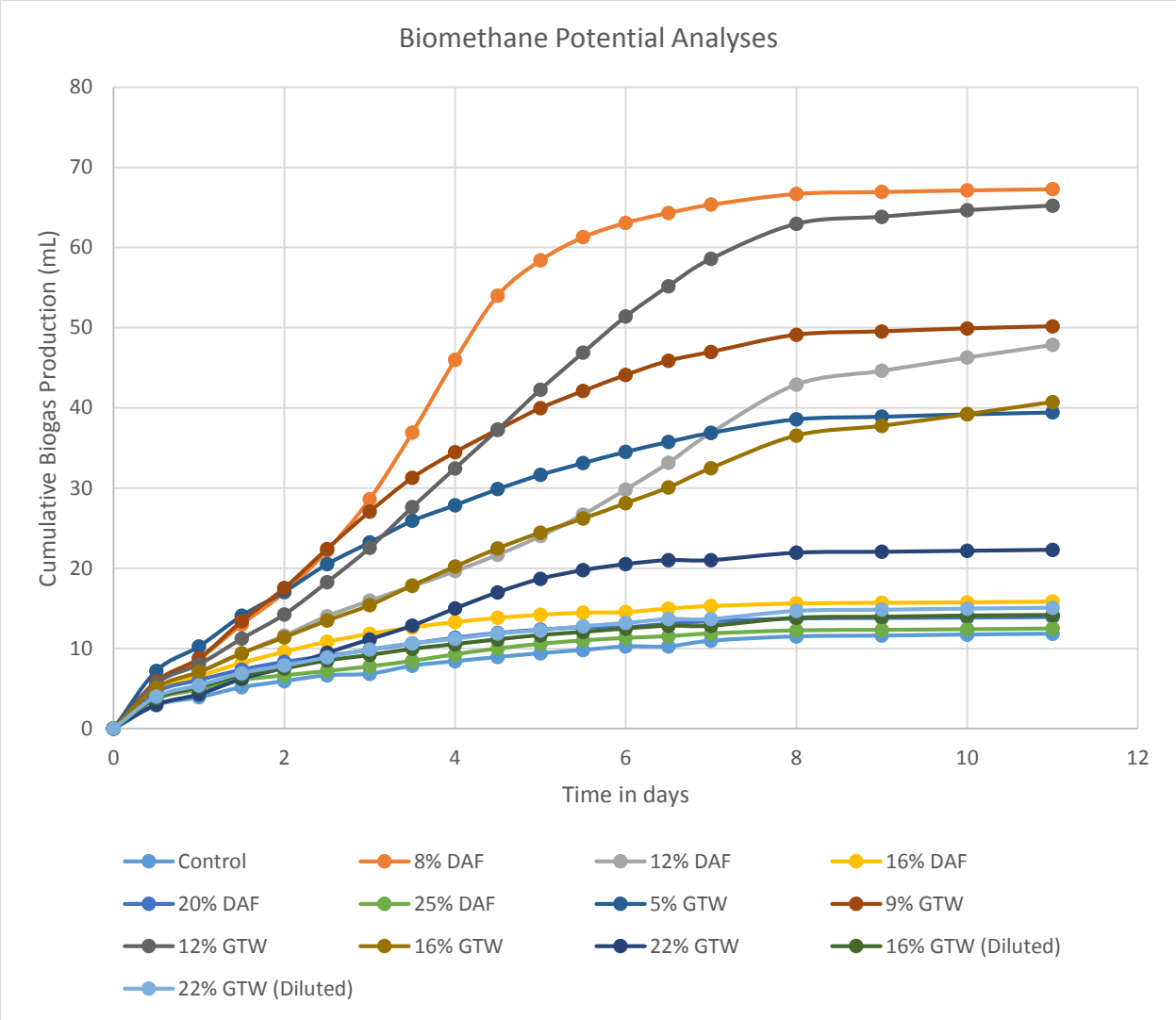


Fig. 3.13. Cumulative biogas production plots

Conclusion

The results showed that addition of HSWs can beneficial to anaerobic digestion and lead to higher methane production. However, not all HSWs are beneficial, depending on the nature of HSWs and can lead to digester failure. The main conclusions made from the study are as follows:

1. The addition of GTW at 16% (v/v) led to digester failure. Utilities need to take this into consideration before adding GTW to their digesters. GTW even at concentrations lower than 16% (v/v) might lead to digester failure as was seen from the overall decrease in alkalinity during phase I.
2. GTW addition was accompanied by a build-up of MT in the headspace gas of dewatered cake solids, suggesting inhibition of the methanogens responsible for the conversion of MT to DMS.
3. Addition of buffer, ($Mg(OH)_2$), did not prevent digester failure. Although the effluent pH of the digesters was initially maintained at 7.0 and even increased to 7.8, the alkalinity of digesters dropped down to 20 meq/L. Thus the addition of buffer only delayed the failure but did not prevent it.
4. The failure of digesters was seen by accumulation of VFAs as high as 35 meq/L and the gas production dropped down to zero. Grease from GTW was accumulated on the inner walls of the digesters.
5. Addition of DAF, whey and juice waste led to 400% increase in the gas production as compared to control in phase III. This shows that these HSWs can be beneficial to anaerobic digestion. DAF from food processor had higher levels of LCFAs as compared to GTW. But the LCFAs from DAF were degraded during digestion as seen in digester 3 throughout the study. Thus DAF addition can prove to be of great importance to increase the methane production.
6. Utilities should run a pilot study before incorporating new wastes in to the feeds of their digesters since the nature of these wastes can vary considerably.

Chapter 4

Conclusion

The study aimed at assessing the feasibility of adding different food processing wastes and fat, oils and grease to a conventional anaerobic digestion process. From the results obtained, it is clear that HSWs have the potential to increase the amount of methane produced during anaerobic digestion. Among the wastes used in this study, the juice and whey waste seem to work well with primary and WAS during co-digestion. Although the DAF had high amounts of LCFAs, they were degraded during digestion and did not inhibit the methanogens. On the other hand, despite the lower LCFA content of the GTW relative to DAF, the co-digestion process was inhibited, resulting in drop in pH to values below 6.0 and accumulation of VFAs in the digesters up to 35 meq/L. Gas production declined to zero and the alkalinity decreased to 20 meq/L during failure. The addition of a buffer ($Mg(OH)_2$), failed to prevent inhibition and only delayed the digester failure. The addition of HSW to digesters did not affect the sludge dewatering characteristics significantly, and the polymer dosage required for the digesters was in the range of 5-10 g polymer/kg dry solids for a capillary suction time of 20 seconds. A cake solids percentage of more than 30% was obtained after dewatering sludge from digester 4, which was receiving GTW, juice waste and whey waste. The overall cake solids data show that addition of HSWs increased the amount of solids in the dewatered cake. The odors of the cake solids, however, might be an issue after adding HSWs. The odors, mainly due to H_2S and MT peaked after 2 to 4 days, but were completely eliminated after a period of 10 to 13 days.

Wastewater treatment facilities need to consider the following points before they decide to add HSWs to their digesters:

1. The nature of HSWs vary in terms of COD, TS and TVS, pH and LCFAs, etc. Utilities need to perform a pilot study to analyze the wastes, especially for the effects of FOG, since FOG can change appreciably, depending on their sources.
2. Addition of GTW at 16% (v/v) resulted in digester failure. This result was confirmed by the BMP analysis. Thus, the utility needs to take this into consideration before incorporating GTW

to the digester feeds. GTW, even at lower doses might result in digester failure eventually, as seen from the overall decrease in the alkalinity during phase I.

3. The plant should try to use GTW at loading rates less than 12% (v/v) or dilute the GTW, which might help in washing out the toxicity present in GTW.
4. Addition of buffer did not prevent digester failure. The data obtained from phase III show that even though the effluent pH increased due to the addition of $Mg(OH)_2$, the digester underwent failure, which was seen by the accumulation of VFAs. pH values alone cannot be used as an indicator of digester stability.
5. The mixture of DAF, whey and juice waste seems to work well even at the highest loading rate, yielding up to 400% increases in gas production as compared to control, as was seen in phase III.

References

1. Ağdağ, O.N. and D.T. Sponza, Co-digestion of industrial sludge with municipal solid wastes in anaerobic simulated landfilling reactors. Process Biochemistry, 2005. 40(5): p. 1871-1879.

2. Alvarez J. M., M.S., Llabres P., Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technology, 2000. 74: p. 3-16.

3. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., van Lier, J. B., Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Science and Technology, 2009. 59(5): p. 927-934.

4. Angelidaki I., A.B.K., Effect of free long chain fatty acids on thermophilic anaerobic digestion. Applied Microbiology and Biotechnology, 1992. 37: p. 808-812.

5. Appels, L., et al., Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 2008. 34(6): p. 755-781.

6. Athanasoulia, E., P. Melidis, and A. Aivasidis, Anaerobic waste activated sludge co-digestion with olive mill wastewater. Water Sci Technol, 2012. 65(12): p. 2251-7.

7. Athanasoulia, E., P. Melidis, and A. Aivasidis, Co-digestion of sewage sludge and crude glycerol from biodiesel production. Renewable Energy, 2014. 62: p. 73-78.

8. Aymerich, E., M. Esteban-Gutierrez, and L. Sancho, Analysis of the stability of high-solids anaerobic digestion of agro-industrial waste and sewage sludge. Bioresour Technol, 2013. 144: p. 107-14.

9. Bailey, R.S. Anaerobic digestion of restaurant grease wastewater to improve methane gas production and electrical power generation potential. in 80th Annual Technical Exhibition and Conference of the Water Environment Federation. 2007. San Diego, CA.

10. Bertin, L., et al., Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. Bioresour Technol, 2013. 128: p. 779-83.

11. Bjornsson L., M.M., Mattiasson B., Evaluation of parameters for monitoring an anaerobic co-digestion process. Applied Microbiology Biotechnology, 2000. 54: p. 844-849.

12. Bolzonella, D., et al., Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochemistry, 2005. 40(3-4): p. 1453-1460.

13. Bond T., B.C.J., Foxon K.M. and Buckley C.A., A critical review of experimental and predicted methane generation from anaerobic codigestion. water Sci Technology, 2012. 65(1): p. 183-189.

14. Bouallagui, H., L. Marouani, and M. Hamdi, Performances comparison between laboratory and full-scale anaerobic digesters treating a mixture of primary and waste activated sludge. Resources, Conservation and Recycling, 2010. 55(1): p. 29-33.

15. Brown, D. and Y. Li, Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresour Technol, 2013. 127: p. 275-80.

16. Bruss J. H., C.J.R., Rasmussen H., Anaerobic storage of activated sludge: effects on conditioning and dewatering performance. Water Sci Technol, 1993. 28: p. 350-357.

17. Buendia, I.M., et al., Feasibility of anaerobic co-digestion as a treatment option of meat industry wastes. Bioresour Technol, 2009. 100(6): p. 1903-9.

18. Callaghan F. J., W.D.A.J., Thayanithy K., Forster C. F., Co-digestion of waste organic solids: batch studies. Bioresource Technology, 1999. 67: p. 117-122.

19. Calli, B., et al., Effects of high free ammonia concentrations on the performances of anaerobic bioreactors. Process Biochemistry, 2005. 40(3-4): p. 1285-1292.

20. Cavaleiro, A.J., M.A. Pereira, and M. Alves, Enhancement of methane production from long chain fatty acid based effluents. Bioresour Technol, 2008. 99(10): p. 4086-95.

21. Chen, Y., J.J. Cheng, and K.S. Creamer, Inhibition of anaerobic digestion process: a review. Bioresour Technol, 2008. 99(10): p. 4044-64.

22. Chynoweth David P., O.J.M., Legrand Robert, Renewable methane from anaerobic digestion of biomass. Renewable Energy, 2001. 22: p. 1-8.

23. Clark, R.H., and Speece, R. E., The pH Tolerance of Anaerobic Digestion, in 5th International Conference on Water Pollution Research. 1970: San Francisco, California.

24. Cuetos, M.J., et al., Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). Biochemical Engineering Journal, 2008. 40(1): p. 99-106.

25. Dai, X., et al., Biodegradation of polyacrylamide by anaerobic digestion under mesophilic condition and its performance in actual dewatered sludge system. Bioresour Technol, 2014. 153: p. 55-61.

26. Esposito, G., Frunzo, L., Liotta, F., Panico, A., Pirozzi, F., Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates. The Open Environmental Engineering Journal, 2012. 5: p. 1-8.

27. F., A., Effects of cation addition on the flocculation behaviour of activated sludge at applied constant shear force, in Department of Civil and Environmental Engineering Water Environment Technology. 2007, Chalmers University of Technology: Göteborg, Sweden. p. 86.

28. Forbes, R.H.H., L.; Adams, G.; Witherspoon, J.; Murthy, S. N.; Card, and R.G. T.; Hargraves, D.; Higgins, M. J. Impacts of In-Plant Operational Parameters on Biosolids Odor Quality: Preliminary Results of WERF Phase 2 Study. in Water Environment Federation and American

Water Works Association Annual Biosolids and Residuals Conference. 2003. Baltimore, Maryland.

29. Fountoulakis, M.S., I. Petousi, and T. Manios, Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Manag, 2010. 30(10): p. 1849-53.

30. G., L., Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek, 1995. 67: p. 3-28.

31. Gavala, H.N., et al., Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Research, 2003. 37(19): p. 4561-4572.

32. Glassman, H.N., Surface active agents and their application in bacteriology. Bacteriology Reviews, 1948. 12(2): p. 105-148.

33. Grady L.C.P., D.G.T., Love N.G., Filipe C.D.M., Biological Wastewater Treatment. Third ed. 2011, New York: CRC press, Taylor and Francis Group.

34. Hansen, K.H., Angelidaki, I., Ahring, B.K., Anaerobic digestion of swine manure: inhibition by ammonia. Water Research, 1997. 32: p. 5-12.

35. He, X., Iasmin, M., Dean, L., Lappi, S., Ducoste, J.J., de los Reyes III, and F.L., Evidence for how fat, oil, and grease (FOG) deposits form in sewer lines. Envionmental Science and Technology, 2011. 45(10): p. 4385-4391.

36. Henderson, C., The effects of fatty acids on pure cultures of rumen bacteria. Agric. Sci. Camb., 1973. 81: p. 107-112.

37. Heukelekian, H., Mueller, P., Sew. Ind. Wastes 1958. 30: p. 1108.

38. Higgins, M.a.N., J., Characterization of Exocellular Protein and Its Role in Bioflocculation. Environmental Engineering, 1997. 123(5): p. 479-485.

39. Higgins, M.J., et al., Cycling of Volatile Organic Sulfur Compounds in Anaerobically Digested Biosolids and its Implications for Odors. Water Environment Research, 2006. 78(3): p. 243-252.

40. Higgins, M.J.M., S. N.; Striebig, B.; Hepner, S.; Yamani, S.; Yarosz, D. P.; Toffey, W. Factors Affecting Odor Production in Philadelphia Water Department Biosolids. in Proceedings of Water Environment Federation Odors and Toxic Air Emissions. 2002. Albuquerque, New Mexico.

41. Holm-Nielsen, J.B., T. Al Seadi, and P. Oleskowicz-Popiel, The future of anaerobic digestion and biogas utilization. Bioresour Technol, 2009. 100(22): p. 5478-84.

42. Hosseini Koupaie, E., et al., Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: process and cost-benefit analysis. Bioresour Technol, 2014. 152: p. 66-73.

43. K., H., Mechanism of Inhibition Caused by Long-Chain Fatty Acids in Anaerobic Digestion Process. Biotechnology and Bioengineering, 1981. 23(7): p. 1591-1610.

44. Kaare Hvid Hansen, I.A.a.B.K.A., Anaerobic Digestion of Swine Manure: Inhibition by Ammonia. Water Research, 1998. 32(1): p. 5-12.

45. KabaraJ. J., V.R., Jie L. K., Antimicrobial Lipids: Natural and Synthetic Fatty Acids and Monoglycerides. Lipids, 1977. 12(9): p. 753-759.

46. Kim, S.H., Han, S.K., Shin, H.S., Kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation and beta-oxidation. Environ. Sci. Health A: Toxic/Hazard. Subst. Environ. Eng., 2004. 39(4): p. 1025-1036.

47. Kobayashi, T., et al., Characterization of start-up performance and archaeal community shifts during anaerobic self-degradation of waste-activated sludge. Bioresour Technol, 2009. 100(21): p. 4981-8.

48. Koster I. W., K.A., Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. Applied and Environmental Microbiology, 1987. 53: p. 403-409.

49. Kroeker, E.J., Schulte, D. D., Lapp, H. M., Methane Production from Animal Wastes II—Process Stability. ASAE, 1976: p. 76-208.

50. Kroeker, E.J., et al., Anaerobic Treatment Process Stability. Water Pollution Control Federation, 1979. 51: p. 718.

51. Kugelman, I.J., and Chin, K. K., Toxicity, Synergism, and Antagonism in Anaerobic Waste Treatment Processes, in Anaerobic Biological Treatment Processes. 1971, American Chemical Society.

52. Lalman J. A., B.D.M., Anaerobic Degradation and Methanogenic Inhibitory effects of oleic and stearic acids. Water Research, 2001. 35: p. 2975-2983.

53. Lansing, S., et al., Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. Bioresour Technol, 2010. 101(12): p. 4362-70.

54. Le Hyaric, R., et al., Pilot-scale anaerobic digestion of screenings from wastewater treatment plants. Bioresour Technol, 2010. 101(23): p. 9006-11.

55. Leiva, M.B., E.H. Koupaie, and C. Eskicioglu, Anaerobic co-digestion of wine/fruit-juice production waste with landfill leachate diluted municipal sludge cake under semi-continuous flow operation. Waste Manag, 2014. 34(10): p. 1860-70.

56. Li, C., P. Champagne, and B.C. Anderson, Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions. Bioresour Technol, 2011. 102(20): p. 9471-80.

57. Long, J.H., et al., Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. Process Safety and Environmental Protection, 2012. 90(3): p. 231-245.

58. Lou, X.F., J. Nair, and G. Ho, Field performance of small scale anaerobic digesters treating food waste. Energy for Sustainable Development, 2012. 16(4): p. 509-514.

59. Luostarinen, S., S. Luste, and M. Sillanpaa, Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. Bioresour Technol, 2009. 100(1): p. 79-85.

60. Lv, W., W. Zhang, and Z. Yu, Evaluation of system performances and microbial communities of two temperature-phased anaerobic digestion systems treating dairy manure. Bioresour Technol, 2013. 143: p. 431-8.

61. Macias-Corral, M., et al., Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. Bioresour Technol, 2008. 99(17): p. 8288-93.

62. Martin, M.A., et al., Semi-continuous anaerobic co-digestion of orange peel waste and residual glycerol derived from biodiesel manufacturing. Waste Manag, 2013. 33(7): p. 1633-9.

63. McCarty, P.L., and McKinney, R. E., Volatile Acid Toxicity in Anaerobic Digestion. Water Pollution Control Federation, 1961. 33: p. 223.

64. McCarty, P.L., Anaerobic Waste Treatment Fundamentals: I. Chemistry and Microbiology; II. Environmental Requirements and Control; III. Toxic Materials and Their Control; IV. Process Design. Public Works, 1964: p. 9-12.

65. McCarty, P.L. Stoichiometry of Biological Reactions. in International Conference Toward a Unified Concept of Biological Waste Treatment Design. 1972. Atlanta.

66. Mikkelsen L.H., K.K., Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. Water Research, 2002. 36: p. 2451-2462.

67. Mondragón F. A., S.P., Cox H. J., Ahring B. K. and Iranpour R., Anaerobic Codigestion of Municipal, Farm, and Industrial Organic Wastes: A Survey of Recent Literature. Water Environment Research, 2006. 78(6): p. 607-636.

68. Monroy Oscar, F.G., Meraz Monika, Montoya Leticia and Macarie Herve, Anaerobic Digestion for Wastewater treatment in Mexico: State of the Technology. Water Research, 2000. 34: p. 1803-1816.

69. Monteith, H.D., and Stephenson, J. P., Mixing Efficiencies in Full-Scale Anaerobic Digesters by Tracer Methods. Journal of the Water Pollution Control Federation, 1981. 53(1): p. 78-84.

70. Muller, C., Lam, P., Lin, E., Chapman, T., Devin-Clark, D., and J. Belknap-Williamson, Krugel, S. Co-digestion at Annacis Island WWTP: metro Vancouver's path to renewable energy and greenhouse gas emission reductions. in Water Environment Federation. 2010. New orleans, LA.

71. Muller, C.D.V., N.; Higgins, M. J.; Novak, J. T. The Role of Shear in the Generation of Nuisance Odors from Dewatered Biosolids. in Proceedings of the 77th Annual Water Environment Federation Technical Exposition and Conference. 2004. New Orleans, LA.

72. Nallathambi, G.V., Anaerobic Digestion of Biomass For methane Production: A Review. Biomass and Bioenergy, 1997. 13: p. 83-114.

73. Nelson, E.D., and Baley, J. F., The Egg is Shape of Things to Come. Water and Sewage Works, 1979: p. 30-32.

74. Neves, L., R. Oliveira, and M.M. Alves, Anaerobic co-digestion of coffee waste and sewage sludge. Waste Manag, 2006. 26(2): p. 176-81.

75. Nielsen P. H., K.K., Disintegration of Activated Sludge flocs in the presence of Sulfide. Water Research, 1998. 32(2): p. 313-320.

76. Nishio, N. and Y. Nakashimada, Recent development of anaerobic digestion processes for energy recovery from wastes. J Biosci Bioeng, 2007. 103(2): p. 105-12.

77. Noutsopoulos, C., et al., Anaerobic co-digestion of grease sludge and sewage sludge: the effect of organic loading and grease sludge content. Bioresour Technol, 2013. 131: p. 452-9.

78. Novak J. T., B.H., Zurow A., Factors influencing activated sludge properties. Environmental Engineering, 1977. 103: p. 815-828.

79. Novak John T., P.C., Higgins Matthew J., Chen Y., Morton R., Gary D., Forbes R., Erdal Z. , WERF Odor Study Phase III: Impacts of the MicroSludge Process on Odor Causing Compounds in Residuals and Biosolids management Conference. 2007.

80. Novak, J.T., Carlson, D.A., The kinetics of anaerobic long chain fatty acid degradation. Water Pollution Control Federation, 1970. 42(11): p. 1932-1943.

81.Novak, J.T., et al., Generation Pattern of Sulfur Containing Gases from Anaerobically Digested Sludge Cakes. Water Environment Research, 2006. 78(8): p. 821-827.

82. Novak, J.T., M.E. Sadler, and S.N. Murthy, Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. Water Research, 2003. 37(13): p. 3136-3144.

83. Owen, W.F., Stuckey, D. C., Healy, J. B., Young, L. Y., McCarty, P. L., Bioassay for monitoring Biochemical Methane Potential and Anaerobic Toxicity. Water Research, 1979. 13: p. 485-492.

84. Pahl, O., et al., Anaerobic co-digestion of mechanically biologically treated municipal waste with primary sewage sludge - a feasibility study. Bioresour Technol, 2008. 99(9): p. 3354-64.

85. Parkin Gene F., O.W.F., Fundamentals of Anaerobic Digestion of Wastewater Sludge. Journal of Environmental Engineering, 1986. 112(5): p. 867-920.

86. Pereira, M.A., Cavaleiro, A.J., Mota, M., Alves, M.M., Accumulation of long chain fatty acids onto anaerobic sludge under steady state and shock loading conditions: effect on acetogenic and methanogenic activity. Water Sci Technol, 2003. 48(6): p. 33-40.

87. Rajagopal, R., D.I. Masse, and G. Singh, A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour Technol, 2013. 143: p. 632-41.

88. Rajeshwari K.V., B.M., Kansal A., Lata Kusum, and K. V.V.N., State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renewable and Sustainable Energy Reviews, 2000. 4: p. 135-156.

89. Raskin, L., Stroot, P. G., McMahon, K.D., Mackie R.I., Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions - I. Digester Performance. Water Research, 2001. 35(7): p. 1804 - 1816.

90. Razaviarani, V. and I.D. Buchanan, Reactor performance and microbial community dynamics during anaerobic co-digestion of municipal wastewater sludge with restaurant grease waste at steady state and overloading stages. Bioresour Technol, 2014. 172: p. 232-40.

91. Razaviarani, V., et al., Pilot-scale anaerobic co-digestion of municipal wastewater sludge with restaurant grease trap waste. J Environ Manage, 2013. 123: p. 26-33.

92. Rinzema A., A.A., Lettinga G., The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. Chem. Technol. Biotechnol., 1989. 46: p. 257-266.

93. Rinzema A., B.M., Knippenberg K. and Lettinga G., Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion. Water Environment Research, 1994. 66: p. 40-49.

94. Romano, R.T. and R. Zhang, Co-digestion of onion juice and wastewater sludge using an anaerobic mixed biofilm reactor. Bioresour Technol, 2008. 99(3): p. 631-7.

95. Rudolfs, W.C., and Amberg, H. R, White Water Treatment II. Effect of Sulfides on Digestion. Sewage and Industrial Wastes, 1952. 24: p. 1278.

96. Salminen E., R.J., Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. Bioresour Technology, 2002. 83: p. 13-26.

97. Sheng, K., et al., Effect of ammonia and nitrate on biogas production from food waste via anaerobic digestion. Biosystems Engineering, 2013. 116(2): p. 205-212.

98. Shilton, A., Powell, N., Broughton, A., Pratt, C., Pratt, S., Pepper, C., Enhanced biogas production using cow manure to stabilize co-digestion of whey and primary sludge. Enviromental Technology, 2013. 34(17): p. 2491-2496.

99. Silvestre, G., et al., Thermophilic anaerobic co-digestion of sewage sludge with grease waste: Effect of long chain fatty acids in the methane yield and its dewatering properties. Applied Energy, 2014. 117: p. 87-94.

100. Sung, S. and T. Liu, Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere, 2003. 53(1): p. 43-52.

101. Swanwick, J.D., Shurgen, D. G., and Jackson, S., A Survey of the Performance of Sewage Sludge Digesters in Great Britain. Water Pollution Control, 1969. 68(6): p. 186-279.

102. Tchobanoglous, G., Burton, F. L., Stensel, D. H., Metcalf and Eddy: Wastewater Engineering, Treatment and Reuse. 4 ed. 2003: Tata McGraw Hill.

103. Torpey, W.N., Loading to Failure of a Pilot High-Rate Digester. Sewage and Industrial Wastes, 1955. 27(2): p. 121-148.

104. Wang, L., T.N. Aziz, and F.L. de los Reyes, 3rd, Determining the limits of anaerobic co-digestion of thickened waste activated sludge with grease interceptor waste. Water Res, 2013. 47(11): p. 3835-44.

105. Ward, A.J., et al., Optimisation of the anaerobic digestion of agricultural resources. Bioresour Technol, 2008. 99(17): p. 7928-40.

106. Yamashiro, T., et al., Anaerobic co-digestion of dairy cow manure and high concentrated food processing waste. Journal of Material Cycles and Waste Management, 2013. 15(4): p. 539-547.

107. Yenigün, O. and B. Demirel, Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry, 2013. 48(5-6): p. 901-911.

108. Zhang, R., et al., Characterization of food waste as feedstock for anaerobic digestion. Bioresour Technol, 2007. 98(4): p. 929-35.

109. Zhen, G., et al., Enhanced dewatering characteristics of waste activated sludge with Fenton pretreatment: effectiveness and statistical optimization. Frontiers of Environmental Science & Engineering, 2013. 8(2): p. 267-276.

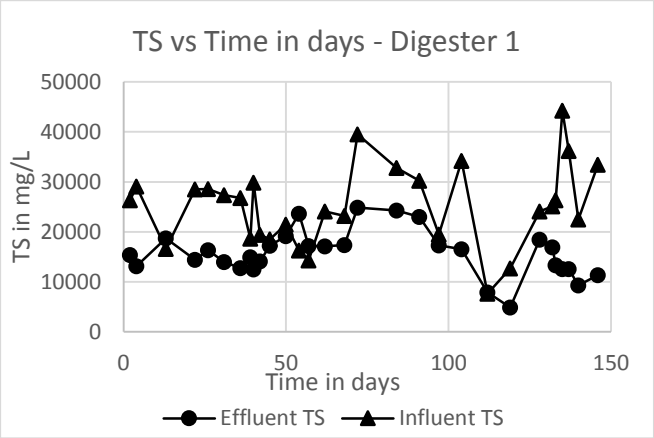
110. Zhu, H., et al., Biohydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges. International Journal of Hydrogen Energy, 2008. 33(14): p. 3651-3659.

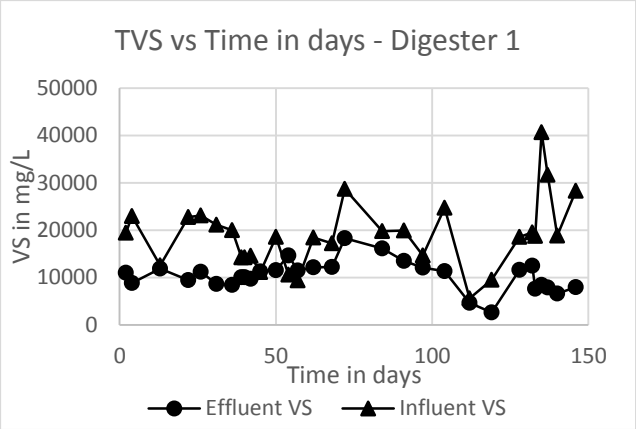
111. Zhu, H., et al., Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. Bioresour Technol, 2008. 99(11): p. 5078-84.

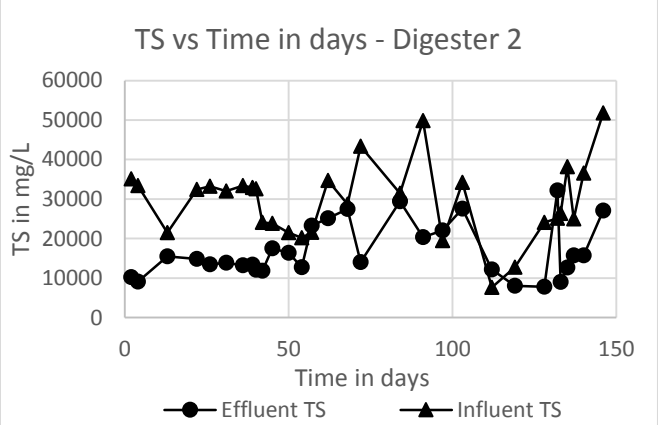
112. Zhu, Z., M.K. Hsueh, and Q. He, Enhancing biomethanation of municipal waste sludge with grease trap waste as a co-substrate. Renewable Energy, 2011. 36(6): p. 1802-1807.

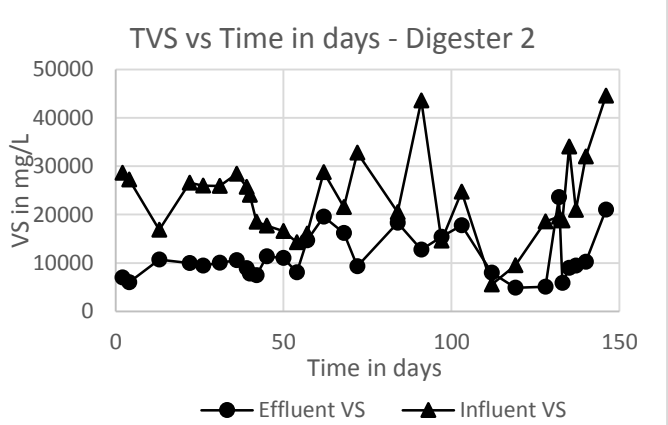
Appendix A

Total and Total Volatile solids









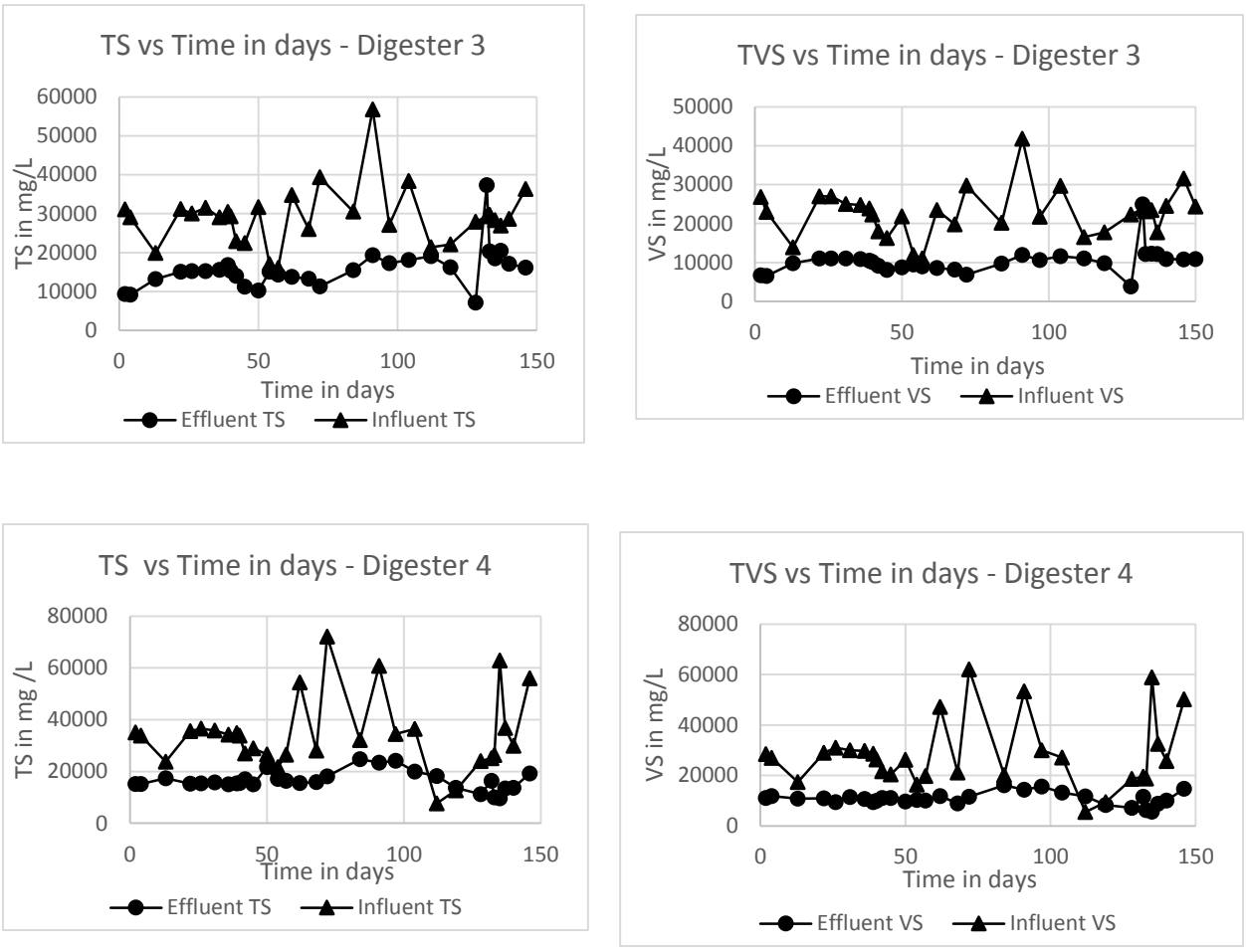


Fig. A.1. Total and total volatile solids

Total Ammonia Nitrogen and Total Kjeldahl Nitrogen

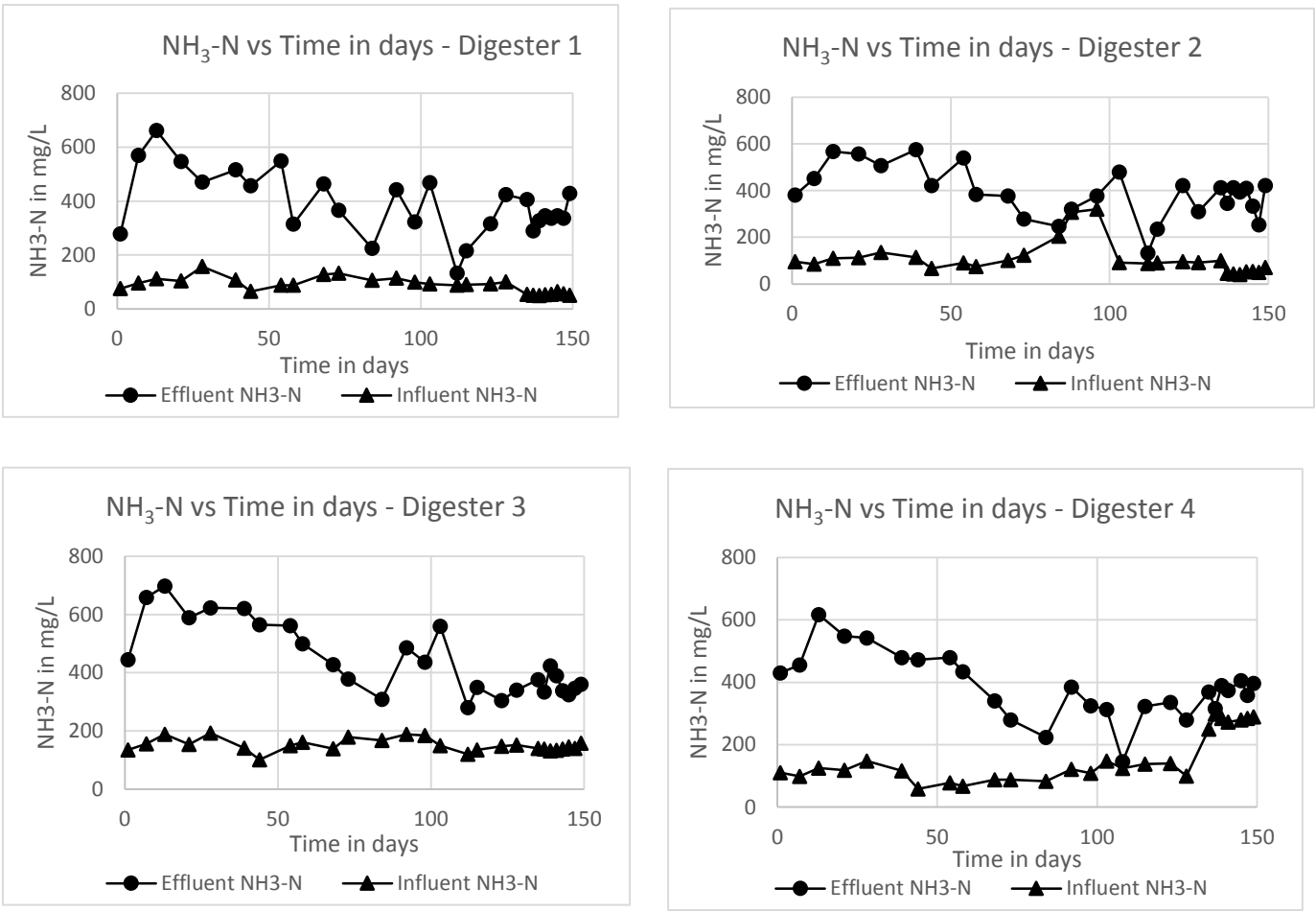
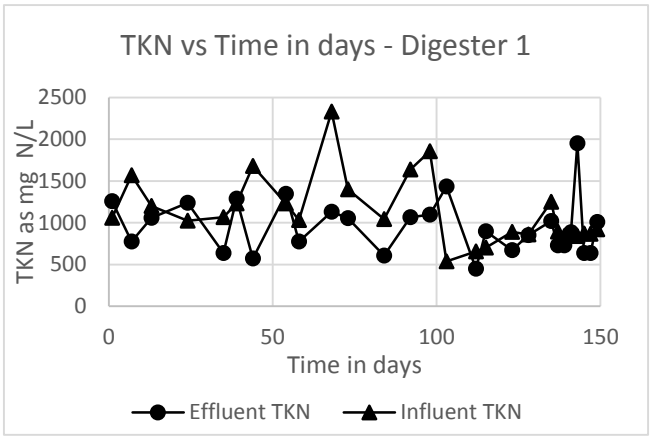
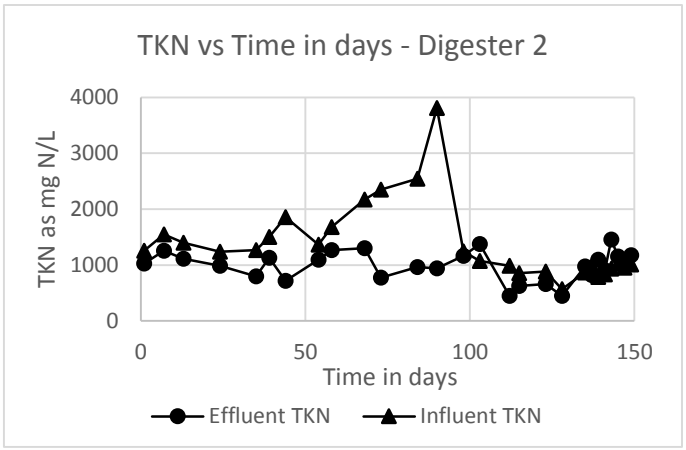
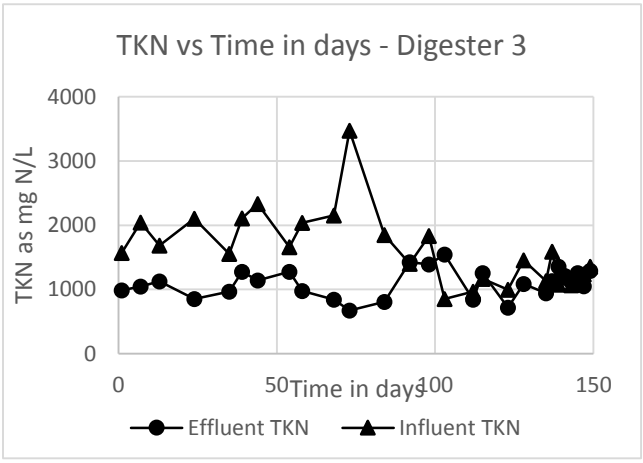


Fig. A.2. Total Ammonia Nitrogen







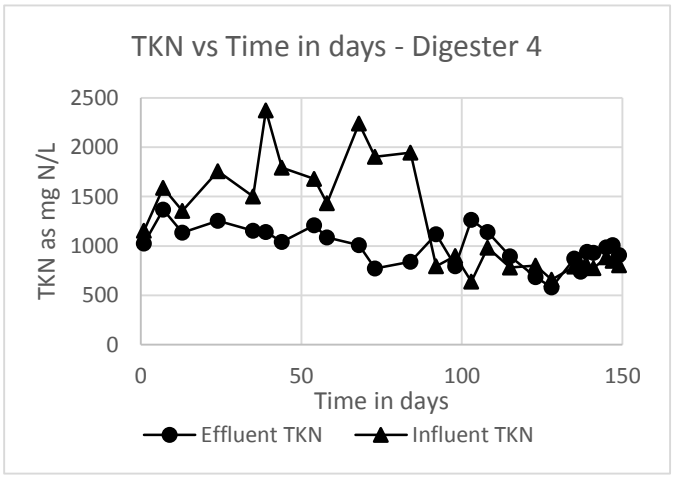
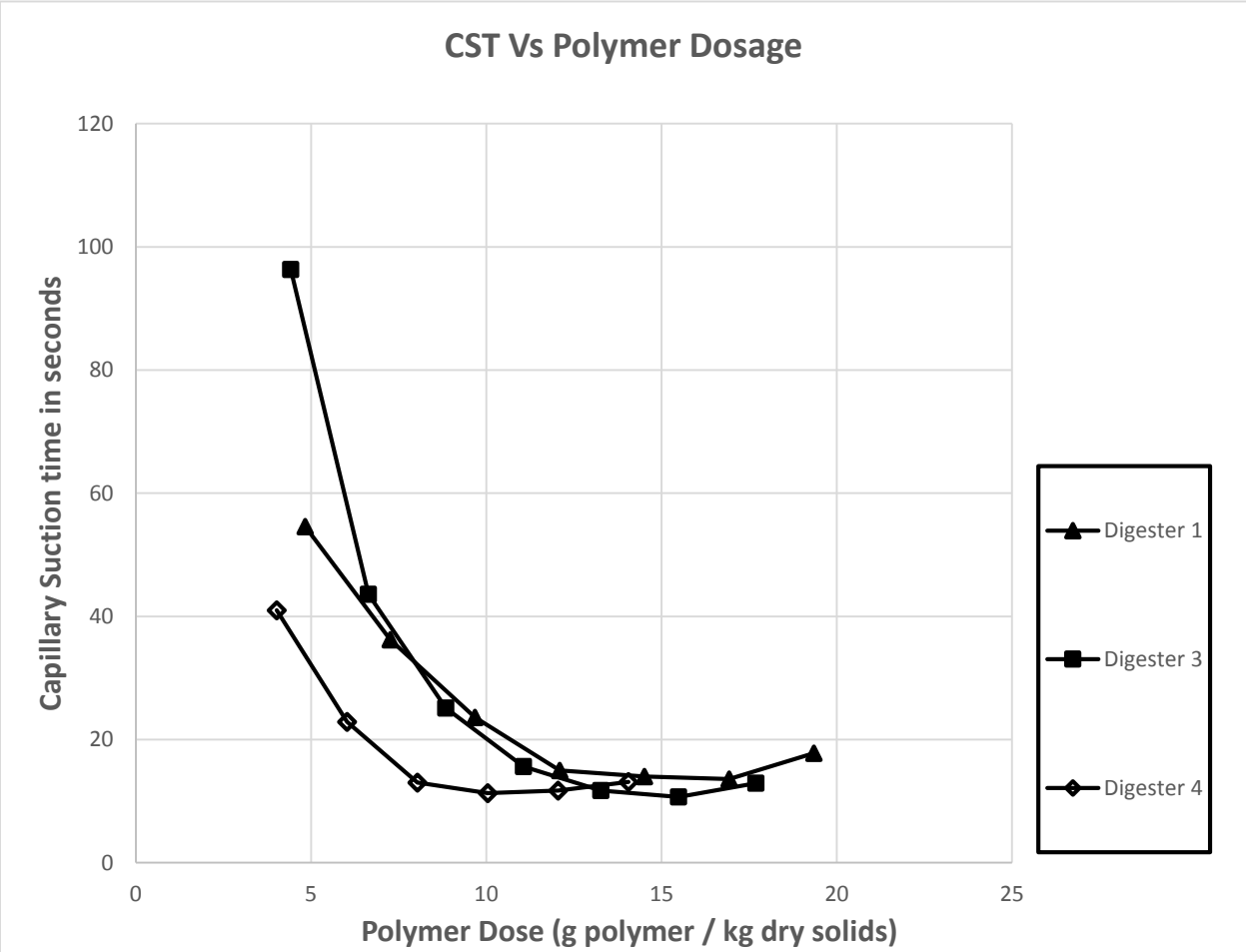


Fig. A.3 Total Kjeldahl Nitrogen

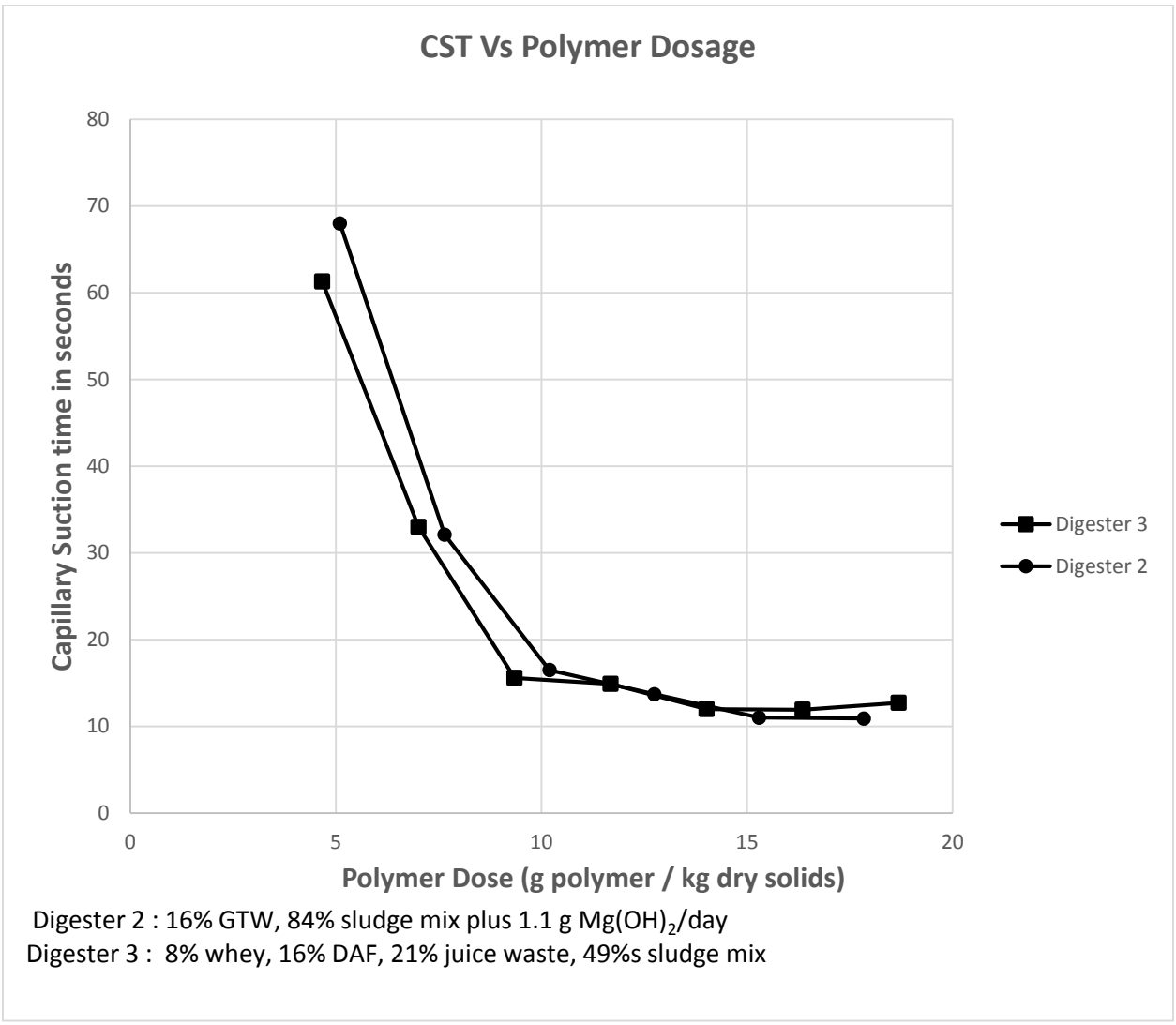
Appendix B

Capillary Suction Time and Biosolids Odors



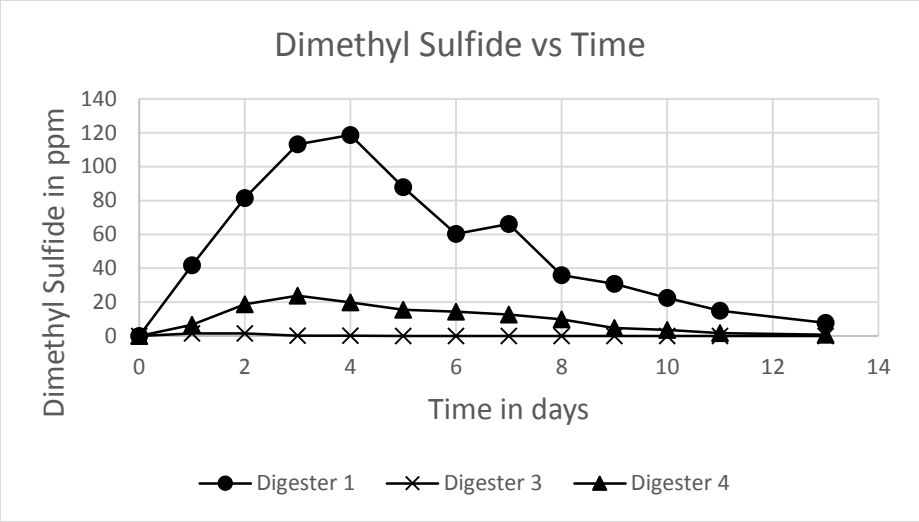
Digester 1 : 100% sludge mix Digester 3 : 6% whey, 12% DAF, 10% juice waste plus 72% sludge mix
Digester 4 : 8% whey, 22% GTW, 10% juice waste plus 60% sludge mix

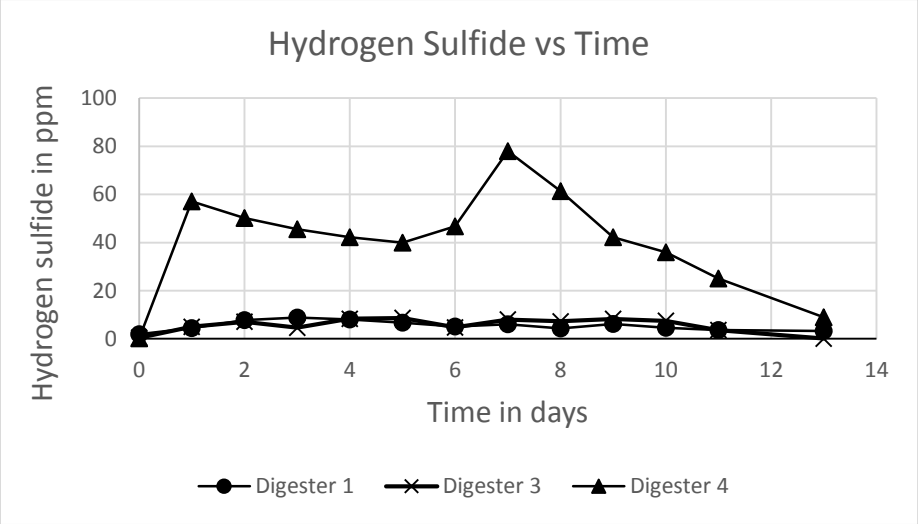
Fig. B.1 CST vs Polymer dosage for phase II



Digester 2 : 16% GTW, 84% sludge mix plus 1.1 g $Mg(OH)_2$/day
Digester 3 : 8% whey, 16% DAF, 21% juice waste, 49%s sludge mix

Fig. B.2 CST vs Polymer dosage for phase III





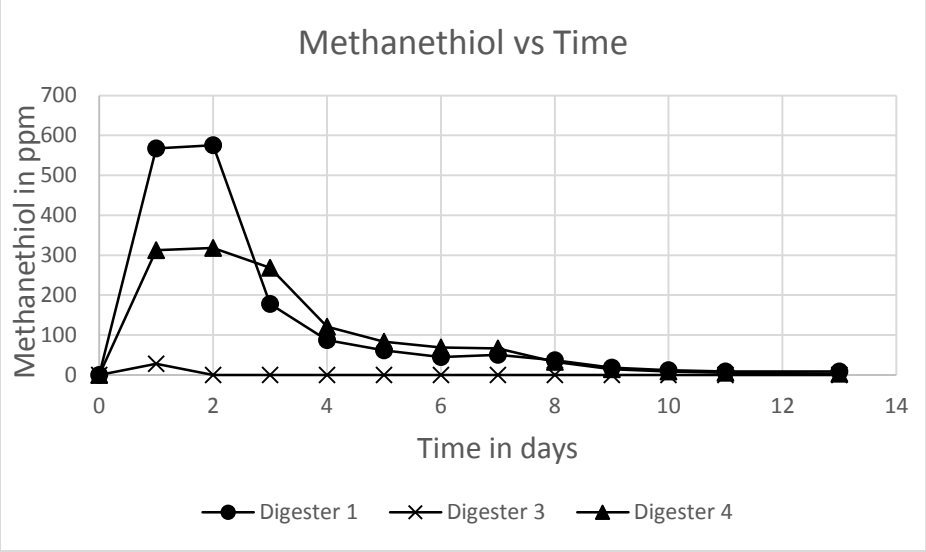


Fig. B.3 VSCs for phase II

Appendix C

Total COD Mass Balance

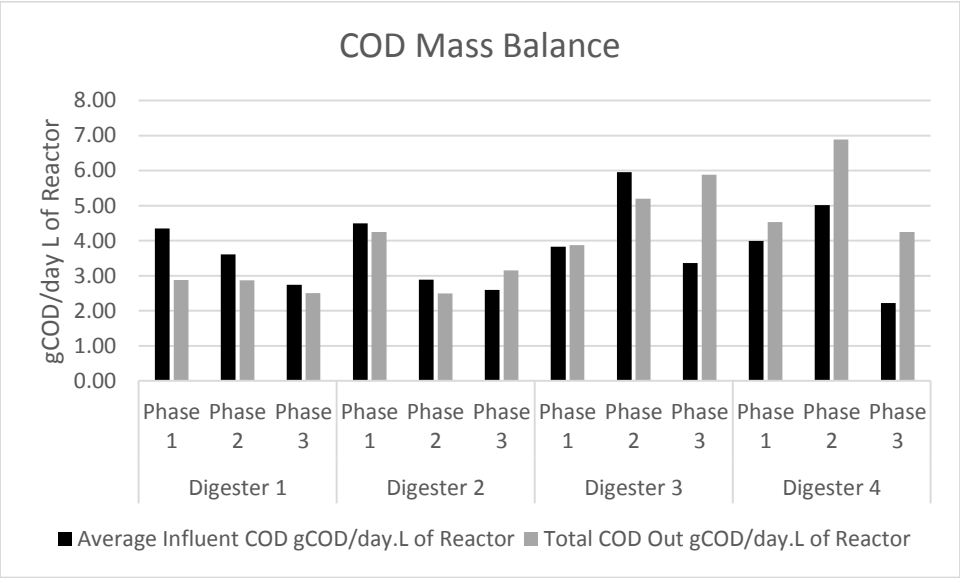


Fig. C.1 COD mass balance in the reactor

The mass balance of COD was carried out by measuring tCOD in the influent and the effluent. The COD in the biogas produced was added to the effluent tCOD. The plot shows that the influent COD and total COD out of the reactor are similar except for phase I in digester 1, phase III in digester 3 and phases II and III in digester 4. There might a leak in the biogas collected in digester 1 during phase I, this could be the reason for the lower COD out (3 gCOD/L day of reactor) as compared to the influent COD which was 4.5 gCOD/L day of reactor. On the other hand, phase III in digester 3 and phases II and III in digester 4 had greater COD out of the reactor (almost tice of the influent) as compared to the influent. The reason for this could be that the COD method failed to detect the actual influent tCOD, leading to lower COD values than those present in the influent.