Modeling Volatile Organic Sulfur Compounds In Anaerobic Digestion

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Anaerobic digestion is a common process for treatment of wastewater sludge from municipal sewage systems. However, odor release is a notorious problem associated with anaerobic digestion. Volatile sulfur compounds (VSCs), including volatile organic sulfur compounds (VOSCs) and hydrogen sulfide, have been reported as the most odorous compounds in digestion emissions, because of their very low odour thresholds and very negative hedonic values. VSCs are also impurities generated in anaerobic digestion which can damage facilities for generation, transportation, storage, and utilization of biogas. VSC generation and conversion have been studied in the fields of food industry, microbiology, natural system, biosolids, etc. However, there has been no comprehensive study on biological generation and degradation kinetics of VOSC or modeling VOSC behaviors through anaerobic sludge digestion. The goal of the present study was to establish a model for VOSC conversions in anaerobic sludge digestion which could facilitate quantitative analysis of VOSC emissions in anaerobic digestion.

VOSCs including MM, DMS, and DMDS and methionine were employed in dosed batch tests. The identified VOSC conversion processes included degradation of MM and DMS, methylation of MM to DMS, DMDS degradation with MM as an intermediate product, and MM generation as the major product of methionine degradation.

The kinetics for the identified VOSC degradation and conversion processes were determined at 35 and 55 °C respectively. Mixed-second order kinetics were found to best fit the conversion processes. Conversion of DMDS to MM was dramatically more rapid at 55 °C as compared to that at 35 °C. However, MM and DMS which were the most frequently detected VOSCs in anaerobic sludge digestion had slower degradation rates at 55 °C. Methylation of MM was also slower at 55 °C. The results suggested that VOSC release might be more problematic at thermophilic temperatures.

The generation of ammonia was monitored simultaneously with MM release from methionine decay. Both accumulation of ammonia-nitrogen and MM-sulfur could be well described by the mixed-second order kinetics. The release of ammonia-nitrogen could be used to predict the trend of MM-sulfur release. Their releases were both faster at 55 °C as compared to that at 35 °C. A model was established based on the identified processes and estimated kinetic constants. The model could well describe the VOSC generation and degradation through methionine incubations at 35 and 55 °C.

To extend the model to VOSC release in anaerobic sludge digestion, mesophilic and thermophilic incubations were conducted with four different sludge samples (activated sludge samples NAS and BAS, primary sludge sample BPS, and mixed sludge sample WMS). The fraction of sulfur degraded in the digestion and released as VOSC was only a small fraction of the total sulfur in the raw sludge (average 3.7%).

The effects of temperature and sludge source on VOSC release patterns were assessed. In incubations that were conducted at 55 °C, all the sludge samples resulted in MM as the dominant VOSC while DMS was released at low concentrations, if it was generated. At 35 °C, in the incubation of the primary sludge sample BPS and the activated sludge sample NAS which was obtained from a wastewater treatment facility without iron addition, VOSC release patterns were consistent with those at 55 °C. In the mesophilic incubation of two sludge samples (BAS and WMS) which contained activated sludge with iron addition (more than 30 mg Fe/g TSS), DMS was released as the dominant VOSC while MM was generated at low concentrations. The results suggested that an unidentified DMS generation mechanism was triggered in the mesophilic incubation of activated sludge in which iron was dosed.

To apply the model which was established based on methionine degradation in sludge digestion, hydrolysis of particulate materials was incorporated. First order kinetics were employed to describe the particulate material hydrolysis. Kinetic constants were estimated for solubilization of particulate COD, mineralization of organic nitrogen, and solubilization of organic sulfur (using methionine as a surrogate of degradable sulfur). The proteinaceous materials (represented by the organic nitrogen) in the activated sludge appeared to be more accessible to degradation as compared to that in the primary and mixed sludge samples. It was found that the same rate constant could be used for hydrolysis of lumped particulate organic matter and hydrolysis of lumped organic nitrogen. In all the sludge incubations at 55 °C, kinetic constants estimated from ammonia-nitrogen release were not significantly different from the kinetic constants estimated for release of methionine-sulfur from particulate materials. At 35 °C, the same conclusion was arrived at for the hydrolysis of the activated sludge sample (NAS).

The model which included hydrolysis of particulate organic sulfur, MM generation from free methionine, and subsequent VOSC conversion was evaluated with results from sludge batch digestions. The model simulations for VOSC behavior in thermophilic batch incubation were able to represent the observed VOSC releases. However, the simulations could not well fit the observed

VOSC release at 35 ° because the model did not include the unidentified DMS generation mechanism.

Monitoring of VSCs in bench-scale digesters was conducted. MM and DMS were the most frequently detected VOSC and their appearance lasted for 2 – 7.5 hours after feeding raw sludge. After that time period, H_2S was the only VSC detected in the digesters. The VOSC conversion model was employed without and with the hydrolysis process respectively to simulate VOSC releases in digesters after feeding methionine and sludge. In the headspace monitoring after methionine addition, the model without the the hydrolysis process was able to represent MM and DMS variations in the headspace of both digesters for 12 hours after methionine was dosed. However, the concentrations of MM and DMS measured 24 hours after methionine addition were much lower than the simulated values. The results suggest that depletion of VOSCs occurred more rapidly in the digesters and that VOSC degradation in the batch tests may have been reduced by a lack of acclimation. The model was employed to simulate VOSC release patterns after raw sludge feeding by incorporating assumptions of constant biogas flow and equilibrium between the sludge and the generated biogas. The measured DMS concentrations were higher than the simulated DMS concentrations and the measured MM concentrations peaked earlier and at higher concentrations than the simulated MM concentrations. The model could not well describe VOSC release after feeding raw sludge. The lack-of-fit of the model may have been due to imprecise estimation of the degradable sulfur in the feed sludge. In addition, in the batch tests and digester operation the ratios of the raw and digested sludge were different. This might have resulted in different concentrations of the microorganisms which mediated biotransformations and hence resulted in different kinetic constants.

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Chapter 1

Introduction

1.1 Background

The element, sulfur, plays an important role in human lives. It is present in two of the 22 ribosomally incorporated protein amino acids, L-cystein and L-methionine, numerous other non-protein amino acids, and secondary metabolites. In addition to its presence in the nutrient sources, sulfur also commonly exists in pharmaceutical and personal care products, such as penicillin.

These inorganic and organic sulfur containing compounds will enter the domestic sewage system, be utilized or degraded by aerobic/anoxic bacteria, and adsorbed or incorporated into wastewater solids through wastewater treatment processes. The sulfur containing compounds that are present in the solids may undergo further degradation through sludge treatment facilities.

Anaerobic digestion is one of the sludge treatment processes which has been successfully used for stabilization and reduction of sludge from wastewater treatment processes. Historically domestic raw sludge from primary sedimentation was the only source of digestion. With the increasing demand for disposal of other types of sludge and the improvement of digestion technology, anaerobic digestion has been gradually applied to excess activated sludge, chemically treated primary sludge, biological sludge from advanced treatments, and industrial wastewater sludge (Parkin and Owen, 1986).

Odor release is a notorious characteristic of anaerobic digestion processes, which arouses public complaint and health concerns of residents. This is because the sulfur containing compounds in the sludge can be anaerobically degraded and generate hydrogen sulfide, low thiols (containing less than 3 carbons) and dialkyl sulfides. These substances have very low odor thresholds and cause discomfort at low concentrations (Gostelow et al., 2001). These low molecular weight sulfur compounds are extremely volatile, and are often referred to as volatile sulfur compounds (VSCs). Based on the presence/absence of carbon in their molecules, VSCs are divided into volatile organic sulfur compounds (VOSCs) and hydrogen sulfide (H_2S). They have been reported as the most odorous compounds in digestion emissions.

Besides causing odor problems, VSCs generated in anaerobic digestion are deleterious to biogas storage and utilization equipment. VSCs are reactive and corrosive and hence can easily damage pipelines, storage tanks, and biogas utilization equipment such as engines and turbines. They can also poison the catalysts employed in biogas scrubbers and fuel cells. When the total concentration of

VSCs in the biogas is higher than 200 ppm (parts per million, volume/volume), the anode of fuel cells will be deactivated (Wheeldon, 2007).

Due to their pernicious characteristics, the existence of VSCs in anaerobic digestion emissions has aroused attention. If the generation and degradation of VSCs in anaerobic digestion can be better understood, predicted, and controlled, then it will be easier to optimize the strategies of odor control, biogas cleaning and utilization and reduce the decision-making and operation costs. However, there has been no comprehensive study that has addressed models which can predict the physico-chemical and biological processes associated with VSCs which may occur during anaerobic sludge digestion.

Some studies on specific VSC generation or degradation have been reported. Although these studies were based on different systems and have different emphases, they together build the fundamentals for a comprehensive conceptual understanding of VSC generation and degradation during anaerobic sludge digestion.

The study of VSCs related to odor problems is not a new topic in the field of oral diseases, food industry, and the paper industry. Trace amounts of VSCs in the mouth have been found to lead to oral problems. Hydrogen sulfide and methyl mercaptan, formed from the bacterial degradation of amino acids, are direct causes of oral malodor (Persson et al., 1990, Yoshimura et al., 2000). On the sites of vegetable storage and paper plants, VSCs have been found to be responsible for irritating odors. They have also been reported to change the flavor and aroma of food during the food production process (Derbali et al., 1998, Kim et al., 2005). In these studies, the generation mechanisms of VSCs and the prohibition thereof were of concern.

VSCs have also been studied for their importance in natural systems. The generation of VSCs (Drotar et al., 1987) in soil (Ruiz-Herrera and Starkey, 1969, Kadota and Ishida, 1972), marine (Visscher and Taylor, 1993b), salt marsh (Kiene and Capone, 1988, Zinder et al., 1977), freshwater sediments (Zinder and Brock, 1978b, Vairavamurthy and Mopper, 1987, Finster et al., 1990,), etc. has been reported to be responsible for aggravated global warming and acid precipitation phenomena. On the other hand, VSCs are indispensable intermediates for sulfur cycling in natural systems (Lovelock et al., 1972, Lomans et al., 2001a, Lomans et al., 2002a, Lomans et al., 2002b). Methyl mercaptan was found to be the primary sulfur-containing compound in emissions from fresh water systems while dimethyl sulfide was regarded as the vital connection between water systems with high salinity and the atmosphere (Lomans et al., 2002a, Lomans et al., 2002b). Different biological mechanisms have been found to be responsible for the variety of VSCs that are observed. Methylation has been found to dominate fresh water systems while algal cleavage of dimethylsulfoniopropionate dominated in marine or other salty water systems (Lomans et al., 2002). Studies on this topic are extensive and valuable references for studying VSCs generation mechanisms during anaerobic sludge digestion. However, caution has to be taken when borrowing theories and principles because the reaction environment in natural systems is quite different from that in sludge digesters.

Previous studies on the VSCs released from biogas plants and biosolids storage provide insight into their behavior in anaerobic sludge digestion systems because of the similar substrates and anaerobic environment. However, instead of identifying the VSC generation mechanisms and predicting their concentrations in the emissions, studies on biogas plants have been more concerned on how to effectively remove VSCs as contaminants in the gas when used as fuel (Chambers and Potter, 2002, Andersson et al., 2004, Suthanthararajan et al., 2004) because new fuel utilization technologies require high purity biogas (Pandey and Malhotra, 1999, Kara et al., 2005). Purifying the biogas can be a costly process and hence has attracted attention. Studies on biosolids storage have explored VSCs generation under anaerobic conditions but have been found to focus on the effect of dewatering techniques on VSC formation (Higgins et al., 2002a, Higgins et al., 2002b, Visan, 2003).

Not only the generation of VSCs but also their consumption is of interest. It has been observed that VOSCs do not always accumulate in natural and some engineered systems, even though they are continuously generated. One important reason for why concentrations can be maintained at an acceptable level is the effect of microbial degradation of VOSCs. By degradation or mineralization mechanisms, VOSCs are converted to H_2S which could be precipitated by metals or removed by gas scouring in digestion systems. This process has been proved to occur in both laboratory and field studies (Bont et al., 1981, Kiene et al., 1986, Maarel and Hansen, 1997, Lomans et al., 2001b, Bok et al., 2006). Digesters that are used for anaerobic sewage digestion are controllable systems. They contain complex bacterial and archaeal groups which can generate and degrade VSCs. VSC-related reactions may be either promoted or restrained in the digesters with a better understanding of the mechanisms.

With an improved understanding of the behavior of VSCs, modeling is an advantageous way to display all the involved biological and physico-chemical processes, simulate system operation, and predict final products. The anaerobic digestion model ADM1 is a widely accepted platform for illustrating and simulating processes occurring under anaerobic conditions. It is open for adding new compounds and processes to expand targets and satisfy new demands. ADM1 simulates the protein degradation process that is associated with VSC generation and the liquid-gas transfer process which could describe the emission of VSCs with enhancement. In addition, the concentration of methanogens, a group of microorganisms vital for VOSC degradation, is estimated in ADM1. Theoretically, ADM1 can be used as an important basis for conducting this study.

ADM1 provides an excellent frame work and modeling platform. However, ADM1 classifies components with respect to details of specific biological reactions. The development of such models requires reproducible experiments to obtain kinetic and stoichiometric data and the use of advanced technologies for feed characterization. In addition, the quantities of sulfur and carbon are of different order of magnitude.

At present, many obstacles are impeding precise and complete description of conversions of sulfur compounds during anaerobic digestion. Standard methods for VSC analysis have not been developed. The mechanisms of VSC generation and transformation have not been proved. Kinetic and stoichiometric data on sulfur conversion reactions are currently not available. In addition the characteristics of sludge samples with respect to sulfur content are only roughly estimated by indirect parameters.

1.2 Objectives

This research aimed to develop a model of the conversion and the fate of VSCs in anaerobic digestion of wastewater treatment sludge samples. To realize this goal, experimental testing and mathematical modeling were conducted for the following specific objectives:

1. Determine the gas-liquid and liquid-solid partitioning coefficients of selected VOSCs and estimate the dependence of the partitioning coefficients on the temperature and sludge concentration.
2. Identify VSC generation and degradation processes in anaerobic digestion by employing an amino acid and selected VOSCs as the substrate, determine the reaction kinetics, estimate the kinetic parameters, and evaluate the effects of temperature.
3. Establish a mathematical model which can describe and predict the behavior of VSCs in anaerobic digestion of simple amino acids.
4. Investigate the variety of VOSCs that are generated from different sludge sources in anaerobic digestion at different temperatures and determine their release rates.

5. Monitor VSC release in gas emissions from semi-continuous digesters while monitoring the sulfur related characteristics of the raw and digested sludge at different temperatures.

All the data used for construction and evaluation of the model was obtained from lab-scale experiments. Objectives 1 – 4 was addressed in batch serum bottles. Objective 5 was addressed in bench-scale reactors.

To support these specific objectives, the following additional objectives needed to be accomplished in advance:

1. Identify the dominant VSCs in anaerobic digestion.
2. Determine the best methanogen-inhibitor to be utilized in the study.
3. Develop the analytical methods for the important sulfur species in sludge and VSCs in the gas phase.

1.3 Structure of the thesis

This research focused on the behavior of VOSCs in anaerobic digestion. Previous studies on physical-chemical and biochemical processes as pertinent to the partitioning and conversion of VOSC under anaerobic conditions, mathematical modeling for the fate of sulfur containing compounds, and analytical techniques for sulfur species are reviewed in Chapter 2. The experimental design and monitored parameters in the present research are summarized in Chapter 3. All the results of the experiments on individual VOSCs, the simple substrate methionine, and sludge samples are discussed in Chapters 4 – 6. The contents of Chapters 4 – 6 and related objectives are listed in table 1-1. At the end, important conclusions of the present research are summarized and recommendations for the further research were given in Chapter 7.

Table 1-1 Chapters and their corresponding objectives

Chapter	Objective	contents
4	1	Determined gas-liquid partitioning coefficients of VOSCs; Assessed the effects of temperature and sludge matrix on VOSC partitioning.
5	2 & 3	Identified VOSC conversion processes; Estimated kinetics and assessed the effect of temperature; Established and verified the model for anaerobic digestion of methionine.
6	4 & 5	Extrapolated the model to sludge digestion by including hydrolysis; Assess the impact of temperature and sludge source on the VOSC release; Evaluated the model application in batch tests and bench-scale digester operation.

Chapter 2

Literature Review

In this chapter, studies on sulfur sources in anaerobic digestion, pertinent conversion processes, and existing sulfur related models were reviewed to build fundamentals for a comprehensive understanding of VSC generation and degradation in anaerobic sludge digestion. Analytical techniques for sulfur species in sludge and for VSC were reviewed and compared to select the feasible analytical method.

2.1 Occurrence of VSC in Anaerobic Digestion

2.1.1 Definition and Introduction

The expression VSCs commonly shows up in reports or articles associated with bad breath (Schmidt et al., 1978, Persson et al., 1990, De Boever, et al., 1994), food storage, food flavor and aroma (Dias and Weimer, 1998, Castillo-Lozano et al., 2008, Sreekumar et al., 2009). The term volatile refers to the fact that these compounds have relatively high vapor pressures and low solubilities. In the range of mesophilic and thermophilic temperatures, VSCs have typically been considered to be sulfur-containing compounds which have less than 3 carbons in a molecule. H_2S, methyl mercaptan (MM), dimethyl sulfide (DMS) are the most frequently reported compounds which contribute to odors.

VSCs are also of interest in aerographical studies because they exist in the atmosphere and play an important role in the global sulfur cycle. VSCs have important environmental functions, related to global warming, cloud formation, and acid precipitation. VSCs in the atmosphere are mostly generated from the ocean and DMS is the major compound (Andreae and Raemdonck, 1983, Osthoff et al., 2009, Todd et al., 2009). DMS has been considered as the most important global warming compound in the ocean (Kloster et al., 2007).

Sulfur containing compounds including VSCs are being referred to with increasing frequency in the field of wastewater treatment because of complaints about odor from people living close to wastewater/sludge treatment facilities (table 2-1). In addition, VSCs existing in the biogas have aroused concern due to the higher purity requirements of advanced biogas utilization technologies such as engines, turbines, and fuel cells.

Table 2-1 Odorous sulfur containing compounds associated with sewage systems (Gostelow et al. 2001)

No.	Compounds	Formula	Boiling point °C	Odor characteristics
1	Hydrogen sulfide	H_2S	-60.28	Rotten eggs
2	Dimethyl sulfide	$(CH_3)_2S$	37	Decayed vegetables, garlic
3	Diethyl sulfide	$(C_2H_5)_2S$	92.1	Nauseating, ether
4	Diphenyl sulfide	$(C_6H_5)_2S$	162-163	Unpleasant, burnt rubber
5	Diallyl sulfide	$(CH_2CHCH_2)_2S$		Garlic
6	Carbon disulfide	CS_2	46	Decayed vegetables
7	Dimethyl disulfide	$(CH_3)_2S_2$	109-110	Putrid
8	Methyl mercaptan	CH_3SH	5.95	Decayed cabbage, garlic
9	Ethyl mercaptan	C_2H_5SH	35	Decayed cabbage
10	Propyl mercaptan	C_3H_7SH	66.2-69.4	Unpleasant
11	Butyl mercaptan	C_4H_9SH	98	Unpleasant
12	tButyl mercaptan	$(CH_3)_3CSH$		Unpleasant
13	Allyl mercaptan	CH_2CHCH_2SH	67-68	Garlic
14	Crotyl mercaptan	$CH_3CHCHCH_2SH$		Skunk, rancid
15	Benzyl mercaptan	$C_6H_5CH_2SH$	194 – 195	Unpleasant
16	Thiocresol	$CH_3C_6H_4SH$	194-195	Skunk, rancid
17	Thiophenol	C_6H_5SH	169	Putrid, nauseating, decay
18	Sulfur dioxide	SO_2	-10	Sharp, pungent, irritating

For this study of VSCs in anaerobic digestion, literature about the problems caused by VSCs, sulfur sources in anaerobic digesters, pertinent microbiological/ physical/ chemical sulfur conversion processes in anaerobic digestion, and the effect of anaerobic digestion operating parameters on VSC conversion were reviewed. Due to the similar anaerobic conditions in oral cavities, cellars for food storage, and underneath water, VSC conversions in the studies of oral diseases, food storage and production, and freshwater/ocean sediments were also reviewed.

In all the mentioned studies, the term VSCs has usually meant H_2S, MM, and DMS. In anaerobic digestion, dimethyl disulfide (DMDS), carbon disulfide (CS_2) and other sulfur compounds are sometimes included. The difference between VSCs and VOSCs is that the former includes H_2S.

2.1.2 VSC in Anaerobic Digestion

The use of septic tanks in domestic sewage treatment began the history of anaerobic digestion (Hobson and Wheatley, 1993). Digestion reduces the volume of sludge, relieves pollution problems, decreases the possibilities of spread of disease by pathogens in wastes, leaves a residual valued as fertilizer and soil conditioner, and provides biogas as an alternative source of energy (Parkin and Owen, 1986, Mata-Alvarez and Llabrés, 2000, Chynoweth et al., 2001).

However, odors have been reported as the main public concern with respect to sewage treatment (Gostelow et al., 2001). Odor problems have received more attention in recent years because of residents complaints, increased public awareness of environment protection, increased expectations on water companies, and more strict environmental legislation (Balling and Reynolds, 1980a and 1980b, Toogood and Hobson, 1987, Pierson and Buckland, 1998).

Anaerobic digestion is believed to cause more serious odor problems than aerobic treatment processes (Lettinga, 1995). Volatile sulfur-containing compounds from anaerobic digestion have been identified as the most odorous compounds related to sewage treatment because of their very low odor thresholds and very negative hedonic values. Even a small amount of VSCs can contribute to odor pollution (Smet and Langenhove, 1998). The most frequently reported odorous sulfur compounds are H_2S, MM, DMS, and DMDS. Odorous properties of these compounds are displayed in table 2-2. The following sections will expand on their generation, degradation and transfer under anaerobic conditions.

Table 2-2: Properties of VSCs of interest

Compounds	Odor threshold[1] (ppbv)	Gas/liquid partitioning coefficient 25°C[2] (mol m^{-3}/mol m^{-3})
H_2S	8.5 – 1000	
MM	0.9 – 8.5	0.10
DMS	0.6 – 40	0.07
DMDS	0.1 – 3.6	0.04

1 (Zwart and Kuenen, 1992); 2 (Przyjazny et al., 1983).

The production of these odorous sulfur compounds is affected by the ambient environment. Therefore, operating conditions such as pH and temperature not only influence the effectiveness and degree of digestion, but also impact generation and emission of odorous compounds.

Digestion with pH values ranging from 5.0 to 7.3 has been evaluated for degradation of pulping effluents. pH was found to affect H_2S release from two aspects. For two phase digestion, the acidogenic phase with a pH value under 5.5 produced the least amount of H_2S because sulfate reduction was reduced with the acid pH value. The neutral condition was observed to be best for reducing H_2S release which might be caused by the sulfide precipitation (Clark and Bruce, 1995, Ghosh et al., 1995).

Thermophilic digestion has been found to cause increased degree and rates of organic matter destruction, improved dewatering characteristics, and increased pathogen reduction of municipal sludge (Gavala et al., 2006, Hartmann and Ahring, 2006a and 2006b). It has also been reported that thermophilic digestion produces less VSCs than mesophilic digestion but an improper start-up strategy might result in higher releases of VSCs. Rapid temperature increases have been found to lead to elevated methyl mercaptan concentrations while slow increases resulted in an increase in hydrogen sulfide emissions (Iranpour et al., 2005).

The presence of heavy metals in digesters is also important because of their potential to precipitate sulfide (Lawrence and McCarty, 1965). Other factors such as SRT and feed characteristics may also affect VSC generation, but detailed studies have not been reported.

2.1.3 Effect of VSC on Biogas Utilization

Biogas is a byproduct from anaerobic digesters which is typically composed of 58~70% methane, 30 ~ 40% carbon dioxide, nitrogen, oxygen, water, VSCs and other impurities. Both methane and carbon dioxide are green house gases and the direct release of biogas to the air may accelerate global warming. The relative abundance of methane in biogas and its clean burning properties make it an attractive fuel. Biogas is more and more frequently used for energy production for both environmental benefits and economic advantages. For example, Tsagarakis and Papadogiannis (2006) reported that a wastewater treatment plant in Iraklio, Greece, was able to produce up to 39% of the total electricity needs of the facility if the biogas utilization equipment was well maintained and operated. Holm-Nielsen et al. (2009) reviewed practices in Germany, Denmark, Austria, and Sweden and reported increased biogas utilization for reducing green house gas emissions. By the end of 2005, about 3000 biogas plants with a total installed electrical capacity of almost 600 MW were in operation in Germany alone (Weilan, 2010).

Besides the availability of biogas utilization technologies and operation efficiency, biogas quality is the most important parameter which influences its utilization. VSCs in biogas are reactive and corrosive to metal pipes, biogas storage tanks, and biogas utilization equipment such as biogas engines and turbine generators. When biogas is used for more efficient electricity generators such as fuel cells, it has to be cleaned-up and reformed. VSCs can poison the catalysts that are used in both reforming and fuel cells. It has been reported that when the total sulfur concentration is above 10 ppmv, the anode of solid oxide fuel cell systems will be deactivated. In addition, when the concentration of VSCs was higher than 200 ppmv, activated carbon treatment could not effectively accomplish the removal to reach a required purity (Wheeldon et al., 2007).

In summary, the presence of VSCs can have negative impacts on biogas transportation, storage, and utilization. The processes which are used for VSC removal are usually costly and may not be effective in removing VSCs when they are present at high concentrations.

2.1.4 Sulfur Sources in Anaerobic Digestion of Wastewater Sludge

2.1.4.1 Sources

The odorous sulfur compounds that are present in digester gases are generated from sulfur containing components in feed sludges through biochemical or chemical reactions. Organic sulfur sources are typically proteinaceous compounds which contain cysteine or methionine, non-protein amino acids such as glutathione, some lipids (sulfolipids) and polysaccharides such as chondroitin sulfate (Landers et al., 1983, Bura et al., 1998). The common inorganic sulfur sources are sulfate, sulfite, and thiosulfate (Sommers et al., 1977).

These sulfur containing compounds originate in foods, fecal matters, pharmaceuticals, and personal care products. They enter the sewage system in discharges from residential, industrial (food, paper, livestock) sources, and seawater and ground water infiltration. For domestic wastewater, approximately 3-6 mg/L of organic sulfur has been reported to exist in the form of proteinaceous materials while about 4 mg/L of organic sulfur has been found to be present as sulfonates, that are derived from household detergents (Boon, 1995, Cheremisinoff, 1998). The quantities of inorganic sulfur, mainly sulfate, vary with the hardness of water, and are typically present at concentrations of 30 ~ 60 mg /L. Infiltration of ground water and seawater or industrial sources may considerably raise concentrations of sulfate (Harkness, 1980).

The conversion of inorganic sulfur compounds (i.e. sulfate) under anaerobic conditions has been well studied (Postgate, 1959, Oremland and Taylor, 1978, Widdel, 1988, Alphenaar et al., 1993, Lens et al., 1995, Visser, 1995, Maillacheruvu and Parkin, 1996, Kalyuzhnyi et al., 1998, Kalyuzhnyi and Fedorovich, 1998, Vincent, 2001, Fedorovich et al., 2003) as compared to the conversion of organic sulfur compounds. Studies on the conversion of sulfur during protein degradation in the medical and food industries have been frequently published (Bonnarme et al., 2000, Kagkli et al., 2006, Landaud et al., 2008, Sreekumar, 2009) but rarely reported in the field of anaerobic sludge digestion. The degradation of complex non-protein sulfur compounds has been seldom studied with the exception of the degradation of dimethyl-sulfonio-propionate in marine systems (Welsha, 2000, Pawlik et al., 2002, Bentley and Chasteen, 2004).

2.1.4.2 Analysis of Sulfur Composition in the Sludge

Analytical methods for quantification of sulfur species in sludges were reviewed. The feasibility of selected methods was tested in the lab.

1. Inorganic Sulfur

Inorganic sulfur in liquid samples, $S_{I,\ liquid}$ included dissolved sulfate and sulfide. The measurement of sulfides in liquids can be conducted by the standard iodometric method 4500-S^{2-}E (APHA, 1992). Sulfate in the liquid can be measured either by ion chromatography method 4500-SO_4^{2-} B, gravimetric methods with ignition or by drying of precipitates, 4500-SO_4^{2-} C and D, or by the standard turbidometric method, 4500-SO_4^{2-} E (APHA, 1992).

For sulfides in liquids, the standard iodometric method is commonly employed for analysis of samples from water/wastewater treatment and environmental samples. In clean water, the precision of this method can reach up to 0.1 mg/L in a 200 ml sample. The pre-treatment for separation of soluble and insoluble sulfides and removal of interfering substances/concentration of sulfide are described in Standard Methods. With pre-treatment, the iodometric method can be employed for wastewater and surface water samples. However, its application to sludge is questionable.

Sludge often contains high concentrations of iron and results in trace amounts of sulfide dissolved in the liquid phase, which requires a very precise measurement. Preliminary testing of the iodometric method was conducted as part of the current study and the following practical problems were encountered:

1) A volume of 200 ml of sample is required by the method: Obtaining 200 ml of filtrate from sludge of high solid concentrations was a time-consuming step. In addition limited volume of sludge samples was available in the batch- and bench-scale tests and it was not practical to obtain 200 ml of filtered sample for titration.
2) Interfering compounds: The iodometric method requires a relatively clean background for a color based titration. Sludge is a complex mixture which contains a variety of organic compounds. Even after flocculation and filtration, the color of the sludge and the dissolved compounds were still problematic.

During the trial test, measurements that were based on the iodometric method showed high variation among replicates of a single sample and inconsistent results before and after sludge sample dilution. The high uncertainty of the results might be caused by the presence of reduced organic compounds and the opaque color of the sludge. It suggested that this method was not feasible for soluble sulfide measurements in the sludge.

For dissolved sulfate, precipitation and gravimetric methods are subject to various interferences, including suspended matter, silica, barium chloride, nitrate, sulfide, and alkali metals. The gravimetric method is suitable for sulfate concentrations above 10 mg/l. The precipitation and turbidimetric methods have interferences from color, suspended matter, and organic materials (Ristow et al., 2005) and are applicable to the range of 1 ~ 40 mg sulfate/l. The sludge samples measured in this study were complex mixtures and contained sulfate concentrations of 0.1 - 10 mg/l. In a trial test, the ion chromatographic method was found to be valid for sludge samples after proper pre-treatment. The ion chromatographic method was employed because it requires a small volume of sample and could measure sulfate concentrations above 0.1 mg/l.

Inorganic sulfur in the solids ($S_{I,\,solid}$) was assumed to include reduced inorganic sulfur (metal sulfide precipitates and a small amount of elemental sulfur) and adsorbed sulfate. A small amount of soluble sulfate may be adsorbed in the solids and can be extracted by $Ca(H_2PO_4)_2$ solution and then analyzed by standard methods (Picot et al., 2001). Reduced inorganic sulfur can be measured by a diffusion method developed by Hsieh and Yang (1989). This method employs a procedure which converts reduced inorganic sulfur species to hydrogen sulfide in a closed container at ambient temperature. The generated hydrogen sulfide is trapped in an alkaline zinc solution by diffusion and then analyzed by iodometric titration (Hsieh and Yang, 1989). This method assumes reduced inorganic sulfur consists of monosulfide (S^{2-}), pyrite (S_2^{2-}), and elemental sulfur (S_0) and can determine each species sequentially. The diffusion method was reported to be advantageous to the traditional active distillation method in the following aspects: ease of analyzing large numbers of samples, no recovery of elemental sulfur during the recovery of pyrite, no extra extraction step for elemental sulfur.

In the current study it was attempted to set up the diffusion method developed by Hsieh and Yang (1989) to measure solid phase inorganic sulfur. However, sludge contained a greater variety of reduced organic sulfur compounds as compared to sediments and estimation of the precipitated sulfide was interfered with by these organic sulfur compounds. Another challenge was the production of the amalgamated zinc reducing agent, which is utilized as a catalyst in the diffusion method. This technique was not employed in this study due to the two challenges.

2. Organic Sulfur

The organic sulfur in sludge samples was assumed to consist of sulfur in the proteinaceous materials and sulfur in synthetic chemicals such as personal care products. It was anticipated that some of the organic sulfur compounds would be non-biodegradable while others would be biodegradable in anaerobic digestion. Proteinaceous sulfur is typically biodegradable and includes the sulfur in the extracellular protein and the protein in the cells. Methods for the measurement of the total organic and proteinaceous sulfur are reviewed.

Instrumental methods for directly determining total organic sulfur were evaluated. Marco et al. (2001) proposed a total organic sulfur measurement by elemental analysis with flame photometry (OEA-FPD). This method is based on the combustion of the sample with oxygen at a high temperature, further reduction of the released gas, and then chromatographic separation and photometric detection. This method is suitable for organic sulfur existing in organic matrices. This method requires complicated equipment setting and has problems such as incomplete combustion and release of inorganic sulfur which are counted as organic species when samples are in inorganic matrices.

Various physical, thermal, and chemical extraction techniques and the subsequent analytical methods have been reported for measurement of protein in sludge samples (Hattingh et al., 1967). The Biuret (Raunkjer et al., 1994), Lowry (Lowry et al., 1951), Modified Lowry (Hartree, 1972), Bradford (Bradford, 1976), and Smith (Smith et al., 1985) methods have been successfully applied to the measurement of protein in sludge samples. However, the amount of sulfur in the protein can not be estimated by these techniques.

To quantify the sulfur in the proteinaceous compounds, not only the total amount of proteinaceous matter but also the portion of sulfur in them needs to be measured. In the food industry, immunology, and microbiology, amino acid analysis is utilized for determination of the amino acid composition of protein and peptides, including the types and amounts of amino acids. Amino acid analysis is realized through four basic steps (Pierce, 2006):

1) Hydrolysis of a protein to individual constituent amino acids: Acid hydrolysis is the most common method.

2) Derivatization of amino acids with colorimetric or fluorescent reagents: Ninhydrin is a routinely used agent whereas o-phthalaldehyde is used when greater analytical sensitivity is required.

3) Separation of amino acids by either ion exchange chromatography or high pressure liquid chromatography.

4) Quantification of target amino acids by relative comparison with standards.

By identifying the amino acid composition, the portion of sulfur in the protein can be calculated.

Measurement of the proteinaceous sulfur was also found to be challenging with existing techniques. At present, no reliable method is available to determine the concentrations of specific proteins or amino acids in sludge as extraction and purification methods have not been developed to separate pure proteins from sludge and impurities will deteriorate the amino acid analysis.

Quantifying sulfur species was found to be challenging after testing some of the reviewed methods, such as the iodometric method and the Hsieh and Yang's diffusion method. For inorganic soluble sulfur, ion chromatography was feasible and employed in the study. For sulfur species in the sludge, an acid digestion method was developed (Appendix A). Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was utilized for measuring the acid-digested samples.

2.2 Anaerobic Generation of VSC

2.2.1 Introduction

In this section, the important mechanisms of VSC generation under a variety of anaerobic conditions are reviewed. Care must be taken when considering sludge digestion. It is possible that some mechanisms reviewed here are not significant in sludge digesters.

The degradation of inorganic and organic sulfur compounds is mediated by different microorganisms and produces different VSCs. The transformation of inorganic sulfur compounds has been well studied and its mechanism will only be summarized here. Organic sulfur compound degradation is more complicated and will be discussed in detail.

The dominant inorganic sulfur compound in sewage systems is sulfate. Inorganic sulfur compounds are usually converted to H_2S. The production of hydrogen sulfide by reduction of sulfates is a prevailing phenomenon during anaerobic digestion. Heterotrophic bacteria which carry out this process are specified as sulfur reducing bacteria (SRB) (Postgate, 1959 and 1984). Usually, SRB and methanogens are simultaneously studied because sulfate reduction and methanogenesis are not mutually exclusive and the two kinds of microorganisms involved coexist in the environmental conditions and utilize similar substrates in meso- and thermophilic anaerobic digestion (Oremland and Taylor, 1978, Lens et al., 1995, Speece, 1996, Weijma et al., 2000).

SRB using various nutrients are active in sewage processes. Usually propionic (CH_3CH_2COOH) and acetic (CH_3COOH) acids and hydrogen (H_2), which are commonly present in anaerobic digestion systems, are regarded as substrates utilized by SRB. Fedorovich et al. (2003) also included butyric acid ($CH_3CH_2CH_2COOH$) by assuming that all the volatile fatty acids (VFA) included in the ADM1 model could be substrates of SRB. The general reactions of different substrate utilizations are expressed by the following equations (Kalyuzhnyi and Fedorovich, 1998):

$\mathbf{C_2H_5COOH + 0.75\ H_2SO_4 \rightarrow CH_3COOH + 0.75\ H_2S + CO_2 + H_2O\ (2\text{-}1)}$

$\mathbf{CH_3COOH + H_2SO_4 \rightarrow H_2S + 2CO_2 + 2\ H_2O\ (2\text{-}2)}$

$\mathbf{4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O\ (2\text{-}3)}$

$\mathbf{C_3H_7COOH + 0.5\ H_2SO_4 \rightarrow 2CH_3COOH + 0.5\ H_2S\ (2\text{-}4)}$

The above four biochemical reactions are assumed to be carried out by different trophic bacterial groups. Biochemical reactions of sulfate reduction with H_2S production described by these equations are generally accepted and estimates of kinetic parameter values have been reported by Maillacheruvu and Parkin (1996), Kalyuzhnyi and Fedorovich (1998), Kalyuzhnyi et al. (1998), and Vincent (2001).

Municipal wastewater and sludge samples contain abundant organic materials but the information on VSC generation from organic sulfur containing compounds is relatively limited. In municipal sludge samples, proteinaceous materials are the major contributors to VSC generation. Therefore this study will focus on the degradation of organic sulfur-containing compounds in anaerobic digestion. Anaerobic degradation of the protein amino acids, cysteine and methionine, was reviewed. Aside from VSC generation from sulfur sources in the influent of digesters, conversion amongst VSC may occur and result in the formation of diverse VSCs and these will be introduced in sections 2.2.3 and 2.2.4.

2.2.2 Degradation of Amino Acids

The pathways of VSC generation have been reported to vary as a function of the nature of the anaerobic conditions that prevail. For instance, sulfide methylation has been reported to dominate in fresh water sediments and produces methyl mercaptan as the main product (Lomans et al., 2001a) while dimethyl-sulfonio-propionate cleavage has been found to be prevalent in marine systems and produces dimethyl sulfide (Lomans et al., 2002b). For anaerobic digestion systems with high amounts of biosolids, such as sludge digestion, biosolids storage, vegetable storage, landfills, etc., where proteins contribute 20-50% of the volatile solids, degradation of amino acids is considered as the most important pathway of VSC generation (Derbali et al., 1998, Higgins et al., 2002a, Anderson et al., 2004, Glindemann et al., 2006, Higgins et al., 2006).

The degradation of proteins to amino acids and, successively, to simpler compounds including VSCs is typically referred to as hydrolysis and is often the first step of anaerobic digestion. The process is mediated by enzymes including protease, peptidase, and lyase (Zinder et al., 1977, Higgins et al., 2002a). The two protein amino acids that contain sulfur are methionine ($C_5H_{11}NO_2S$, CH_3-S-$(CH_2)_2$-CH(NH_2)-COOH) and cysteine ($C_3H_7NO_2S$, HS-CH_2-CH(NH_2)-COOH) and their degradation is directly associated with generation of VSCs.

2.2.2.1 Degradation of Methionine

The degradation of methionine is believed to be catalyzed by inner and external enzymes. The pathways of methionine degradation are different between bacteria and yeasts. Four different pathways of degradation of methionine have been summarized from studies in microbiology, cheese ripening, sediments, and anaerobic digestion.

Pathway 1 is extrapolated from a pathway which was originally observed under aerobic conditions (Bonnarme et al., 2000, Bonnarme et al., 2001). However it has been generally accepted to be active in anaerobic digestion (Segal and Starkey, 1969, Kadota and Ishida, 1972). It includes two steps: oxidative deamination and demethiolation that are mediated by various bacteria, actinomycetes, and filamentous fungi (Miwatani et al., 1954, Kallio and Larson, 1955, Ruiz-Herrera and Starkey, 1969, Segal and Starkey, 1969). Certain bacteria that have been isolated from soil have been reported to employ methionine as the sole organic substrate. The two-step degradation process is displayed in Figure 2-1. The demethiolation step requires oxygen and a source of energy however certain facultative bacteria have been reported to employ this pathway in the absence of oxygen (Ohigashi et al., 1951, Miwatani et al., 1954). When oxygen is not present, the degradation pathway can be initiated by an aminotransferase. Transamination occurs, instead of deamination, with the presence of an amino acceptor (e.g. α-ketoglutarate), yielding L-glutamic acid and α-ketomethionine (Gao et al., 1998).

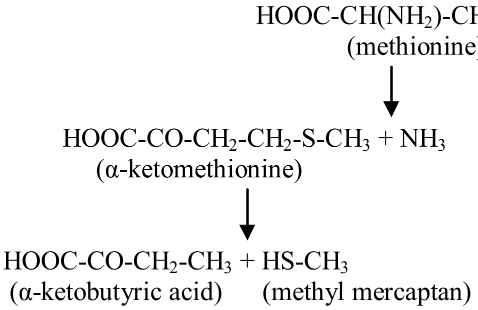

Figure 2-1 Two step degradation of methionine (Kadota and Ishida 1972)

Pathway 2 has been observed in studies of sediments under anaerobic conditions (Zinder and Brock, 1978a). In these studies the formation of VSCs has been reported to result from direct cleavage of methyl mercaptan from the methionine to produce homoserine ($HO_2CCH(NH_2)CH_2OH$) (Zinder et al., 1977, Zinder and Brock, 1978a and b):

Methionine → homoserine + CH_3SH (2-5)

After the cleavage, homoserine is degraded into volatile fatty acids and ammonia. This pathway can be easily applied to exhibit the formation of methyl mercaptan under anaerobic conditions and used in mathematic models.

Pathway 3 may occur coincidentally with Pathway 1 (Kadota and Ishida, 1972). It is not commonly recognized because the specific reaction conditions have not been clarified. In this pathway, a methyl group is cleaved from methionine instead of methyl mercaptan:

Methionine → homocysteine + CH_3 carrier (2-6)

Homocysteine → homoserine + Cysteine (2-7)

Cysteine → homoserine + H_2S (2-8)

By this mechanism, H_2S will be produced instead of methyl mercaptan during degradation of methionine. It is however not considered a dominant mechanism for degradation of methionine under anaerobic conditions (Kadota and Ishida, 1972, Zinder and Brock, 1978a).

Pathway 4 is a one-step degradation of L-methionine by the versatile enzyme L-methionine γ-lyase. This is a pyridoxal 5'-phosphate-dependent enzyme catalyzing the α, γ elimination of methionine to form α-ketobutyrate, methyl mercaptan, and ammonia (Gao et al., 1998, Bonnarme et al., 2000, Hullo et al., 2007).

$$\mathbf{L-methionine \xrightarrow{L-methionine\ \gamma-lyase} \alpha - ketobutyrate + HSCH_3 + NH_3} \text{ (2-9)}$$

It is currently believed that the Pathways 2 and 4 best describe the anaerobic degradation of methionine. Methyl mercaptan has generally been considered as the sole direct sulfur product of anaerobic degradation of methionine (Kadota and Ishida, 1972, Ferchichi et al., 1986, Higgins et al., 2006). However, Pathway 2 was developed on the basis of sediments and still needs proof by further studies under anaerobic sludge digestion conditions. In addition, dimethyl disulfide has been reported to be produced with methyl mercaptan from methionine by some special bacteria (Hayward et al., 1977, Sreekumar et al., 2009). Whether Pathways 2 and 4 are active in anaerobic digestion of sewage sludges and whether methyl mercaptan should be considered as the only volatile sulfur product needs verification.

2.2.2.2 Degradation of Cysteine

The pathways of degradation of cysteine are better understood as compared to the degradation of methionine. Normally, cysteine is considered as an organic precursor of hydrogen sulfide under anaerobic conditions (Miwatani et al., 1954, Kadota and Ishida, 1972, Persson et al., 1990, Derbali et al., 1998). The reaction is expressed by the following equation:

$$\mathbf{Cysteine \rightarrow homoserine + H_2S} \text{ (2-10)}$$

However, direct degradation of cysteine to generate H_2S is not the only pathway for cysteine metabolism. In pure microbiological and cheese-ripening bacterial studies, it has been reported that cysteine can be transformed to methionine with mediation by cysteine desulfhydrase, cystathionine γ-synthase, and aminotransferase. The generated methionine was then degraded to generate VOSC (Hullo et al., 2007, Sreekumar et al., 2009).

2.2.3 Methylation

Vairavamurthy and Mopper (1987) studied an iron-poor marine system and the results suggested that abiotic nucleophilic addition of sulfide to unsaturated bonds of organic molecules was the major chemical pathway for methyl mercaptan and dimethyl sulfide production. However, production of methyl mercaptan and dimethyl sufide by biological methylation has been observed in sulfur cycling in natural systems has been accepted as a more significant mechanism (Lovelock et al., 1972, Drotar et al., 1987, Lomans et al., 2001b). It is believed that methylation also happens in anaerobic sewage sludge digestion systems, since dimethyl sulfide has been detected in the emissions of sewage digesters (Iranpour et al., 2005, Higgins et al., 2006).

Methylation of sulfide was first assumed to result from the enzymatic activities of aerobic bacteria. It has been reported to occur widely in water, sediment, vegetation, soil, and marine algae cultures (Drotar et al., 1987). S-adenosylmethionine was the effective reactant in the methyl transfer and dimethyl sulfide was the major sulfur compound product. Some possible pathways that follow such a sequence include (Drotar et al., 1987, Hullo et al., 2007):

H_2S + S-adenosylmethionine → CH_3SH + S-adenosylhomocysteine (2-11)

CH_3SH + S-adenosylmethionine → CH_3SCH_3 + S-adenosylhomocysteine (2-12)

However, it was also reported that methyl mercaptan may have been produced from the breakdown of methionine which was triggered by the presence of sulfide and methylation may not have occurred (Drotar et al., 1987).

Studies carried out during the 1990's in Denmark have identified additional substrates that are preferred for methylation. Non-motile, rod-shaped bacteria isolated from fresh water sediments, marshes, and marine environments could utilize a small and almost identical range of substrates for methylation. The bacteria could not be isolated by usual enrichment procedures. A freshwater and a marine strain were isolated by direct dilution in liquid media supplemented with 3mM 3,4,5-trimethoxybenzoate (TMB) and syringate were designated TMBS4 and SA2, respectively. Methyoxylated aromatic acids and trihydroxybenzenes were identified as the preferred compounds for methylation of sulfide (Finster et al., 1990, Bak et al., 1992). Metabolic products included acetate and small amounts of butyrate (butyrate was only produced by SA2). Dimethyl sulfide has been observed as the dominant sulfur containing product of methylation with sufficient methyl providers. Syringate, identified as a typical methylating compound by Finster and Bak (1992), has been widely used in recent research on methylation of sulfur compounds in different media such as biosolids, freshwater sediments, and marine water (Lomans et al., 1997, Lomans et al., 2001a, Lomans et al., 2002b, Higgins et al., 2002b, Higgins et al., 2006). In these reactions hydrogen sulfide reduces the methyl group on an aromatic ring and forms methyl mercaptan and dimethyl sulfide (R = aromatic residue) through the following reactions (Bak et al., 1992):

$R\text{-}O\text{-}CH_3 + H_2S \rightarrow R\text{-}OH + CH_3SH$ (2-13)

$R\text{-}O\text{-}CH_3 + CH_3SH \rightarrow R\text{-}OH + (CH_3)_2S$ (2-14)

Sum: $2\ R\text{-}O\text{-}CH_3 + H_2S \rightarrow 2\ R\text{-}OH + (CH_3)_2S$ (2-15)

In freshwater and marine sediments, methylation occurs when lignin exists as source of methoxylated aromatic compounds and sulfide is available, with DMS or MM produced (Bak et al., 1992). However, in anaerobic sewage digesters, lignin is typically not significantly degraded and hence methoxylated aromatic compounds, which themselves are usually considered inert are not abundant for methylation. The occurrence of methylation in the anaerobic digestion processes needs to be verified while the pertinent sulfur containing reactants and products need to be identified.

Further study on TMBS 4 has supported the possible occurrence of methylation of sulfide in systems with little degradation of lignin through enhancement of methyl transfer in the presence of elevated concentrations of protein. The specific rate of activation was found to increase with protein concentration in a non-linear manner and reached a constant level 20 nmol/min/mg at protein concentrations ≥10 mg/ml (Kreft and Schink, 1993). The dependence of the reaction on protein concentration is believed to be complex and needs further study.

Most studies on methylation of sulfide have focused on methoxylated aromatic compounds but some studies have shown the possibility of methylation by alcohols. In studies of VOSC degradation, transient accumulation of DMS has been found during degradation of MM (Lomans et al., 1999, Stets et al., 2004, Leerdam et al., 2006). Methylation of MM to DMS was assumed to occur with methanol as the reactant instead of methoxylated aromatic compounds. This methylation is a reversible process (Leerdam et al., 2006). However, this methylation pathway needs to be verified and could not be simply extrapolated to sulfide methylated to MM at the presence of methanol. Sulfide was reported to stimulate the degradation of methanol by Zandvoort (2005) rather than be methylated.

The occurrence of methylation of H_2S and MM and methyl provider in anaerobic sludge digestion need further study. The reviewed studies indicate methylation by methoxylated aromatic compounds prevails in other anaerobic conditions such as sediments, and could be promoted by proteins in one study based on sludge digestion. Compared with methoxylated aromatic compounds, methanol and other alcohols and the methionine derivative – S-adenosylmethionine more commonly appear in digesters or as intermediates during the digestion process. The significance of this kind of methylation in sludge digestion is not clear. The possibility of methylation at thermophilic temperature needs verification because methylation was reported to occur between 15 ~ 37°C in all the cited studies.

2.2.4 Formation of DMDS

Dimethyl disulfide has been identified in saline marshes (Adams et al., 1981), soils (Guenther et al., 1989), coastal zones (Mello et al., 1987), municipal waste systems, and industrial wastes (Sivela and Sundman, 1975) however a biological formation mechanism was not verified in these studies. Tomita et al. (1987) isolated more than twenty strains of DMDS-forming bacteria in an activated sludge and it was proved that DMDS could be formed by biological oxidization under aerobic conditions.

The formation of DMDS from abiotic oxidation of MM has also been observed in the presence of transition metals such as Cu(II) and Fe(III). This oxidation occurs under both aerobic and anaerobic conditions to different degrees. Usually, less than 30% of MM will be oxidized under anaerobic conditions but addition of ascorbate highly promotes the transformation (Chin and Lindsay, 1994). Metal catalysis of DMDS formation is expressed in equations 16 and 17 (ex., Fe(III) as the oxidizing metal):

$$\mathbf{CH_3SH + Fe(III) \rightarrow CH_3S\cdot + Fe(II) + H^+ \quad (2\text{-}16)}$$

$$\mathbf{CH_3S\cdot + CH_3S\cdot \rightarrow CH_3SSCH_3 \quad (2\text{-}17)}$$

In studies on the formation of sulfur compounds under anaerobic conditions, the concentrations of DMDS were usually at very low levels and only increased when large amounts of MM accumulated with exposure to oxygen (Lomans et al., 2002a, Higgins et al., 2006). DMDS could also form as an artifact during the process of VSC sampling or analysis (Visan, 2003). The potential for formation of DMDS by metal catalysis in anaerobic sewage digestion requires further investigation.

2.2.5 Section Summary

On the basis of previous studies of the formation of sulfur compounds under anaerobic conditions, the following processes are considered as significant biological mechanisms for anaerobic sulfur conversions:

1) Formation of sulfide from inorganic sulfur compounds mediated by SRB;

2) Formation of MM and H_2S from the amino acids, methionine and cysteine;

3) Formation of MM and DMS from H_2S by methylation;

4) Formation of DMDS by metal oxidization of MM.

The conversion of inorganic sulfur compounds by SRB has been well studied whereas the other mechanisms are still under further exploration. Different environmental conditions of various anaerobic systems may result in different conversion mechanisms and products (Rosenfeld et al., 2001, Cheng et al., 2005). Processes that have been observed in other anaerobic systems may be inhibited or promoted in sludge digesters.

2.3 Anaerobic Degradation of VSC

2.3.1 Introduction

The production of VSCs is a continuous process but their concentrations in digestion emissions are often not high. One potential reason for this behavior is that only limited amounts of sulfur compounds enter the system. Another reason is that VSCs, including VSCs produced during digestion and VSCs existing in the feed sludge, are consumed by chemical oxidation, physical adsorption, precipitation and biodegradation in anaerobic digesters. There are different mechanisms for degradation of VOSCs due to different active microorganisms in different environments (Visscher and Taylor, 1993b, Lomans et al., 2002b).

In anaerobic digesters, the microorganisms involved in VSC degradation are believed to include methanogens and SRB. Biodegradation of low molecular weight (MW) VOSC is likely a significant removal process and generates inorganic sulfur compounds. In a study of VOSC biodegradation, only MM, DMS, and DMDS have been reported to be degraded by methanogens or SRBs. Higher thiols such as ethanethiol and propanethiol were not anaerobically degraded by them (Leerdam et al., 2006). A *Thiobacillus* ASN-1 was isolated that could oxidize a series of sulfur containing compounds including ethanethiol, propanethiol, diethyl sulfide, etc., using nitrate or oxygen as the electron acceptor (Visscher and Taylor, 1993a).

More complex VSCs (table 2-1) beside MM, DMS, and DMDS have been found in sewage systems and may enter digesters with the sludge. No reports of the mechanisms and pathways of their degradation have been found. Some of these complex VSCs were regarded as VSCs that are resistant to anaerobic digestion. In addition, these complex VSCs will not usually occupy significant percentages as the dominant sulfur compounds in emissions.

2.3.2 Degradation by Methanogens

2.3.2.1 Pathway

Methanogens have been reported to be involved in the degradation of MM, DMS, and DMDS with the formation of methane (Zinder and Brock, 1978a, Kiene et al., 1986) and the processes of degradation of organic VSCs are illustrated in Figure 2-2.

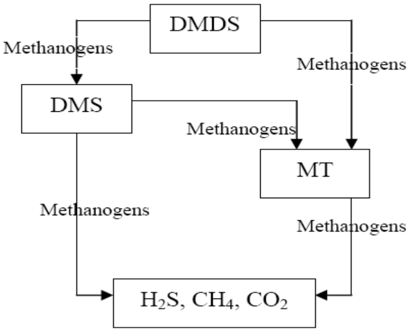

Figure 2-2: Degradation of organic VSCs by methanogens

Pure cultures of methanogens which can grow on MM (Finster et al., 1992, Chen et al., 2005, Bok et al., 2006) or DMS (Bont et al., 1981, Kiene et al., 1986, Finster et al., 1992) as the sole substrate have been isolated. The degradation of MM by methanogens can be expressed by the following equation:

$4\ CH_3SH + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ H_2S$ (2-18)

The degradation of DMS which involves MM production can be either written as a two-step process or combined in one overall reaction:

$(CH_3)_2S + 2\ H^+ + 2e^- \rightarrow CH_4 + CH_3SH$ (2-19)

$CH_3SH + H_2O \rightarrow 0.5\ CH_4 + 0.5\ CO_2 + H_2S + 2H^+ + 2e^-$ (2-20)

SUM: $2\ (CH_3)_2S + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ H_2S$ (2-21)

DMDS can be rapidly reduced to MM with small amounts of DMS and hence DMDS is not considered as the direct precursor of methane (Lomans et al., 2002b). Catalyzed by a DMDS reductase that uses nicotinamide adenine dinucleotide as the reductant, DMDS is cleaved into two molecules of methyl mercaptan (Smith and Kelly, 1988).

The degradation of DMDS has the same products as the degradation of DMS and MM; the general reaction can be written as described in equation 2- 21. Because DMDS may inhibit methanogens (Kiene et al., 1986), it is usually assumed that all DMDS is quickly converted to MM and DMS (Wu, 2004) or DMDS does not exist in the anaerobic conditions of digester system (Higgins et al., 2006). If so equation 2- 22 can be excluded from biochemical processes during anaerobic digestion.

$$\mathbf{2\ (CH_3)_2S_2 + 2\ H_2O \rightarrow 3CH_4 + CO_2 + 4H_2S\ (2\text{-}22)}$$

2.3.2.2 Inhibition of Methanogens

Methanogens have been identified as the most important microorganisms which degrade VOSCs under a variety of anaerobic conditions. To verify the role of methanogens in degradation of VOSCs and estimate VOSC generation kinetics, inhibiton of methanogens was employed. The literature about the inhibitors of methanogenesis and the mechanisms of their inhibition has been reviewed. Important inhibitors and their impact on the biochemical methanogenesis process will be introduced.

Methanogens are sensitive to their environment. Many factors such as high concentrations of heavy metals, low pH, high alkalinity, a high concentration of fatty acids, etc. can reduce or inhibit their activity (Speece, 1996). However, the previously-mentioned inhibiting factors were not appropriate for the purposes of this study because of the following reasons:

1) These factors do not only affect methanogens but also have an influence on other microorganisms.
2) Methanogens can acclimate to certain extreme conditions and after that, the inhibiting effect will be weakened.
3) Some of the inhibitors such as long chain fatty acids can gradually degrade.

An inhibitor which specifically affects methanogenesis and is resistant to degradation was a requisite. It was also important that the inhibitor would not generate VSCs under the digestion conditions.

The biochemical mechanisms of methanogenesis will be introduced to help understand the function of different inhibitors. Methane generation from substrates such as acetate and methyl mercaptan can be summarized by two reactions:

Catalyzed by corrinoid enzyme, a methyl group is transferred from the substrate to coenzyme M (H-S-CoM, 2-thioethanesulfonate) to form methyl-coenzyme M; methyl-coenzyme M reacts with coenzyme B (H-S-CoB, 7-thioheptancylthreonine-phosphate) to generate heterodisulfide CoM-S-S-CoB and methane (R.K. Thauer, 1998).

Coenzyme M and methyl-coenzyme M have only been found in methanogenic archaea. The same holds true for the enzymes which catalyze the methyl group transfer and the formation of heterodisulphide. Inhibitors that target these enzymes will specifically inhibit methanogensis rather than other biochemical processes.

Compounds which can inhibit the corrinoid enzyme or are analogs of coenzyme M/coenzyme B are able to inhibit methanogenesis. Studies related to the typical inhibitors reported in the literature including iodopropane (IP), 2-bromoethanesulphonate (BES), and 7-(methylthio) heptanoyl-*O*-phospho-l-threonine are further reviewed. BES is the inhibitor which has been widely used in anaerobic studies in microbiology and environmental engineering however it contains sulfur. IP and 7-(methylthio)heptanoyl-*O*-phospho-l-threonine were of interest because they do not contain sulfur and could be feasible for this study.

7-(methylthio)heptanoyl-*O*-phospho-l-threonine is an analog of coenzyme B and has been reported to be a potent inhibitor for methanogenesis. However, it is not a commercial product and has to be synthesized in the lab. In addition, it is only able to inhibit methanogenesis in cell extracts or when the enzyme is purified (Ellermann et al., 1988).

IP is a corrionid enzyme inhibitor which can prevent methylation of coenzyme M and cease methanogenesis. B-cyclodextrin was reported to be used as an inactive carrier of IP to overcome its volatile and liquid nature (Mohammed, 2003). In the preliminary tests conducted in the current study, IP was dissolved in organic solvents such as methanol and acetone and dosed as an inhibitor in digested sludge samples. However, unidentified volatile compounds were generated after the addition of IP into the sludge and their presence affected the measurement of VOSCs. Therefore, IP was not chosen as the inhibitor in this study.

BES has been widely used in anaerobic studies, especially when hydrogen rather than methane was the desired product of digestion. It has proven to be a robust methanogenesis inhibitor which can be effective in complex matrices such as municipal sludge. However, there were 2 concerns about using BES as the inhibitor in this study:

1) Based on the biochemistry, BES addition will result in the accumulation of methyl-coenzyme M. Methylation of H_2S to generate MM and DMS could be promoted by the abundant methyl-coenzyme M.

2) BES contains a sulfonic radical which might be gradually degraded and release VSC during anaerobic incubation.

In preliminary tests (Appendix A), addition of BES did not result in more VSC generation and therefore it was chosen as the methanogen inhibitor in this study when VOSC degradation was intended to be prevented.

2.3.3 Degradation by SRB

Although methanogens are believed to be the most dominant microorganisms responsible for the degradation of DMDS, MM, and DMS, SRB are also believed to play an important role in this biodegradation when sulfate is present (Roelofs, 1991, Lomans et al., 2001b). High sulfate concentrations tend to inhibit methanogens and meanwhile enhance the degradation of VOSCs by SRB (Lomans et al., 2002a). However, so far, only three SRBs have been isolated from a thermophilic digester, which have the ability of degrading MM and DMS (Tanimoto and Bak, 1994). The reactions and stoichiometry are described by equations 2-23 and 2-24:

$$\mathbf{4\ CH_3SH + 3\ SO_4^{2-} \rightarrow 4\ HCO_3^- + 7\ HS^- + 1.25\ H^+ \quad (2\text{-}23)}$$

$$\mathbf{2\ (CH_3)_2S + 3\ SO_4^{2-} \rightarrow 4\ HCO_3^- + 5\ HS^- + 3\ H^+ \quad (2\text{-}24)}$$

Compared to methanogens, it is believed that SRB are not the dominant microorganism in the degradation of VOSCs because they strictly require sulfate as an electron acceptor and they are not prevailing in all the anaerobic conditions at different temperatures.

2.3.4 Section Summary

On the basis of the literature reviewed in sections 2.2 and 2.3, the possible conversions of VSCs during anaerobic digestion are illustrated in Figure 2-3.

For anaerobic conditions, methanogens and SRB are considered as the microbial populations responsible for degradation of MM and DMS. SRB are typically less active than methanogens in anaerobic systems (Wu, 2004). However, considering that methanogens are more sensitive to environmental conditions, various inhibitors, and organic loads, SRB may be more active for degradation of VOSCs under some conditions in anaerobic digesters. In this study, methanogens are considered as the major microorganisms mediating VOSCs degradation and biogas analysis will be conducted to ensure the activity of methanogens. Conventional parameters such as pH, COD, and ammonia will also be measured to monitor the anaerobic digestion.

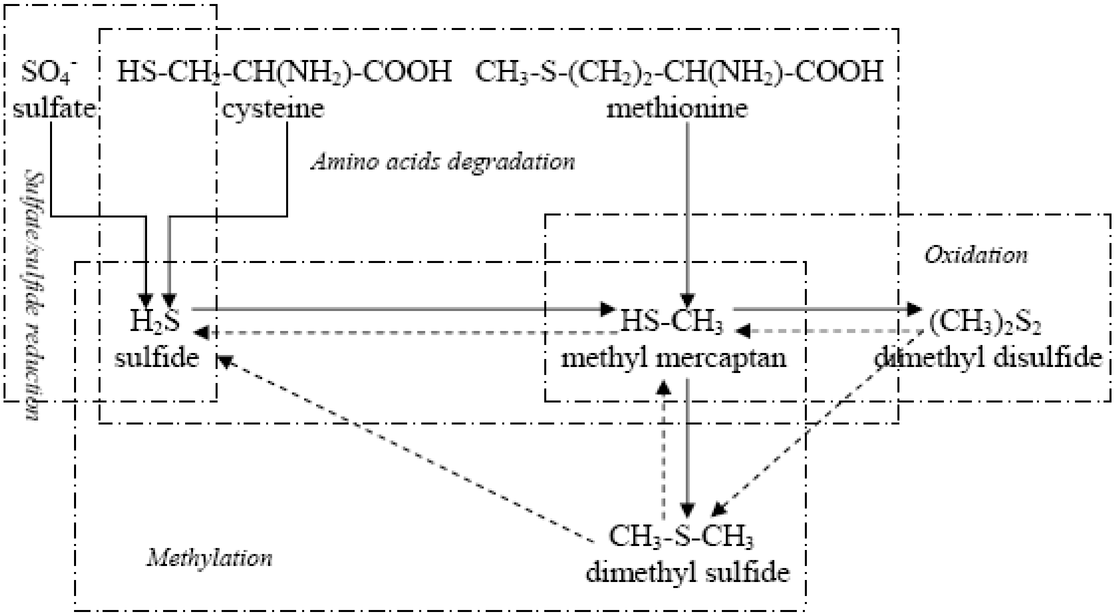

Figure 2-3 Potential VSC conversions during anaerobic digestion (Solid arrows are generation processes and dashed arrows are degradation processes)

2.4 Physico-Chemical Processes

Physico-chemical processes do not directly produce or consume VSCs but may influence biochemical processes and affect the emission of VSCs as odorous compounds. Following the classification utilized by the International Water Association (IWA) (Batstone et al., 2002), there are three types of physico-chemical processes that can be ranked according to their relative kinetic rates: liquid-liquid processes (rapid), liquid-gas processes (rapid/medium), and liquid-solid processes (medium/slow).

2.4.1 Liquid-Liquid Processes

Liquid-liquid processes typically refer to ion association-dissociations. Hydrogen sulfide has polarity and is a weak acid in aqueous solutions. The chemical forms of H_2S in the liquid will satisfy the dynamic equilibrium between the acid-base pairs (Equation 2-25 and 2-26). pKa_1 and pKa_2 are 7.04 and 11.96, respectively. Sulfide is emitted with gas and gives off odor in the form of H_2S. Therefore, pH that is affected by the balance of weak acids and bases in the anaerobic digesters will influence H_2S emissions.

$\mathbf{H_2S \xrightarrow{K_{a1}} H^+ + HS^-}$ **(2-25)**

$\mathbf{HS^- \xrightarrow{K_{a2}} H^+ + S^{2-}}$ **(2-26)**

MM is also a weak acid and could be dissociated. However, it has a very low K_a value (pKa = 10.5) and only significantly dissociate when pH is above 9.5 (Worsnop et al., 1995). Dissociation of MM is negligible under the anaerobic conditions which commonly have pH values between 6.5 ~ 8.5.

2.4.2 Liquid-Gas Processes

The emission of VSCs from the liquid phase to the gas phase is a liquid-gas mass transfer process. Most VSCs are only slightly soluble in the water and at equilibrium their behaviour will follow Henry's Law. The difference between the concentration of a VSC in the liquid and the equilibrium concentration calculated by the Henry' law is the driver for liquid-gas mass transfer.

Henry's Law describes the relation between the partial pressure of the gas and the concentration dissolved in the liquid. At a constant temperature, the partial pressure can be converted to the concentrations of the gaseous compound through the ideal gas law. Therefore, the gas-liquid distribution coefficient, defined as equation 2-27, can be used instead of the Henry's Law coefficient to describe the gas-liquid distribution (Przyjazny et al., 1983).

$$k_{liquid-gas} = C_l / C_g \tag{2-27}$$

Where C_l and C_g are concentrations in the liquid and gas phases after distribution and $k_{liquid\text{-}gas}$ is the distribution coefficient.

A headspace method was employed by Przyjazny et al. (1983) to determine the distribution coefficients of 12 VOSCs and their dependence on temperature. VOSCs were introduced into the headspace device from a standard VOSC solution and the VOSC concentrations in the gas phase were measured at equilibrium. A mass balance (Equation 2-28) was utilized to calculate the distribution coefficient (Przyjazny et al., 1983).

$$C_T V_{Solution} = C_g V_g + C_l V_l \tag{2-28}$$

Where C_T and $V_{Solution}$ are the concentration and volume of the standard solution for VSC addition, V_g and V_l are volumes of gas and liquid phases.

2.4.3 Liquid-Solid Processes

For VSCs in anaerobic digesters, there are two potential liquid-solid processes: precipitation and sorption. Precipitation typically refers to precipitation of sulfide by heavy metals while sorption refers to adsorption of VOSCs to solids in the sludge.

Heavy metals can chemically combine with dissociated sulfide ion or hydrogen sulfide, to form insoluble sulfides, and realize the transfer of S from the liquid to solid phase. This process is described by Equation 2-29. The formation of insoluble metal sulfides is the main sink of generated sulfide during anaerobic digestion; hence, it will reduce the occurrence or degree of methylation reactions. Kinetics based on different assumptions (Musvoto et al., 2000, Vlyssides et al., 2007) have been reported in modeling the metal sulfides precipitation. With a focus of VOSC, this study did not include the precipitation processes for sulfide.

$$\textbf{Heavy metal}^{2+} + \mathbf{H_2S} \rightarrow \textbf{Metal Sulfides}\downarrow + \mathbf{2H^+} \quad \textbf{(2-29)}$$

Sorption by biosolids can remove volatile compounds from the liquid phase and reduce gas phase emissions. The degree of sorption determines the distribution of volatile compounds between solid and liquid phases. Sorption equilibrium is often assumed to occur instantaneously. A partitioning coefficient, K_p, is typically used to express this distribution in linear models however the values of partition coefficients for VOSCs have been rarely reported. In this study, partitioning of VOSCs between solid and liquid phases will be assessed. K_p values will be estimated if the effect of sorption of VOSCs is significant.

2.5 Modeling of VSC Conversion

2.5.1 Introduction

Using models to simulate and predict performance is commonly employed in the field of engineering and is an effective method for research and analysis. The main goal of this study was to develop a quantitative model for predicting the behavior of volatile sulfur compounds during anaerobic digestion of sludges from municipal wastewater treatment processes. Data collected from experiments will be used for construction of a model and to verify the sulfur conversion processes.

Several mathematical models have been developed and successfully used for describing anaerobic digestion. ADM1 (anaerobic digestion model 1) is representative of these models (Batstone et al., 2002, Batstone et al., 2006, Kleerebezem and Loosdrecht, 2006a) and has been extended to describe specific biological processes beyond those traditionally considered as part of the anaerobic digestion process (Eladawy and Ogurek, 2006, Parker and Wu, 2006, Tugtas et al., 2006). An extension of ADM1 to include sulfate reduction has been reported (Fedorovich et al., 2003). Another extension of ADM1 for the emission of odorous compounds including VSC conversion processes has been proposed (Wu, 2004) but has not been verified by experimental data.

ADM1 and its extended models describe anaerobic digestion and some sulfur conversion processes in substantial detail; however, their complexity highly limits it application in engineering industry. The structure and functional biochemical processes of ADM1 and the two extended models are important references for modeling in this study but will not be imitated due to their complexity.

2.5.2 ADM1 and Its Extensions

ADM1 (Batstone et al., 2002) is a structured model that is a practical and extendable platform for simulation of sulfur conversions during anaerobic sewage digestion because the concentrations of substrates and related microorganisms mediating sulfur conversions can be calculated. An overview of the structure and kinetics of ADM1 has been briefly reviewed.

2.5.2.1 Structure of ADM1

ADM1 includes 19 biochemical processes based on substrate-uptake to describe the disintegration of composite, hydrolysis of particulate and high molecular substrates, digestion of soluble materials, and decay of biomass. Physico-chemical processes in ADM1 include acid-base dissociation/association and liquid-gas transfer of the main gas products, hydrogen, carbon dioxide, and methane; liquid-solid precipitation is not included. The structure of biochemical processes and typical COD flow are illustrated in Figure 2-4.

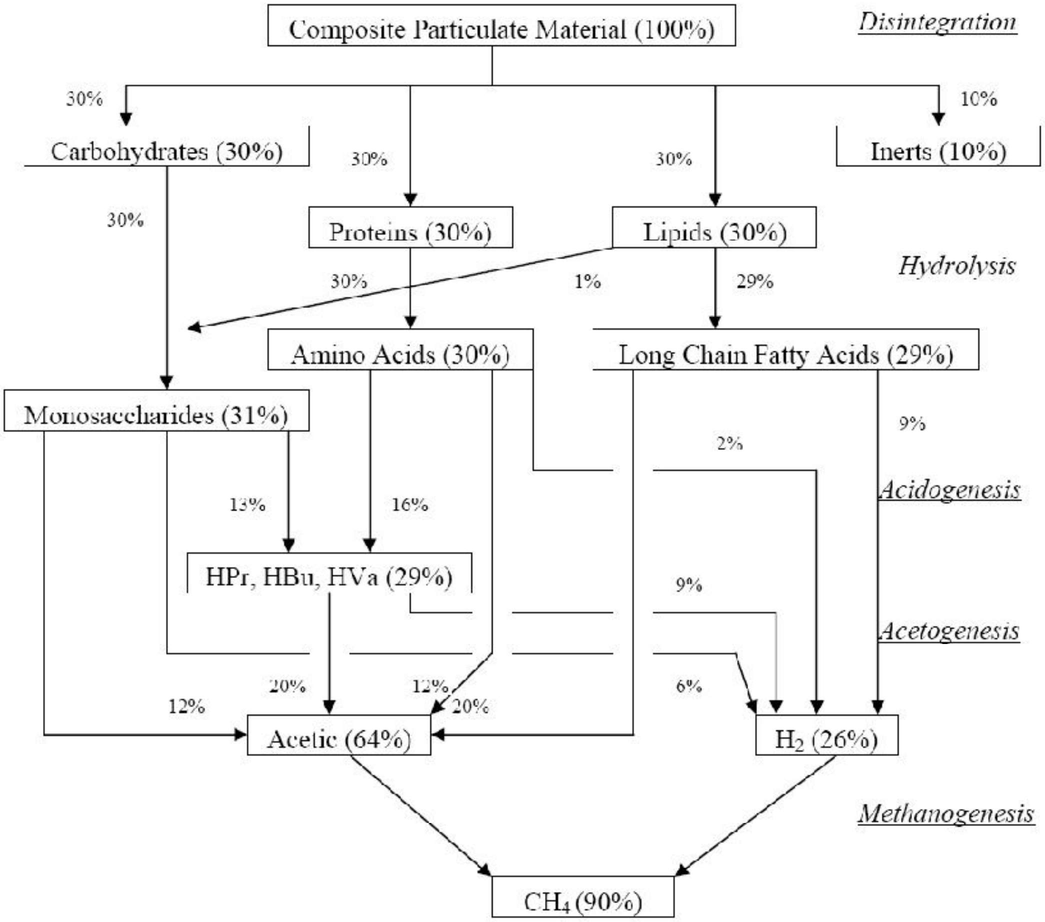

Figure 2-4 Structure of biochemical processes used in ADM1 (Batstone et al., 2002)

2.5.2.2 Kinetics of ADM1

In ADM1, the four stages of biochemical reactions that are mediated by different microorganisms include: hydrolysis and disintegration, acidogenesis, acetogenesis, and methanogenesis. Disintegration and hydrolysis are attributed to extracellular processes and described by first order kinetics (Equation 2-30 and 2-31) while others are growth related and are described by Monod kinetics (Equation 2-32). The decay of microorganisms is expressed by first order kinetics (Equation 2-33).

$$r_{dis} = k_{dis} X_C \tag{2-30}$$

Where,

r_{dis}= the rate of disintegration (kg COD m^{-3} d^{-1})

k_{dis} = first order disintegration coefficient of complex particulate (d^{-1})

X_C = concentration of complex particulate (kg COD m^{-3})

$$r_{hyd} = k_{hyd} X_i \qquad \textbf{(2-31)}$$

Where,

r_{hyd} = the rate of hydrolysis of particulate component i (kg COD m^{-3} d^{-1}), i represents carbohydrates, proteins, or lipids

k_{hyd}= first order hydrolysis coefficient (d^{-1}), same value used for carbohydrates, proteins, and lipids

X_i= concentration of particulate component i (kg COD m^{-3})

$$r_i = k_{m,i} \frac{S_i}{K_{S,i} + S_i} \qquad \textbf{(2-32)}$$

Where,

r_i = Monod uptake rate of component i (kg COD m^{-3} d^{-1})

$k_{m,i}$ = Monod maximum specific uptake rate (d^{-1})

S_i= concentration of substrate i (kg COD m^{-3})

$K_{S,i}$ = half saturation constant of substrate i (kg COD m^{-3})

$$r_{dec,i} = k_{dec} X_i \qquad \textbf{(2-33)}$$

Where,

$r_{dec,i}$= the decay rate of biomass i (kg COD m^{-3} d^{-1})

k_{dec}= first order decay coefficient, same value for all biomass, 0.02 d^{-1}

X_i= concentration of biomass i (kg COD m^{-3})

ADM1 introduces pH inhibition to all intercellular processes, hydrogen inhibition to acetogens, and free ammonia inhibition to aceticlastic methanogens.

2.5.2.3 Extension of ADM1 with Sulfate Reduction

The extension of ADM1 with sulfate reduction (ADM1-SR) (Fedorovich et al., 2003) incorporated four reactions (Equation 2-1 ~ 4) into ADM1 to describe sulfate reduction processes. In this model, SRB do not compete with fermentative bacteria in the degradation of sugars and amino acids but do compete with acetogenic bacteria and methanogenic archaea for volatile fatty acids, ethanol, acetate, and hydrogen (Fedorovich et al., 2003). Kinetic expressions were extended to include inhibition by sulfide in its undissociated form.

2.5.2.4 Extension of ADM1 with Odorous Compounds

The extension of ADM1 to address odorous compounds (ADM1-OC) expanded ADM1 with additional biochemical and physico-chemical processes (Wu, 2004). 14 biochemical processes including conversions of VOSCs and sulfate reduction were introduced. Physico-chemical processes were also extended to include H_2S dissociation to HS^- (liquid-liquid), emission of VSCs and volatile organic acids (liquid-gas), sulfide precipitation, and VOSC sorption on biosolids (liquid-solid). Modifications of kinetic expressions with sulfide inhibition were also applied.

The biochemical processes describing sulfur conversions in ADM1-OC are consistent with the literature review in this study. Interactions of these processes with the processes presented in ADM1 are displayed in Figure 2-5. However, ADM1-OC has not been verified with experimental data.

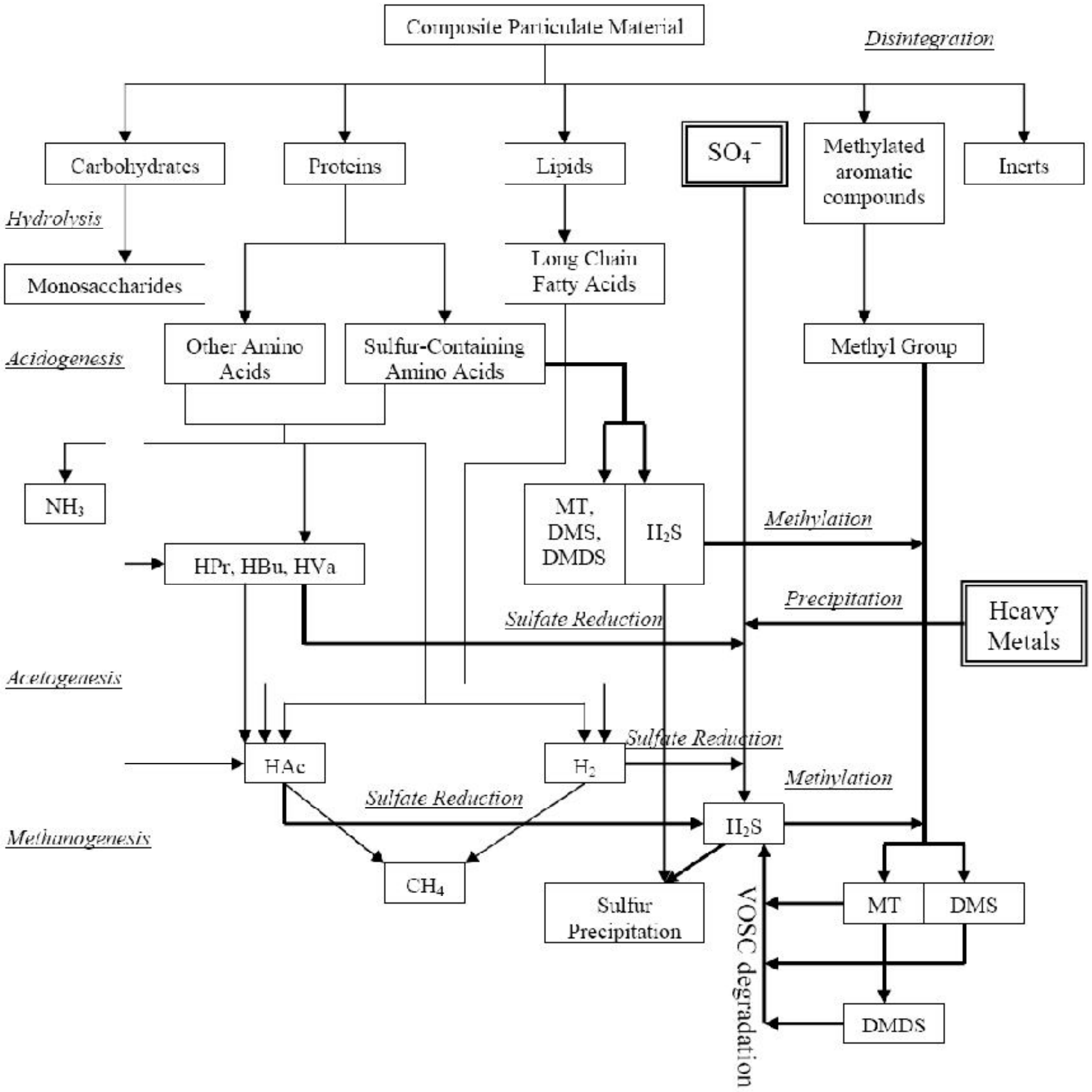

Figure 2-5 Digestion with conversion of VSCs (Wu, 2004)

2.6 Quantitative Analysis of VSCs

2.6.1 Characterizing Analysis of VSCs

Since the 1980's, a number of studies on VSC analysis have been published. The applications of VSC analysis have progressed from the food industries to environmental samples, and, subsequently, to sewerage treatment and biosolids. Gas chromatography has been widely employed as the analytical equipment for VSC measurements. However, correct quantitative measurement of VSCs is still a challenge and no standard method is available. The analysis of VSCs produced through anaerobic digestion has proved to be challenging.

Analytical challenges are associated with the properties of VSCs and the matrices in which they are present. VSCs are highly reactive compounds which can be oxidized through metal-catalyzed reactions and irreversibly adsorbed during the analysis process (Wardencki, 1998, Mestres et al., 2000). Deactivation of vessels, pipes, and columns used in the procedures is an effective method to prevent loss of VSCs. Matrix interference, especially for liquid-based samples, also causes difficulty for analysis of VSCs. In addition, the concentrations of VSCs in digester gases may vary over a broad range according to the operating conditions of the digesters and hence a linear relationship between concentrations and responses can not be ensured by detectors. Due to the importance of VSC measurement in this study, the methods and procedures of VSC sampling, storage, and analysis have been reviewed.

2.6.2 Sampling and Storage

2.6.2.1 Vessel Preparation

Considerable loss of VSCs from samples may occur due to irreversible adsorption on the surface of glassware in the analytical system, especially when concentrations of VSCs are at low levels such as 10^{-6} and 10^{-9} (v/v). Deactivating the contact surfaces of vessels by either physical or chemical procedures effectively prevents this problem (Farewell and Gluck, 1980, Lee and Wright, 1980). Physical procedures are the easiest deactivation methods and have been widely applied for a long time. All the glassware are thoroughly cleaned and conditioned by sequential soaking and rinsing steps. Chromerge and HCl solutions are the most common glass cleansers used in succession (Farewell and Gluck, 1980).

Chemical procedures involve modifying the surfaces of glassware to make them non-reactive. Deactivating agents are divided into four categories on the basis of the principle of surface modification (i.e. surface active agents, non-extractable films of polar compounds, siloxane polymers, and silanization agents). Silanization is commonly utilized to prevent glassware from reacting with sulfur compounds with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) most commonly employed for this purpose (Wardencki, 1998).

2.6.2.2 Sampling Techniques

For gas samples, if the concentrations of VSCs are above the detection limit of the chosen detector, direct injection into GC is the best procedure because unexpected reactions with sorbents or extraction solution are avoided (Lau, 1989). If the concentrations of VSCs are at a lower level, pre-concentration is required to achieve a precise and reliable measurement. Different pre-concentration techniques have been developed for different applications but most fall into the following categories:

1) Metal sorption: This technique is based on the ability of certain metals such as gold, palladium, and platinum to sorb sulfur gases. However, collection efficiencies are usually very low (less than 50%) (Kagel and Farewell, 1986).

2) Porous sorbent sorption: This method is one of the simplest but efficient methods and is based on the adsorption of VSCs on porous sorbents such as Tenax and molecular sieve 5A (Deprez and Franzmann, 1986, Davison, 1994,). Artifact formation and loss reactions that can be catalyzed by coating materials and trace metals can be substantial problems (Rudolph et al., 1990).

3) Cryogenic trapping: This technique is realized by low temperature VSC trapping in open or packed tubes (Tangerman, 1986). The trapping equipment is installed on the injection port of the gas chromatograph (GC) (Ramstad et al., 1995). Temperature and flow control are the most important operational factors in this process (Kohno and Kuwata, 1991, Wardencki, 1997).

4) Solid phase microextraction (SPME): This is a new and advantageous alternative to common sorption techniques. This solvent-free technique is easy to operate and has the ability to preserve VSCs (Mestres et al., 2000, Lestremau et al., 2004c,). However, the extraction efficiency is affected by sampling time, moisture, and temperature (Nielsen and Jonsson, 2002, Lestremau et al., 2004b). The main disadvantage of this technique is the production of artifacts that can be created during sampling (Lestremau et al., 2004a).

For VSCs in liquid and solid samples, extraction or isolation procedures are employed upstream of gas chromatography for separation (Mestres et al., 2000).

1) Liquid extraction: Organic solvents, especially azeotropic mixtures, provide efficient extraction of sulfur compounds. However, liquid extraction has serious disadvantages,

including the need for toxic solvents, sample dilution, and time. In addition, liquid extraction is subject to losses of sulfur compounds with low boiling points (Mestres et al. 2000).

2) Static headspace: In a closed system VSCs are collected from the space above the liquid or solid sample during a moderate heating process (Nedjma and Maujean, 1995). This is an efficient method for sulfur compounds with intermediate boiling points less than 120°C (Jacobsson and Falk, 1989, Christensen and Reineccius, 1992).

3) Dynamic methods: The purge-and-trap method (PT) is the most commonly used dynamic method. In a closed system, volatilized sulfur compounds (from the liquid or solid sample) are carried by a gas flow to a trap, where they are adsorbed and concentrated (Simo et al., 1993). Usually PT equipment needs to be installed on the injection port of the GC (Leck and Bagander, 1988).

4) SPME: As it is used for preconcentration of gas samples, it is also applicable to liquid samples (Pelusio et al., 1995). Matrix interference is the main problem associated with directly inserting SPME fibres into liquid samples (Mestres et al., 2000).

2.6.2.3 Storage Stability

Immediate analysis after collection can effectively avoid losses and transformation of VSCs. However, if immediate analysis is not possible, limits should be placed on storage time and conditions such as container material, sample matrices, moisture, and temperature. Tedlar bags and glass bottles are usually utilized as storage containers. Teflon and Tedlar bags can keep VSCs for two weeks even at ppb concentrations. However, SO_2 and H_2S can be lost from these bags over a period of hours to days (Lau, 1989). For glass bottles, moisture, temperature, and matrices can influence VSC storage stability for both gas samples and extracted samples (Devai and Delaune, 1994). The presence of moisture significantly reduces H_2S and MM concentrations. Storage at a temperature of 0 - 4°C prolongs storage time as compared to ambient temperatures. Freezing can maintain the stability of VSCs for two weeks (Tangerman, 1986). Matrix effects include the effects of the sample source and composition. Samples from liquids with high salinity keep their stability less than 48 hours (Simo et al., 1993).

2.6.3 Analysis

2.6.3.1 Analytical Techniques

Gas chromatography (GC) is the most commonly used technique for analysis of VSCs due to its sensitivity, reliability, and separation capability. Also, GC is widely compatible with various detectors and hence it was employed in this study.

The selection of the column is based on its separation capability and on the column and packing materials due to the possible loss of VSCs by adsorption and catalytic reactions. Poly-tetra-fluoroethylene (PTFE) is extremely non-reactive and heat-tolerant and is the most popular material used as packing materials for columns in analysis of VSCs as it has proved to be efficient for analysis of VSCs in various matrices (Graedel et al., 1981, Deprez and Franzmann, 1986, Leck and Bagander, 1988, Caron and Kramer, 1989, Lau, 1989, Simo et al., 1993, Perssoon and Leck, 1994,). The development of fused-silica capillary columns has provided broader and easier applications to VSC analysis. Thick films of neutral materials facilitate low concentration levels and provide better separation. Commercial columns are available for a wide range of VSCs in different sample matrices (Wardencki, 1998, Mestres et al., 2000, Nielsen and Jonsson, 2002).

2.6.3.2 Detectors

Different detectors have been employed for VSC analysis and are widely reported. Sulfur selective detectors are preferred because they can reduce analysis time and avoid contamination and loss of analytes.

The flame photometric detector (FPD) is a relatively inexpensive device that is robust for VSC analysis. It has been the most widely used sulfur-selective detection method (Mestres et al., 2000, Lestremau et al., 2004c). Problems associated with FPD are the quench effect associated with hydrocarbons (Liu and Fu, 1988) and the non-linear (exponential) relationship (Wardencki, 1998, Mestres et al., 2000) between VSC concentrations and responses. The sulfur chemiluminescence detector (SCD) is a recent and attractive alternative to FPD. SCD not only has good selectivity to sulfur compounds, but also provides a linear and uniform response to VSCs (table 2-3) and provides low detection limits (10^{-13} g) (Shearer and Skelton, 1994, Ivey and Swan, 1995). SCD is an excellent detection method for complex matrices and trace VSCs concentrations whereas FPD functions well under optimized analytical conditions (Macak et al., 1984, Inomata et al., 1999, Firor and Quimby, 2001).

Other universal detectors such as the atomic emission detector (AED) (Quimby and Sullivan, 1990), the Hall electrolytic conductivity detector (HECD) (Caron and Kramer, 1989), and the mass spectrograph (MS) are also applied to analysis of VSCs (Thornton et al., 1990, Bianchi et al., 1991, Eisele and Berresheim, 1992, Yokouchi et al., 1993) and have been shown to be versatile for the qualitative and quantitative analysis of VSCs. Electron capture (ECD), flame ionization (FID), and photoionization (PID) detectors have also been applied to analysis of VSCs at moderate concentration levels (Wardencki and Zygmunt, 1991).

Table 2-3: Characteristics of detectors (Wardencki and Zygmunt 1991)

Detector	Detection limit (g)	Selectivity S/C	Linear concentration range (decades)	Temperature limit (°C)
FPD	10^{-11}	$10^3 – 10^6$	3	420
ECD	Variable up to 10^{-15}	Variable	4	420
SCD	10^{-13}	$10^6 – 10^7$	3 – 4	420
AED	10^{-12}	10^4	3 – 4	350
HECD	10^{-11}	$10^4 – 10^6$	3 – 5	400
PID	10^{-12}	Poor	6	350
MS	10^{-11}	Specific	5	350

At present, FPD, SCD, and MS are the most widely used detectors. SCD and MS both have a linear relationship between sulfur concentrations and responses for a relatively broad concentration range. FPD is inexpensive and still preferred for its sensitivity to sulfur and stability of the non-linear response.

2.6.3.3 Standards Generation

Generation of a wide range of mixture concentrations is required to cover both the linear and non-linear parts of the calibration curves (Konieczka et al., 1992). Thus, preparation of standard gaseous mixtures is important. The obtained mixtures must fulfill a number of practical requirements, including stability, sufficient quantities, and have the desired level of accuracy (Namiesnik, 1984, Wardencki, 1998). Thermal decomposition (Konieczka et al., 1991, Konieczka et al., 1996), simple dilution (Nielsen and Jonsson, 2002), and dilution by permeation (Eisele and Berresheim, 1992, Perssoon and Leck, 1994) have been used for producing VSC mixtures:

1) Thermal decomposition: This method is usually used for preparing standard thiol mixtures. The principle is to synthesize a compound chemically bonded to the silica gel surface and then heat the modified gel and decompose the thiol at a specific temperature. The thiol

release varies with temperature, flow-rate of dilution gas, generation time, and mass of silica gel. This method is preferred for preparation of standard mixtures of compounds that are unstable, toxic, reactive, or malodorous but its operation is complicated and time-consuming (Konieczka et al. 1996). It is however not suitable for preparation of gas mixtures with multiple VSCs.

2) Simple dilution: This method is performed by injection of pure VSC into a glass bulb or steel cylinder which contains dilute gas. Both the accuracy and reproducibility of preparation of the mixtures greatly depend on the manner of injection. Devices with various injection methods are commercially available (Konieczka et al., 1991).

3) Permeation methods: All permeation methods are based on Fick's diffusion law. Permeation tubes now are the most commonly used permeation device. The problems associated with permeation tubes are the long initial induction period, high cost, limited life time, and difficulty of calibrating the tubes. Permeation methods are versatile and easy to be automated. They are widely used for preparation of VSC mixtures (Konieczka et al., 1991).

2.7 Summary

At the present, there are no comprehensive studies on the behavior of VOSCs in the anaerobic digestion of municipal sludge. Kinetic information of VOSCs conversion under anaerobic conditions is rarely reported. The present research aims to establish a mathematic model which can describe the fate of VOSC in anaerobic sludge digestion and predict VOSC concentrations in the biogas release. Therefore, studies related to distribution processes and biochemical kinetics of VOSC as well as laboratory analysis and mathematical models employed for VOSC quantification were the focus of the literature review.

Occurrence of VSCs prevails in anaerobic conditions. With focus on anaerobic processes for municipal sludge digestion, dominant VSC types and their effect on biogas utilization were introduced. The physical and chemical processes, biochemical pathways, and microorganisms involved in generation and degradation of the dominant VOSCs (MM, DMS, and DMDS) which were observed in anaerobic environments were demonstrated. By reviewing studies about VSC generation and degradation in diverse anaerobic environments, a VSC conversion frame was built up. This frame provides a comprehensive and fundamental understanding on the behavior of VSC.

To identify physico-chemical characteristics of VSC, verify occurrence of VSC conversion pathways, and monitor VSC variation in municipal sludge digestion, potential experiment scheme, selection of methanogen-inhibitor, and feasibility of analytical methods were discussed and assessed by critical literature review and preliminary experiments. Information collected and analyzed in this chapter helped to determine experimental plan and approach which were utilized in this study.

Modeling which includes framing biological processes pertinent to VSC conversion in sludge digestion, value estimation for kinetic parameters, and model evaluation is the goal of this study. The anaerobic model ADM1 and its extensions which address sulfur behavior were reviewed. The kinetics utilized in these models for biological processes were introduced. They are important reference for establishing VSC conversion model in this study.

Chapter 3

Experimental Plan and Approach

This chapter presents the experimental plan for the research, which included bench-scale digester set up and operation and batch testing. The parameters monitored in the experiments are introduced and are classified into two categories: conventional parameters and sulfur related parameters. The conventional parameters included biogas volume, methane and carbon dioxide concentrations, COD, TS/VS, TSS/VSS, TKN and ammonia, and pH values. The sulfur related parameters included VSC concentrations in the gas phase and sulfur species in the sludge. The definition, function, and significance of each category of parameters, as well as their experimental materials and approaches are introduced.

3.1 Experimental Plan

3.1.1 Bench-Scale Digesters

3.1.1.1 Reactor Set-up

Two bench-scale anaerobic digesters were set up in the lab. The mesophilic reactor was operated at 35°C while the thermophilic reactor was operated at 55 °C. Each reactor was cylindrical, fabricated from opaque plastic and had a capacity of 20 L. The reactor set-up is illustrated in Figure 3-1. Heat-tracing cord, connected to an automatic temperature control, was wound around the reactor to heat the sludge in the reactor. The reactor and heat trace were wrapped with insulation to assist with maintaining the temperature constant. A dial thermometer was plugged into the reactor to monitor the temperature.

The biogas was released from the reactor through a gas release port and flowed through a low flow rate gas flow meter (Triton-WRC, model 181). A 5 L Teflon gas bag was connected between the gas release port and the flow meter to buffer pressure changes and avoid pulling of air into the reactor during sludge wasting.

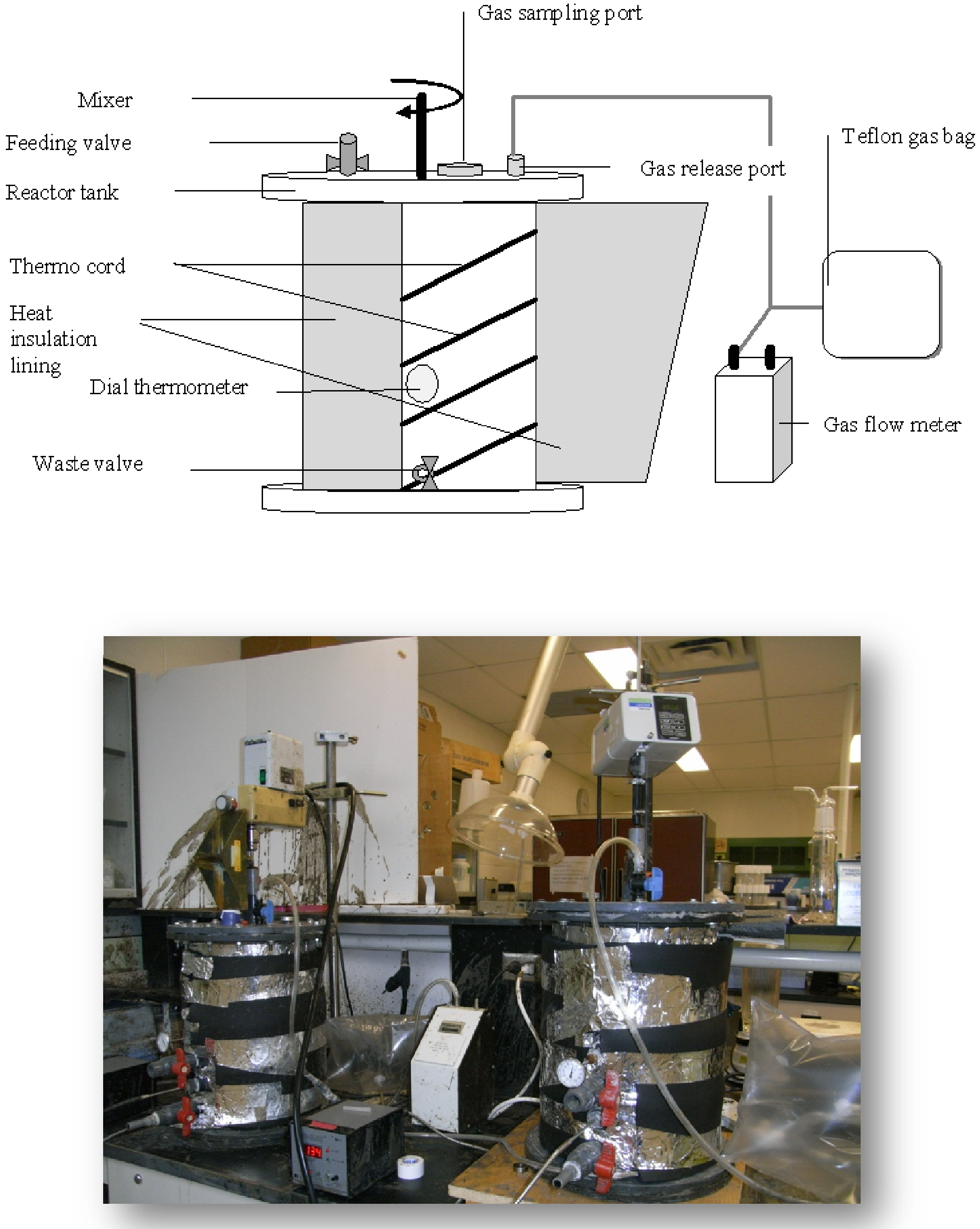

Figure 3-1 Bench scale anaerobic digester

When the reactors were initially set up, 18 L of sludge that was obtained from the mesophilic (35°C) digesters of the Waterloo Wastewater Treatment Plant (WWTP) was loaded into each reactor. The temperatures of the reactors were adjusted to 35°C and 55°C, respectively. After operating the reactors at the target temperature for 1 day regular wasting and feeding was applied to both reactors. The feed (raw) sludge consisted of a mixture of primary and secondary sludge that was obtained from the Waterloo WWTP. The raw sludge was collected once every week and stored in a fridge at 4°C. From Monday to Thursday, 1 L of digested sludge was wasted from the reactor and then the same volume of raw sludge was pumped in. On Friday, the waste/feed sludge volume was 1.5 L. This semi-continuous feeding scheme gave each reactor an average hydraulic/solids retention time between 22-23 days.

After operating for 90 days, the two bench-scale reactors were deemed to have reached steady state. Two intensive reactor monitoring experiments were conducted. The first one was conducted before batch tests to validate the stability of the inocula source. After validating the steady state of the two reactors, the digested sludge was then employed as the seed sludge source for serum bottle tests throughout the research. Another intensive monitoring was conducted at the end of this study, whose results were used for analysis of sulfur species and model evaluation.

The average operating parameters of the two digesters at steady state are listed in table 3-1. The numbers/ranges listed in table 3-1 are the average values/data ranges obtained from the first intensive reactor monitoring. The first intensive reactor monitoring was conducted for 4 months: liquid phase analyses were conducted once every week while gas analysis was conducted on all the week days. The steady state of both reactors was validated before utilizing the digested sludge as inocula in batch tests in the rest of this study.

Table 3-1 Operational parameters for bench scale digesters

Parameters	Mesophilic	Thermophilic
Temperature	35±0.1 °C	55±0.1 °C
pH	7.7±0.3	7.8±0.4
Total alkalinity (mg $CaCO_3$ /L)	3655 ± 208	3302 ± 346
Ammonium/ammonia (N mg/L)	875±115	910±180
Gas production	308±32 ml CH_4/g removed COD	311±35 ml CH_4/g removed COD
VSS reduction (%)	38±15%	42±10%

3.1.1.2 Reactor Monitoring

This research sought to develop a model describing VSC generation and degradation in anaerobic digestion based on kinetic data collected through batch tests. The two reactors were used to provide stable mesophilic and thermophilic inocula for the batch tests. In addition, the feed sludge, digested sludge samples from both reactors, and the generated biogas samples were monitored to enrich the steady state database which was used to evaluate the model application. The parameters that were monitored for this purpose are listed in table 3-2. During the intensive monitoring (5 months), the sludge characteristics were analyzed once every two weeks while the biogas property was measured on all the week days.

Table 3-2 Parameters monitored during reactor operation

	Parameter
Biogas properties (daily on week days)	Volume of the biogas
	Biogas composition: methane (CH_4), carbon dioxide (CO_2)
	VSC impurities: H_2S, MM, DMS, DMDS
Influent and effluent sludge characteristics (weekly)	Chemical oxygen demand (COD): total COD, soluble COD, flocculation and filtration COD
	pH values
	Solids: total solids and volatile solids (TS/VS), total suspended solids and volatile suspended solids (TSS/VSS)
	Total Kjeldahl nitrogen (TKN): total TKN, soluble TKN, Ammonia
	Sulfur species: total S, soluble S, precipitated S

3.1.2 Static Headspace Test

Partitioning coefficients of selected VOSCs in the sludge were determined in this study to facilitate subsequent quantification of the VOSCs in the sludge based on their measured quantities in the gas phase. A static headspace method was developed to estimate the partitioning coefficients and to assess the effect of temperature and solids concentration in the sludge on phase partitioning. The static headspace method and the pertinent biomass deactivation method are described in this section.

3.1.2.1 Experiment Scheme and Objectives

1. Partitioning in Water-Gas System

Partitioning coefficients were determined in deionized water at temperatures within the range of 12 – 58 °C. This phase of the study estimated the dependence of the partitioning coefficients on temperature and the results were compared with the literature to verify the reliability of the headspace gas chromatographic method.

2. Partitioning in Sludge-Gas System

Partitioning coefficients between gas and a sludge matrix (digested sludge obtained from the bench-scale digesters and diluted to two different solids concentrations) were determined at temperatures within the range of 12 – 58 °C. Specifically, partitioning coefficients in sludge samples with a variety of different solids concentrations were measured at 35 and 55 °C, the temperatures employed in subsequent mesophilic and thermophilic anaerobic digestion studies, respectively. The objective of this phase was to estimate the effect of temperature and solids concentration on partitioning when a complex sludge matrix was employed.

3.1.2.2 Static Headspace Method

The liquid-gas partitioning coefficients in the gas-liquid systems were determined in either 1000, 500 or 250 ml glass serum bottles (effective volumes were about 1050, 560, and 330, respectively) which were sealed with screw caps and PTFE foil. The caps were equipped with a gray bromobutyl septum for headspace gas sampling. Bottle capacities were determined individually. The diluted sludge was obtained from the bench-scale digesters, deactivated and stored in the fridge one day before the partitioning tests. Deionized water was produced in the lab. The deionized water/diluted sludge was added into the serum bottles to make up about 35 - 60% of the volume. Due to the reductive and reactive characteristics of VOSCs, the solutions (water or diluted sludge) were flushed with pure nitrogen for at least 2 minutes to evacuate oxygen and other gaseous impurities prior to introduction of VOSCs.

Before addition of VOSCs, the bottles were placed into an incubator/refrigerator for 120 minutes to warm up or cool down the contents from room temperature to the set temperature. When the set temperature was lower than the room temperature, pure nitrogen was injected into the headspace in advance to avoid low pressures that would be caused by gas contraction. After the 120 minute temperature adjustment, the headspace gas of each bottle was released through a manometer to adjust the pressure in the bottle to 1 atmosphere.

Analytical reagent grade MM, DMS, and DMDS were injected into the serum bottles, individually, for the partitioning tests. Then the bottles were shaken and placed in the incubator/refrigerator to reach an equilibrium state. It was found in the primary tests that the concentrations of VOSC became constant 45 minutes after injection, which suggested that equilibrium state was reached. However, in the study of Przyjazny et al. (1983), a prolonged equilibrating time (90 minutes) was utilized. To ensure the equilibrium state and comparable results to Przyjazny et al.'s study, 90-minute equilibrating time was employed. The measurements of the headspace gas were carried out in replicate bottles which were controlled at the target temperature ± 0.1 °C. At equilibrium the gas phase was sampled by a 0.5 ml gas tight syringe with a PTFE pump. Samples were analyzed chromatographically by the GC-PFPD method described in section 3.2.

The distribution between the gas and liquid phases and the corresponding partitioning coefficient was determined using a mass balance approach. The liquid-gas partitioning coefficient was defined as the ratio of the concentration of the VOSC in the liquid phase to its concentration in the gas phase (Equation 3-1).

$$\boldsymbol{k = \frac{C_l}{C_g} = \frac{(n - C_g \times V_g)/V_l}{C_g}} \qquad \textbf{(3-1)}$$

Where C_l and C_g were the equilibrium concentrations of VOSC in the liquid and gas phases, respectively, n was the total mass of VOSC (mole) added into the bottles, and V_l and V_g were the volumes of the liquid and gas phases.

The measured concentrations of VOSC were reported as volume fractions ($\frac{V}{V}$), which represented the ratio of the volume of VOSCs to the volume of the headspace. The gas pressure in the headspace was controlled at atmosphere pressure (total pressure was 1 atm) so the measured concentration also represented the partial pressure of the VOSC in the headspace. According to the idea gas law, the concentration of VOSC in the gas phase can be expressed by Equation 3-2.

$$\boldsymbol{C_g = \frac{n_g}{V_g} = \frac{P}{RT}} \qquad \textbf{(3-2)}$$

Where n_g was the mass of VOSC (mole) in the gas phase, R was the ideal gas law constant (0.082 $L \cdot atm \cdot K^{-1} \cdot mol^{-1}$), T was the temperature of headspace gas (K), and the P was the measured VOSC partial pressure (atm).

With the measured VOSC concentration in the headspace gas and the estimated liquid-gas partitioning coefficient, the solid-liquid coefficient was able to be calculated if it was applicable. It was assumed that VOSC were adsorbed by the suspended solids, the adsorption surface area was directly proportional to the mass of the suspended solids, and the volume of the solids was negligible when compared with the total volume of the sludge. The specific concentration of VOSC adsorbed on the suspended solids would be proportional to the concentration of VOSC in the bulk liquid when the partitioning equilibrium was reached (Equation 3-3).

$$\boldsymbol{k_s = \frac{m}{C_l}} \tag{3-3}$$

Where k_s was the solid-liquid partitioning coefficient (l/g), m was the specific concentration adsorbed on the suspended solids (mol VOSC/g-solids), and C_l was the concentration of VOSC in the bulk liquid.

The mass balance for the distribution of VOSC in the three phases could be expressed by Equation 3-4:

$$\boldsymbol{n = n_g + n_l + n_s = \frac{pV_g}{RT} + C_l V_l + m \cdot TSS \cdot V_t} \tag{3-4}$$

Where n was the total mass of VOSC dosed into the serum bottle (mol), n_g, n_l, and n_s were the VOSC distributed in the gas, liquid, and solid phases after equilibrium was reached (mol), V_t was the total sludge volume (≈ the liquid phase volume V_l), TSS was the total suspended solid concentration. C_l can be calculated by Equation 3-1.

At the equilibrium state, a higher k_s suggested significant adsorption of VOSC on the suspended solids, which would result in a lower VOSC concentrations in the headspace. In contrast, when k_s was extremely small, it suggested that the amount of VOSC adsorbed on the suspended solids or distributed in the solid phase was low. Under the latter condition, only the liquid-gas partitioning was important for the VOSC distribution.

3.1.2.3 Biomass Deactivation

Sludge is a complex aqueous matrix composed of organic and inorganic solids, various dissolved inorganic salts and organic matter. Each individual soluble or insoluble component of the sludge may influence the partitioning coefficients. For example, suspended solids, ionic concentration, or short chain fatty acids, which impact adsorption, salting out, or azeotropy, may either increase or decrease the solubility of VOSC in a sludge sample. However, all the individual effects on partitioning may not be directly additive. To quantify the VOSC partitioning, it was important to assess their overall influence that would be caused by the combination of different factors in the sludge.

It was difficult to simulate sludge by any synthetic mixture in the lab because of its complexity. Hence dilutions of digested sludge were used to estimate partitioning coefficients in the second stage of the partitioning experiments. Sludge contains abundant microorganisms which can mediate the transformation and degradation of VOSC hence it was necessary to deactivate the biomass before using it in the partitioning experiments.

A sludge deactivation procedure that involved heating 1 L of digested sludge at 80 °C for 30 minutes was set up in the lab. This method was adapted from a thermal method that has been previously employed for extraction of extracellular polymer substrates (Goodwin and Forster, 1985). It was reported that this temperature and duration was able to effectively deactivate the microorganisms without destroying the cells. However, it was found that thermal deactivation itself was not able to completely deactivate microorganisms in preliminary experiments. A small amount of methane was still produced if the thermally treated sludge was placed in an incubator with a temperature of 35 or 55 °C. Hence, chemical deactivation was employed in sequence with the thermal method. After the sludge cooled down, sodium azide was added at a concentration of 1% in the sludge. The sludge was then placed in a refrigerator (4 °C) overnight to ensure an effective and "complete" deactivation. The deactivated sludge was used within 48 hours of being taken out of the refrigerator as it was anticipated that the activity of the microorganisms would revive gradually, and hydrolysis and other processes would become significant within 72 hours after the deactivation.

3.1.3 Batch Tests under Controlled Conditions

3.1.3.1 Experiment Objectives

In this research, the following information was of interest:

1) Dominant VOSC generated during anaerobic sludge digestion,
2) Kinetics of VOSC generation and degradation,
3) Effect of temperature on the kinetics,
4) Effect of sludge source on the species of VOSC produced and their release pattern in anaerobic digestion at different temperatures.

To obtain the target information, dynamic data were collected with time through batch tests that were conducted under controlled conditions. The digestion temperature, initial concentrations of VOSCs, sludge source, and methanogen-inhibitor dose were controlled in the batch tests. Three groups of batch tests were designed and conducted to obtain the previously described information. They included incubations that involved raw sludge samples, VOSC dosed sludge samples and amino acid dosed sludge samples.

3.1.3.2 Incubation Protocol

Although different substances were utilized for the different experimental objectives in the three groups of batch incubations, their incubations followed a similar protocol. The protocol, including the bottle preparation, pH adjustment, oxygen evacuation, and bottle replication for sampling, is described in this section.

All the batch tests were conducted in KIMAX-35 glass serum bottles with a total volume of 328.7 ml. A total volume of 200 ml of the mixed incubation liquid was added to each bottle and hence a headspace with the volume of 128.7 ml remained (Figure 3-2). The contents of the mixed liquid were dependent on the purpose of the batch test and will be described in following sections in which the individual batch tests are discussed.

Considering the importance of maintaining a neutral pH for anaerobic digestion, bicarbonate was used as a pH buffering compound when a high organic load was employed in the serum bottles. To avoid any toxic effects that might be caused by cations, half of the bicarbonate was dosed as the sodium salts while another half as the potassium salts. The dosed concentration of each bicarbonate salt was 1.6 g/l (including the mass of the cation).

After addition of various liquid components into the serum bottles, the mixed liquid was purged with pure nitrogen for 1 – 2 minutes to evacuate the oxygen. Black polypropylene screw caps with gray bromobutyl septa were employed to ensure sealing and sample access with syringes.

The serum bottles were placed in an isothermal incubator or water bath for continuous incubation at a constant temperature. During the incubation, the bottles were placed upside down to avoid gas leaking under high pressures that might result from biogas generation (Figure 3-2).

When only gas samples were to be analyzed in the batch tests, duplicate serum bottles with the same contents were prepared. When liquid sample were required through the incubation process, 5 – 10 replicate bottles with the same contents were prepared at the outset of the test. The replicate bottles were gradually sacrificed for analysis of the liquid samples.

Figure 3-2 Serum bottle and their set-up in the isothermal incubator

3.1.3.3 Experiment Design

1. Raw Sludge Incubation

The raw sludge incubation was designed to identify the variety of VSCs produced and the generation and degradation patterns of VOSC in anaerobic sludge digestion, to confirm the important role of methanogens in VOSC responses, and to assess the effects of temperature and sludge source on VSC generation. Different incubation temperatures (35 and 55 ºC) and different sludge sources were utilized in this group of batch tests. The sludge sources are listed in table 3-3.

Table 3-3 Sludge sources for raw sludge digestion

Raw sludge	Source
Secondary sludge	Pilot SBR in New Hamburg WWTP
Excess secondary sludge	Skyway WWTP, Burlington
Primary sludge	Skyway WWTP, Burlington
Mixture of primary and secondary sludge	Waterloo WWTP

Methanogens have been considered as the functional microorganisms which mediate VOSC degradation. To confirm that methanogens play such an important role in VOSC degradation in both mesophilic and thermophilic anaerobic digestion, methanogen-inhibited incubations were conducted along with non-inhibited incubations. The generation patterns of VSC under the two conditions were compared. The methanogen inhibitor which was utilized in this study was 2-bromoethanesulfonic acid (BES).

Raw sludge usually had a total COD value 30000 ~ 50000 mg/l and required a long incubation time to totally digest the biodegradable organic materials. An incubation time of 13 ~ 50 days was employed. For the different purposes, two series of batch tests were conducted.

In the first series of tests, sludge from the Waterloo WWTP was utilized (table 3-4). In the incubations without methanogen-inhibition, the speciation of generated VSCs was identified and their concentration variation through the digestion was monitored to understand the generation and degradation patterns under the two temperatures. In the incubations with methanogen-inhibition, the VSC releases at the two temperatures were quantified. BES was dosed to a final concentration of 25 m mol/l as the methanogen-inhibitor.

In this series of batch tests, both VSCs and methane were measured with the latter conducted to monitor methanogen activity. Headspace sampling was conducted 4 ~ 6 times on the first day, once every day in the first 7 days, and then once every 2 ~ 7 days in the following incubation. Liquid phase sampling was conducted once every 2 days in the first 8 days and then once every 3 ~7 days after. This sampling strategy was due to the increased VSC release and biogas generation at the beginning of incubation.

The sludge characteristics, including the conventional parameters and sulfur species, were also monitored with time through the incubation. The liquid was sampled by sacrificing the serum bottles and analyzed for conventional parameters and sulfur composition. COD and solids measurements were used for mass balance calculations. TKN and ammonia measurements were used to explore the relationship between nitrogen and sulfur release. Sulfur species analyses were used to characterize the variation of the sulfur composition in the sludge through digestion.

Table 3-4 Waterloo WWTP sludge incubation

Methanogen inhibition	Temperature
+	-
-	-
+	+
-	+

Methanogen inhibition: + with inhibition; - without inhibition. Temperature: + 55 ºC; - 35 ºC.

In the second series of tests, sludge samples from the New Hamburg and Skyway WWTP were incubated with inhibition of methanogens. BES was dosed to a final concentration of 25 m mol/l as the methanogen inhibitor (BES dosage was increased to 37.5 m mol/l for the incubation of primary sludge obtained from Burlington to achieve complete inhibition). The VSC variety, release rate, and release capacity were quantified. These studies facilitated an assessment of the effect of sludge source on VSC release.

In the incubation, the gas sampling strategy was similar as the batch tests conducted with the raw sludge from the Waterloo WWTP. The liquid was sampled by syringe for ammonia analysis. Before and after the incubation, the conventional parameters, sulfur composition, and iron concentration were analyzed for the sludge.

2. VOSC Dosed Incubation

The second group of batch tests examined VOSC degradation in dosed sludges. This group of batch tests was designed to determine the kinetics that best described VOSC degradation and to estimate the kinetic parameters at 35 and 55 °C (Figure 3-3).

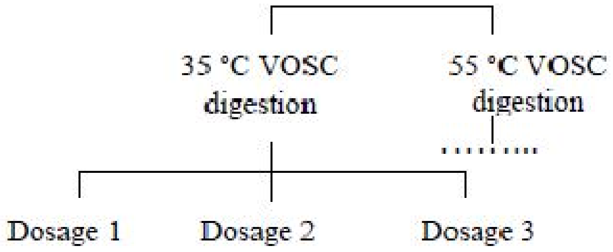

Figure 3-3 Experiment design for VOSC degradation (repeating branchs of the tree were omitted in the figure)

The VOSC that were identified in the raw sludge incubations (MM, DMS, and DMDS) were dosed individually and incubated with the inocula. 98% DMS (liquid), 99% DMDS (liquid), 98% MM (high pressure gas) were utilized in this part of experiment. Pure VOSCs were injected into the serum bottles individually with Hamilton syringes to avoid the impact of organic solvents on the analyses.

Different amounts of VOSC were dosed into the bottles to generate a range of initial concentrations. The dosages of each VOSC utilized in the batch tests are listed in table 3-5. The concentration of the dosed VOSCs in the bottle headspaces were monitored with time. The dynamic responses of concentrations with time were used for estimation of kinetics.

Table 3-5 Initial dosage of individual VOSC in batch tests

Unit: mg S/g VSS	Mesophilic			Thermophilic	
Initial dosage level	High	Mid	Low	High	Low
DMS	5.20	2.92		2.13	1.22
MM	4.67	3.11	1.30	1.84	0.92
DMDS	2.94	1.27	0.89	1.27	0.38

Methanogens are believed to be responsible for the degradation of VOSC and hence it was hypothesized that the presence of acetate as a competing substrate might influence the rate of VOSC degradation. Hence, dosed VOSC tests were conducted with and without acetate to explore the effect of an external carbon source on VOSC degradation. For the VOSC dosed batch tests, four types of serum bottles were prepared and their contents were shown in the table 3-6.

Table 3-6 Contents of serum bottles in VOSC dosed incubation

Serum bottle type	Contents		
	Diluted anaerobic digested sludge	Acetate (1m mol)	VOSC
Inocula	+	-	-
Inocula with acetate addition	+	+	-
VOSC dosed	+	-	+
VOSC dosed with acetate addition	+	+	+

+ represents addition of the corresponding compound/sludge
– represents absence of the compound/sludge

Due to the relatively high biodegradability of VOSC, the incubation times were much shorter as compared with the raw sludge incubation. An incubation time of 2 ~ 7 days was employed, and depended on the VOSC and incubation temperature.

3. Amino Acid Dosed Incubation

VOSCs are known to be formed from proteinaceous materials. This group of batch tests was designed to explore the generation of VOSC from amino acids and to estimate the kinetic parameters of this process at 35 and 55 °C. Methanogens were inhibited to allow for identification of the VOSC that were generated directly from the amino acids. Incubations without methanogen inhibition were also conducted to monitor the general pattern of generation and degradation of VOSC from anaerobic digestion of amino acids.

Cysteine ($HO_2CCH(NH_2)CH_2SH$) and methionine ($HO_2CCH(NH_2)CH_2CH_2SCH_3$) are the most significant amino acids which contain the element sulfur. According to the literature review, normally, the anaerobic degradation of cysteine will generate H_2S as the sulfur-containing final product. It could generate other VSC only when it is converted to methionine through complicated biochemical processes. Based on the results of a preliminary test (data not shown in the thesis), it was confirmed that H_2S was the predominant VSC generated in cysteine tests. Hence, considering the purposes of this group of incubations, cysteine was not employed to explore the VOSC species generated in anaerobic digestion.

In contrast to cysteine, degradation of methionine has been reported to generate VOSC rather than H_2S. Therefore, methionine was chosen as the substrate in this group of batch tests. A stock solution of methionine with a concentration of 500 m mol/l was prepared. The stock solution was added to the dosed serum bottles to obtain a final concentration of 5 or 2.5 m mol/l for the mesophilic and thermophilic incubation, respectively. The experimental plan for the methionine-dosed tests assessed the impacts of methanogen-inhibition and temperature on VOSC formation (experiment design was same as described in table 3-4). In the batch tests with methanogen inhibition, BES was dosed to a final concentration of 15 m mol/l as a methanogen inhibitor.

For the methionine dosed incubation, 5 replicates were prepared for each test condition and the incubations lasted 17 and 28 days for the mesophilic and thermophilic incubations, respectively. The gas phase of all the bottles was sampled 2 times every day for the first 3 days, then once every 1 to 3 days until the end of the incubation. One serum bottle of each condition was sacrificed for each analysis of the liquid phase. The frequency of the liquid phase analysis was reduced from once every 2 days to once every 5 days, over the duration of the incubation.

3.2 Experimental Analysis

3.2.1 Conventional Parameters

Conventional analyses that describe biogas properties and sludge characteristics including generated gas volume, methane and carbon dioxide concentrations, COD, TS/VS, TSS/VSS, TKN and ammonia, and pH values were conducted in this research. They were employed to directly/indirectly describe the digestion conditions, sludge characteristics, and methanogen activity. They were also used to calculate a carbon related mass balance and to evaluate the effects of external factors on digestion. Moreover, in this research, by exploring their correlation with the sulfur related parameters through digestion, the use of conventional parameters as surrogates for sulfur related parameters was assessed.

In this section, the role of these conventional analyses for anaerobic digestion and their correlation with the sulfur measurement will be explained. The analytical methods will also be briefly introduced.

3.2.1.1 Aquatic Analysis

1. COD

COD is a common chemical measurement in the water/wastewater field, which indirectly represents the amount of organic matter present in a sample. The COD of a sludge can be classified into total COD, soluble COD, and ff COD (flocculated and filtration COD) (Mamais et al., 1993) by the solubility of the organic matter.

The difference in the total COD of a sludge sample before and after anaerobic digestion reflects the quantity of the organic compounds which are degraded during its retention in the anaerobic reactors. When combined with the measurement of methane (the only additional COD outlet from the digester except sludge waste) production, COD measurement allows for mass balance checks for carbon conversion.

In this study, soluble COD was defined as organic matter which is able to pass through filters with a pore size of 1.2 µm after centrifugation, and likely included dissolved and colloidal compounds. The ff COD measurement includes the organic matter which is able to pass through a filter with a pore size of 0.45 µm after colloidal matter is flocculated. Samples were flocculated by adding 0.4 ml of a 70 g/l aluminum sulfate hexahydrate solution to a 15 ml filtered (1.2 µm) sample. ff COD reflects the readily degradable organic matters including short chain fatty acids in the sludge (Mamais et al., 1993). The extent of contribution of soluble and ff COD to the total COD may affect the rate of methane production and the degradability of the sludge within a limited retention time.

The COD analysis in this project followed a method modified from the standard method 5220 D, Closed reflux, colorimetric method (18th edition, 1992) with the potassium dichromate and concentrated sulfuric acid as the oxidants. The sludge sample which was utilized for total COD measurement was homogenized before dilution and analysis were employed. The reflux time was extended from 2 hours to 3.5 hours.

2. TKN and Ammonia

TKN measurements describe the sum of the organic nitrogen and ammonia in a sludge. One of the important functions of anaerobic digestion is to convert organic nitrogen to inorganic nitrogen to realize stabilization/mineralization. The difference between the quantities of TKN and ammonia in a sample is the organic nitrogen. Degradable organic nitrogen usually exists in proteinaceous materials. Proteinaceous materials are also the major source of sulfur in sludges. Hence, measurement of TKN and ammonia allows for estimation of the extent of nitrogen mineralization and made it possible to assess the correlation between the ammonia and sulfur release from the organic matter.

The principle of TKN measurement is to digest the samples with sulfuric acid at high temperatures to decompose the organic nitrogen to ammonium. The TKN digestion method employed in this study was developed at the Wastewater Technology Center (Burlington, Canada) and adjusted in the lab. To prepare the TKN digestion solution, 40 gram potassium sulfate and 2 ml Selenium oxychloride (97%) were dissolved in 250 ml concentrated sulfuric acid and diluted to 500 ml with deionized water after the acid solution cooled down. Ammonia/ammonium reacts with phenate to produce a blue compound, which allows an automatic colorimetric analysis at 660 nm (Figure 3-4). Nitroprusside was used as a reaction catalyst.

Figure 3-4 Automatic ammonia analyzer

3. pH Value

The pH value is important for anaerobic digestion because the activities of anaerobic microorganisms are pH sensitive. For instance, methanogens are only active within a very narrow pH range and a neutral environment can maximize their activity. Therefore, measuring pH is the easiest way to monitor the digester or batch incubation to assess whether the digestion is progressing properly. In addition, pH value determines the dissociation of hydrogen sulfide and other weak acids and the dissolution of metal salts in the liquid phase. In this study, pH values were measured by a pH meter, Orion model 710A, with Ag/AgCl as reference electrode filling solution. Calibration with standard pH solutions was conducted before analysis of real samples. Fresh sludge samples (without dilution or homogenization) were utilized in the pH analysis.

4. Solids

TSS is a measure of particulate matters present in a sample and is the mass obtained by separating particles from a water sample using a filter (pore size, 1.2µm). The difference of the solid mass between TSS and the residue of TSS after combustion at 500± 50°C is defined as VSS, which is an approximation of the concentration of organic particles in the sludge. VSS concentrations were used to normalize data when comparing different samples in this study.

TS measurements include both dissolved and suspended solids in a sludge sample. VS measurements represent the portion lost during combustion of TS and are an approximation of the dissolved and suspended organic solids. The differences between TS/VS and TSS/VSS measurements represent the dissolved solids and dissolved organic solids respectively. To measure TS, a well-mixed sample was evaporated in a weighed dish and dried to a constant weight at approximately 105 °C. The increase in weight over that of the empty dish represented the total solids. The dried residue was then combusted to a constant weight at 500 ± 50°C for VS estimation.

The solids analysis in this project followed methods from Standard method 2540 B, D, and E for total, total suspended, and volatile solids, respectively (18th edition, 1992). The temperature employed for the volatile solids ignition was 540 °C. In this study, the solid residue after combustion was collected and digested for the precipitated sulfur test.

3.2.1.2 Biogas Analysis

1. Gas Volume

For the biogas generated from the bench-scale digesters, low flow rate gas volume meters (Triton-WRC, model 181) were used for on-line gas flow recording. The low flow rate gas volume meter was designed to measure small volumes of gas production over an extended period of time. A volume of gas is measured through an automatically siphoning measurement chamber. Each time the chamber siphons, the display increments 1 volume of gas. The chamber volumes were between 54 and 57 ml and each unit was individually calibrated.

For the biogas generated during batch incubations, the gas in the serum bottles was released through a manometer device (Figure 3-5) after a period of incubation time, until the pressure in the serum bottle was equal to the atmosphere pressure. The volume of the water in the manometer which was replaced by the released gas was recorded.

Figure 3-5 Manometer device for gas volume measurement

2. Biogas Composition

Biogas generated from the digesters had a high methane content (55 ~ 75%) with carbon dioxide representing a majority of the remaining gas. The methane production is an index of the biogas quality and the activity of methanogens. It was calculated as the product of the released biogas volume and the percentage of methane.

In this study, biogas samples with a volume of 1 ml were analyzed by a SRI 310C gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a 6'×1/8"OD Porapak column (80/100 mesh) (Figure 3-6). The temperatures of the injector and column were 30°C.

Figure 3-6 GC-TCD for methane and carbon dioxide analysis

3.2.2 VSC Measurement

3.2.2.1 Instrument

The sulfur in a sludge sample may be released as VSCs through the process of anaerobic digestion. Although the concentrations of VSCs in a biogas are usually present at a level of parts per million or even parts per billion, their existence in the biogas can arouse serious odor problems and cause damage on biogas utilization equipment. They were the primary target compounds in this research and hence accurate measurement was important.

Based on the literature review (section 2.7), a gas chromatograph analytical method was employed. The GC employed in this study was equipped with a capillary column (VB-1, 100% dimethylpolysiloxane, 30m × 0.32 mm, 0.4 μm) and a pulsed flame photometric detector (PFPD) (Figure 3-7).

Figure 3-7 GC-PFPD for VSC analysis

Manual injection of a 0.5 ml gas sample was performed with a Hamilton® Luer tip gas tight glass syringe (PTFE seal) and the injector temperature was 140 °C. The GC oven temperature was initially held at 37 °C for 5 minutes and then ramped at 30 °C per minute until 140 °C where it was held for 5 minutes. The total analysis time was approximately 13.4 minutes. It took about 10 minutes for the column to cool down to 37 °C and another 2 minutes to be stabilized before the subsequent injection.

3.2.2.2 Standard and sample preparation

HPLC or GC grade pure VOSCs (methyl mercaptan (gas), dimethyl sulfide, dimethyl disulfide, carbon disulfide, dimethyl mercaptan, propyl mercaptan) were purchased. Standard hydrogen sulfide gas was obtained from the Safety Office at the University of Waterloo and stored in a glass bulb (used within 24 hours).

HPLC grade nitrogen gas was utilized for dilution of the pure VSCs and for biogas samples containing VSCs of a concentration higher than 25 ppmv. Glass bulbs with calibrated volume and plastic syringes with volumes of 10 and 50 ml were used for dilution.

3.2.3 Sulfur Species in Sludge

3.2.3.1 Categorization

To better model the conversion of sulfur from organic to inorganic forms, from solid to liquid phases (or reverse), and from liquid to gas phases, a detailed quantitative description of sulfur fractions was developed. Taking feed sludge as an example, the sulfur fractions were classified as described in Figure 3-8.

The total sulfur in the sludge included the sulfur in the liquid and solid phases. The sulfur in each phase was assumed to include organic and inorganic species. The dissolved inorganic sulfur in the sludge was assumed to include sulfate and soluble sulfides. The inorganic sulfur in the solid phase was assumed to consist of heavy metal sulfides that had precipitated. The organic sulfur was assumed to include complex sulfur and low molar weight sulfur compounds. The low molar weight organic sulfur in this study was defined as the target volatile sulfur compounds (MM, DMS, and DMDS). The other organic sulfur compounds such as sulfur-containing amino acids or peptides were considered as complex sulfur. Through this definition, each fraction could be either directly measured or indirectly estimated.

The measured sulfur species in this study includes total S, S in liquid phase (soluble S), inorganic soluble sulfur, and inorganic sulfur in the solid phase (precipitated S). Dissolved sulfide is usually neglible due to the high iron concentration in the sludge (1000 ~ 2500 mg/l) and hence the sulfate concentration was used to represent the inorganic soluble sulfur.

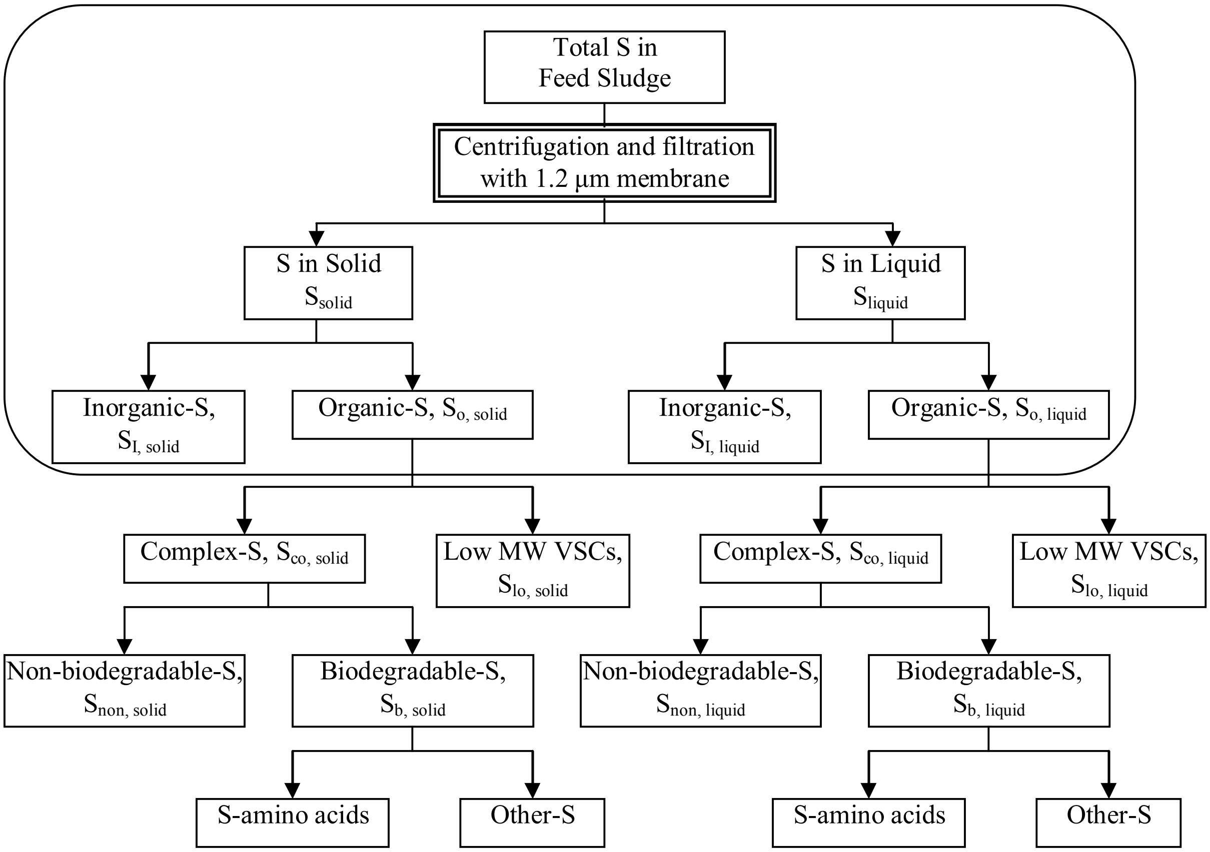

Figure 3-8 Sulfur fractions in feed sludge

3.2.3.2 Sulfur Species Analysis

1. Sulfate Concentration

Sulfate represents the majority of the inorganic sulfur containing compounds in the raw municipal sludge. Liquid samples for sulfate analysis were prepared through a two-step filtration. The sludge samples were firstly centrifuged and filtered through the 1.2 μm glass membrane and then filtered through a 0.45 μm Nylon syringe filter. The modified standard method 4500-SO_4^{2-}, Ion Chromatography method (18th edition, 1992) was employed for analysis.

An ion chromatograph (Dionex) equipped with an AS3500 auto sampler, an AG9-9HC guard column, an AS9-HC separation column, and a CDM-II conductivity detector was employed in the sulfate analysis. The eluent was 9mM Na_2CO_3 solution which was delivered at a rate of 1 ml/min at a pressure between 2000 to 2500 kPa. The regenerant solution was 13mM H_2SO_4 while the autosampler rinse solution was 25% (v/v) isopropyl solution.

2. Total and Soluble S Concentrations

Sludge samples were homogenized for total S analysis while the filtrates collected after sludge centrifugation and filtration (1.2 μm) were used for soluble S analysis. A microwave enhanced acid digestion was adapted (described in Appendix B) from USEPA 3050B and USEPS 3051A for sample pre-treatment. The reduced sulfur compounds in the sludge and filtrate samples were oxidized by concentrated nitric acid (68 ~ 70%) at a temperature between 150 ~ 250 °C and a high pressure (highest to 8000 kPa) in a Parr® microwave bomb (Figure 3-9). The digested solution was diluted with deionized water and measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Figure 3-10).

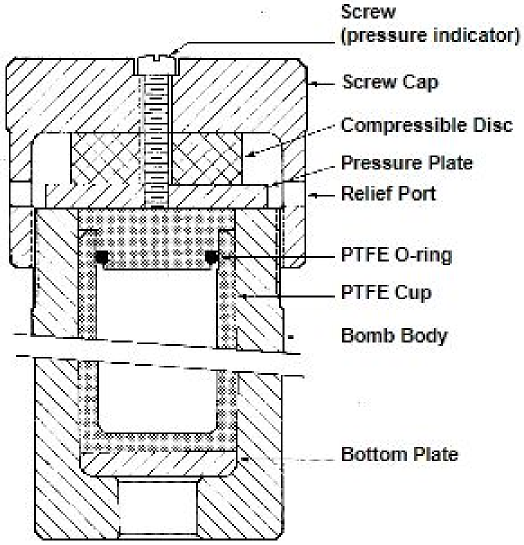

Figure 3-9 Microwave bomb used for the microwave enhanced acid digestion (Parr® microwave bomb operation instruction)

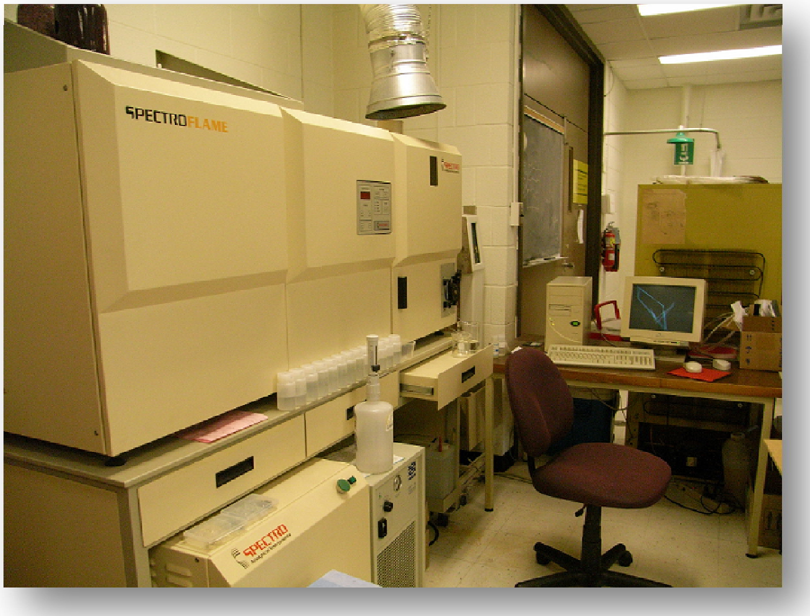

Figure 3-10 ICP-AES used for sulfur species analysis

3. Precipitated S Concentrations

The residue after drying and combustion of the sludge was converted to powder and used for precipitated S analysis. The microwave enhanced acid digestion process was employed but the reagents consisted of a mixture of concentrated nitric acid (68 ~ 70%) and concentrated hydrochloric acid (36.5 ~ 38%) at a ratio of 1:5 (v/v) (Appendix B). ICP-AES was employed to analyze the diluted digested solutions.

3.2.3.3 Quantification of Sulfur Fractions

Preliminary testing revealed that the methods developed in this study were able to reliably measure total sulfur (S_{total}), sulfur in liquid (S_{liquid}), and precipitated sulfur (S_{preci}, $\approx S_{I,solid}$) in sludges (data shown in Appendix B). The soluble inorganic sulfur ($S_{I,liquid}$) in the sludge was assumed to be mainly sulfate, which was analyzed by ion chromatography in this study. Direct measurement of the organic sulfur species was a challenge when characterizing the sulfur contents in the sludge. However, this component could be estimated as the difference between the total and inorganic sulfur. The relationships can be described by equations 3-5 to 3-7. Using these relationships the quantities of sulfur in the solid phase, the organic sulfur in the liquid phase, and the organic sulfur in the solid phase were calculated.

$$s_{total} = S_{liquid} + S_{solid} \tag{3-5}$$

$$S_{org,liquid} = S_{liquid} - S_{I,liquid} \tag{3-6}$$

$$S_{org,solid} = S_{solid} - S_{I,solid} \tag{3-7}$$

Chapter 4

Phase-Partitioning of VOSC in Sludge

In this study it was not possible to measure the concentrations of the VOSCs in the liquid or solid phases. Hence, these substances were initially measured in the gas phase and the concentrations in the other phases were then estimated on the basis of inter-phase partitioning coefficients. The research described in this chapter aimed to determine the physico-chemical properties of the VOSCs as pertinent to their partitioning in sludge. The liquid-gas partitioning coefficients of MM, DMS, and DMDS were determined. The effects of temperature and the sludge concentration on partitioning were assessed.

4.1 Introduction

In this study, VOSCs were studied as important intermediates/end products of the anaerobic degradation of sulfur containing compounds. A GC-PFPD method (described in Chapter 3) was developed for the VOSCs in the gas phase. Ideally, VOSCs in the liquid phase would also have been quantified directly. However, direct analysis of VOSCs in the liquid phase was found to be challenging due to the low concentrations of VOSCs under consideration and the presence of various interfering compounds in the complex sludge matrix. Reliable analytical methods for measurement of the VOSCs that were present in the sludge were not available. Therefore, the concentrations of VOSCs in the sludge were indirectly determined by measuring their concentrations in the gas phase and then calculating these concentrations on the basis of liquid-gas partitioning coefficients (i.e. Henry's Law).

The Henry's law coefficient is dependent on the characteristics of both solvent and solute and can be affected by temperature, pH value, ionic concentration, and so on. A number of studies describing partitioning of VOSC in fresh and ocean water and its dependence on various factors (mainly temperature and ionic strength) have been reported. However, the partitioning characteristics of VOSC in sludge have not been reported. As compared to fresh water or ocean water systems, the sludge matrix is much more complicated because of the higher solids concentrations and the high concentration of soluble organic compounds.

Liquid-gas partitioning over the range of conditions that are observed in sludge digestion systems were examined. Anaerobic sludge digestion can be conducted under cryophilic (10 – 15 ºC), mesophilic (30 – 40ºC), and thermophilic (50 − 70ºC) temperatures. Temperature is known to significantly impact liquid-gas partitioning (Przyjazny et al., 1983, Iliuta and Larachi, 2007) and hence this range of temperatures was explored.

The liquid-gas partitioning of compounds that dissociate in water may be affected by the pH of the solution. Of the VOSCs selected in this study, MM is a polar compound and has a dissociation coefficient (K_a) of 10^{-10} M. The partitioning coefficient of MM would increase with an increase in pH. However, the variation of the partitioning coefficient of MM was found only significant when pH value was higher than 9.5 (De Bruyn et al., 1995). Anaerobically digested sludge normally has a pH value between 7 and 8.5. In the sludge with such a pH range, the dissociation of MM is assumed to have little effect on its partitioning coefficient. The other VOSCs selected in this study are non-polar compounds and hence their dissociation in water is negligible.

Other factors related to the matrix and texture of the sludge may also affect the Henry's law coefficients. A typical digested sludge in this study had a total solids concentration of 2 – 2.5%. The suspended organic solids will provide adsorbent particles and collection surface for collecting VOSCs as adsorbates. In a closed static partitioning system with gas, liquid, and solid phases, adsorption of VOSC on the solids will reduce VOSC concentrations in the gas phase as compared to the system with the same total VOSC quantity but which only has gas and liquid phases. Digested sludge also contains dissolved inorganic salts and organic compounds. Elevated concentrations of dissolved salts will lead to a higher ionic strength in the sludge. When more water molecules are associated with salt ions, the number of water molecules available to interact with VOSC will be reduced and hence decrease the solubility of VOSC. This process is called salting out (Iliuta and Larachi, 2005 and 2007). In addition, soluble organic matter such as short chain fatty acids and alcohols may mix with VOSC and form azeotropes, which can affect the volatility and partitioning of VOSC (Denyer et al., 1949).

In summary, various factors could affect the partitioning of VOSCs in the sludge during anaerobic digestion. In this study, the combined effect of all the factors on the VOSC partitioning in the sludge was of interest. The partitioning of VOSCs in the water-gas system and sludge-gas system was studied. The dependence of the VOSC partitioning coefficients on the temperature was assessed. To eliminate the effect of biological reactions, the sludge used in the tests was deactivated. The static headspace method and the biomass deactivation approach which were introduced in Chapter 3 were employed in this part of the study. The partitioning coefficients were calculated by Equations 3-1 and 3-2.

4.2 Results and Discussion

4.2.1 Water-Gas Partitioning

The liquid-gas partitioning coefficients of MM, DMS, and DMDS were estimated in a water-gas system at temperatures of 12, 25, 35, and 58 °C. Because all the bottles were flushed with nitrogen before sealing, the headspace gas phase consisted of nitrogen. The liquid phase consisted of deionized water that was generated in the lab.

The experiment conducted to assess DMS partitioning will be described in detail as a representative of VOSC partitioning tests. Partitioning tests for MM and DMDS were conducted by following the same procedure and their coefficients were calculated in the same way. Partitioning of DMS was conducted in 500 ml serum bottles. A volume of 200 ml of DI water was added into each bottle and 359 ml headspace was left. A volume of 0.5 µl HPLC grade DMS liquid was injected into each bottle. The bottles were shaken and then placed into the isothermal chambers at different temperatures. At each temperature, 4 replicate bottles were prepared. After 90 minutes when the equilibrium between liquid and gas phases was reached, the gas sampled from the headspace of each bottle was analyzed for DMS concentration.

With the measured concentrations of DMS in the headspace at each temperature, partitioning coefficients were estimated by the calculations presented in table 4-1. The quantity of DMS in the headspace n_g could be estimated from its concentration (p) and the headspace volume (V_g): $n_g = C_g \times V_g = \frac{p}{RT} V_g$. The difference between the total dosed DMS quantity n and n_g was the DMS quantity in the liquid phase, n_l. The liquid-gas partitioning coefficient k could be calculated by Equation 3-1: $k = \frac{C_l}{C_g} = \frac{n_l/V_l}{n_g/V_g}$. Average values and standard deviations of the coefficient that were estimated from the replicates were calculated. The relative variations which represented the analytical error of the estimated partitioning coefficients were between 2 ~ 12%.

Table 4-1 Calculation sheet for the DMS partitioning coefficient

Sample replicate	T (°C)	Concentration p (ppm)	In gas n_g (10^{-7} mol)	In liquid n_l (10^{-6} mol)	k	AVE	std	RV (%)
12(1)	12	30.55	1.69	5.28	20.20			
12(2)	12	26.37	1.34	5.31	25.49			
12(3)	12	28.61	1.47	5.30	23.28			
12(4)	12	30.88	1.70	5.28	19.98	22.24	2.64	11.87
25(1)	25	53.72	2.48	5.20	13.52			
25(2)	25	56.37	2.97	5.15	11.17			
25(3)	25	53.28	2.81	5.17	11.85			
25(4)	25	55.18	2.91	5.16	11.42	11.99	1.06	8.83
35(1)	35	76.96	3.93	5.05	8.30			
35(2)	35	75.41	3.85	5.06	8.48			
35(3)	35	76.41	3.90	5.06	8.36			
35(4)	35	74.18	3.79	5.07	8.63	8.44	0.15	1.74
58(1)	58	165.42	7.86	4.66	3.83			
58(2)	58	163.77	7.78	4.67	3.87			
58(3)	58	183.44	8.71	4.58	3.39			
58(4)	58	176.38	8.38	4.61	3.55	3.66	0.23	6.30

n_g, the quantity of VOSC in the gas phase;

n_l, the quantity od VOSC in the liquid phase;

k, the VOSC liquid-gas partition coefficient;

AVE, average value of the partition coefficient replicates;

std, standard deviation of the partition coefficient replicates;

RV, relative variation $RV=\frac{std}{AVE}\times 100\%$.

Calculation sheets of MM and DMDS were prepared in the same format and are shown in Appendix C. The measured partitioning coefficients of MM, DMS, and DMDS at each temperature are shown in Figure 4-1. The error bars show the maximum and minimum values of the partitioning coefficients estimated at each temperature. A higher partitioning coefficient value represents a lower fraction partitioned to the gas at the specific temperature. From Figure 4-1, it can be seen that among the 3 VOSCs, DMDS had the highest partitioning coefficient while MM had the lowest one. With the increase of temperature, all the partitioning coefficients of the 3 VOSCs decreased. The partitioning coefficient of DMDS had the most significant variation with temperature while the partitioning coefficient of MM had the least significant variation with temperature.

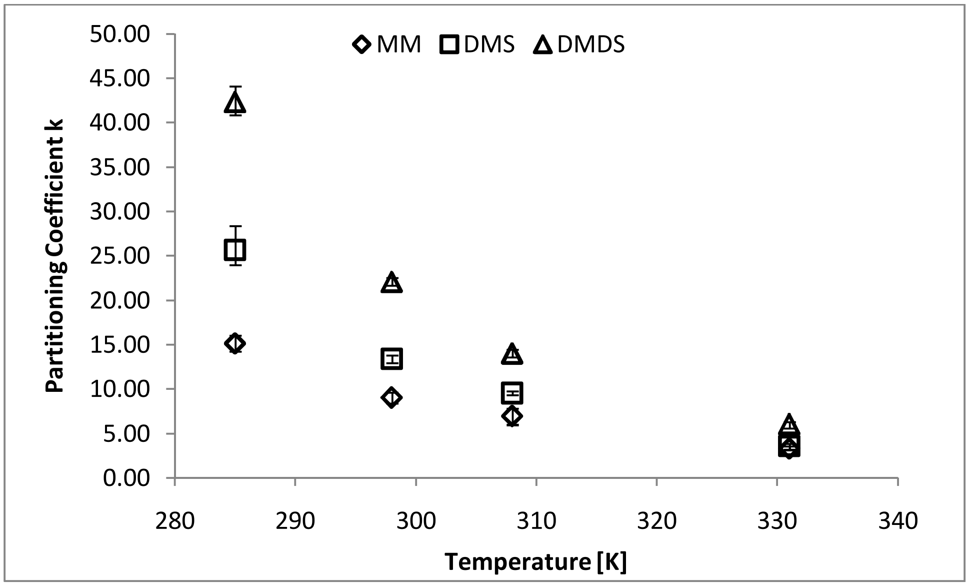

Figure 4-1 Partitioning coefficients of MM, DMS, and DMDS

Thermodynamically, it would be expected that there would be a linear relationship between log k and $\frac{1}{T}$ (Equation 4-1) (Przyjazny et al., 1983). The average values of the measured replicates at the four temperatures were used to fit the linear regression curve by means of the least-square method (Figure 4-2). The resulting regression parameters and the values of the coefficient of determination, r^2, for MM, DMS, and DMDS were summarized in Table 4-1. All the r^2 values for the regression lines were higher than 99%. With the regression parameters a and b, the partitioning coefficients can be calculated for any temperature within the range of 12 – 58 °C.

$$\boldsymbol{log k = a\frac{1}{T} + b} \qquad \textbf{(4-1)}$$

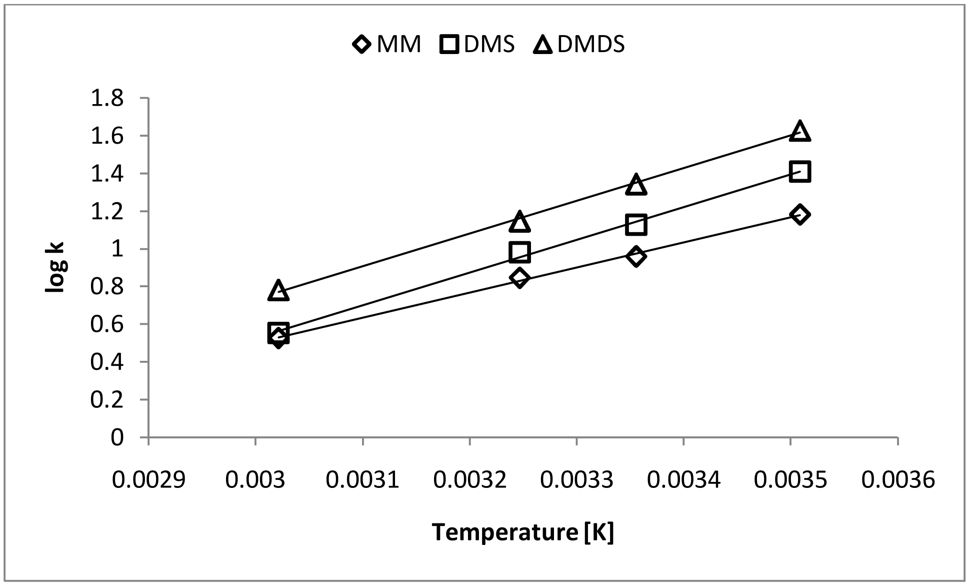

Figure 4-2 Temperature dependence of liquid-gas partitioning coefficients

Table 4-2 Regression parameters for VOSC partitioning in water

	Regression parameters		
Compound	a	b	r^2
MM	1216.5	-3.0989	0.9976
DMS	1594.4	-4.2557	0.9974
DMDS	1551.5	-3.8379	0.9968

The estimated values obtained from this study were compared to the values calculated by the empirical formula published in the study of Przyjazny et al (1983). The differences between the values measured in this study and the values calculated by empirical formulas were not significant for the selected compounds. With the estimated standard deviation, hypothesis tests relying on the student's distribution were conducted. The calculated t-test values were smaller than t value (df=3, P=0.99) in the student's distribution table, 5.841. Statistically, the measured values were same as the literature values. It suggested that the method utilized in this study was reliable.

Table 4-3 Liquid-gas partitioning coefficient in water-gas system

Temperature (K)	Number of samples	k for MM				
		Mean value	RV % [a]	Literature value [b]	Difference % [c]	t-test values [d]
285	4	15.63	14.47	15.48	0.9	0.129
298	4	8.89	5.06	9.63	7.7	3.308
308	4	7.07	9.43	6.87	2.9	0.600
331	4	3.87	4.49	3.41	13.5	5.295
Temperature (K)	Number of samples	k for DMS				
		Mean value	RV % [a]	Literature value [b]	Difference % [c]	t-test values
285	4	22.24	11.87	24.60	9.6	1.790
298	4	11.99	8.83	13.81	13.2	3.434
308	4	8.44	3.74	9.16	7.8	4.530
331	4	3.66	6.30	3.91	6.4	2.183
Temperature (K)	Number of samples	k for DMDS				
		Mean value	RV % [a]	Literature value [b]	Difference % [c]	t-test values
285	4	43.51	10.74	40.12	8.4	1.451
298	4	21.82	5.43	22.37	2.5	0.928
308	4	14.91	10.95	14.76	1.0	0.184
331	4	7.64	7.84	6.24	22.4	4.675

[a] RV was the relative variation, the ratio of the standard deviation to the mean value;

[b] The literature values were calculated by using the empirical formulas reported by Przyjazny et al. (1983);

[c] The difference was calculated as $\frac{|experiment\ value - calculated\ value| \times 100}{calculated\ value}$;

[d] t was calculated as $t = \frac{\bar{x} - \mu_0}{s/\sqrt{n}}$, degree of freedom is n-1.

4.2.2 Sludge-Gas Partitioning

The partitioning of VOSCs in a sludge-gas system was investigated to identify whether adsorption of VOSC was significant in the sludge and whether VOSC partitioning was different from the partitioning in the water-gas system. The partitioning coefficients of VOSC in a sludge-gas system which utilized a digested sludge with 4.4 g/l VSS were estimated at 13, 22, 35, and 57 °C. Then the partitioning tests were repeated with a sludge with 16.3 g/l VSS at 12, 25, 35, 58 °C. In both tests, 3 replicate bottles were prepared for VOSC partitioning at each temperature. The calculation of partitioning coefficients followed the same procedure exhibited in table 4-1 (calculations are shown in Appendix C).Within the utilized temperature range, the linear models for the relationships between log k and 1/T in the two sludge samples were regressed and compared with the linear model estimated in the water-gas system. At the end, the partitioning coefficients of VOSC at 35 and 55 °C in sludge samples with different VSS concentrations were estimated to verify values calculated by these regression models.

The sludge-gas partitioning tests were conducted with deactivated sludge. After being removed from the refrigerator, deactivated sludge was loaded into the serum bottles, flushed with the nitrogen gas, and placed into an isothermal chamber to warm the liquid to the set temperature. Before VOSC addition into the bottles, the headspace gas was sampled and analyzed for the background VOSC concentrations.

It was noticed that MM was generated from the deactivated sludge within 60 minutes of incubation when the temperature was above 35 °C. The released amount of MM gradually increased with the incubation time. The sludge deactivation procedure included heating and chemical addition. Cells of the microorganisms in the deactivated sludge might be more vulnerable to hydrolysis and other actions of chemical/biological agents. The biodegradable proteineous materials from the cells would decay and release MM. Due to this reason the measured quantities of MM in the gas phase could be higher than the dosed quantity of MM and hence the partitioning coefficients of MM obtained at temperatures higher than 35 °C were considered to be invalid. The average values of partitioning coefficients for MM in the sludge-gas systems are exhibited in Figure 4-3. The partitioning coefficients estimated in the water-gas system are also shown in Figure 4-3 for comparison. The error bars represent the maximum and minimum estimates for the partitioning coefficients.

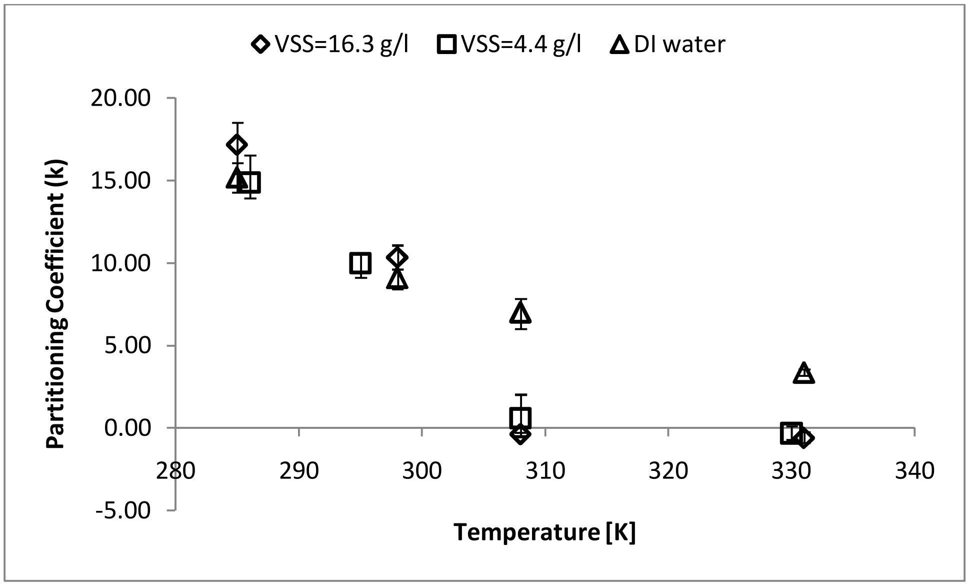

Figure 4-3 Partitioning coefficients of MM in the sludge-gas system

DMS and DMDS were not detected in the headspace before VOSC addition and concentrations of the dosed DMS and DMDS maintained a constant level in the headspace within 1.5 – 5 hours after their addition. The partitioning coefficients of DMS and DMDS in the sludge-gas systems (with VSS values of 16.3 and 4.4 g/l) were estimated within the temperature range of 12 – 58 °C. Figure 4-4 and 4-5, respectively, show the variation of the partitioning coefficients of DMS and DMDS (average and extreme values) with the temperature in sludge-gas systems. Partitioning coefficients estimated in the water-gas system are also shown in the two figures for comparison.

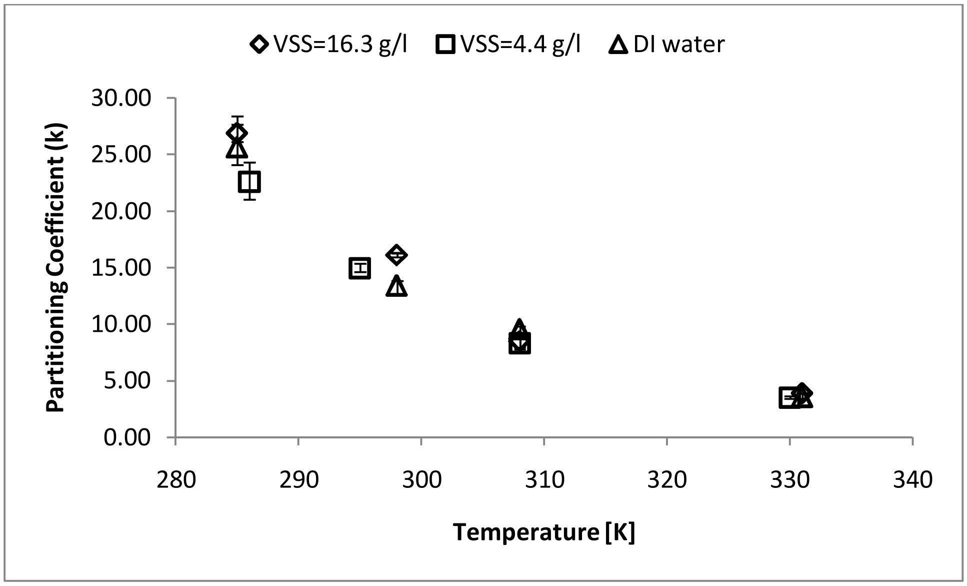

Figure 4-4 Partitioning coefficients of DMS in the sludge-gas system

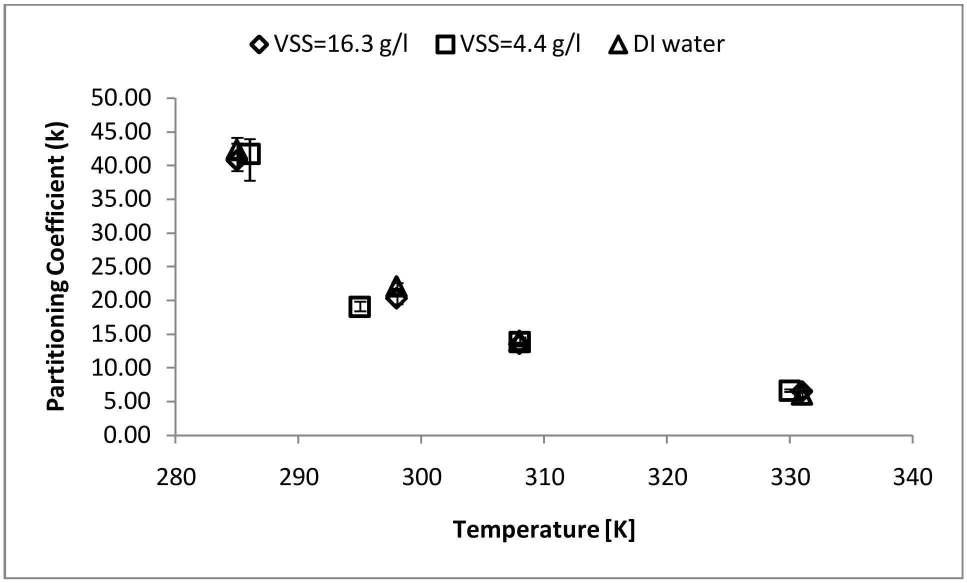

Figure 4-5 Partitioning coefficients of DMDS in the sludge-gas system

Similarly to the water-gas system, a plot of log k vs 1/T had a linear relationship for DMS and DMDS partitioning in the sludge-gas systems. The regression lines obtained from both sludge-gas systems and the water-gas system are shown in Figure 4-6 and 7. By visual comparison, the three regression lines for DMS partitioning in Figure 4-6 are close to each other. The same also applies to the regression lines for DMDS partitioning shown in Figure 4-7. The results suggest that partitioning of DMS and DMDS in sludge-gas systems is not different from their partitioning in water-gas system. Solids and soluble compounds in the sludge did not exhibit significant effect on VOSC partitioning in liquid-gas systems. The parameters and r^2 values estimated for the regression models which describe the variation of VOSC partitioning coefficients with temperature in sludge-gas systems are listed in table 4-4.

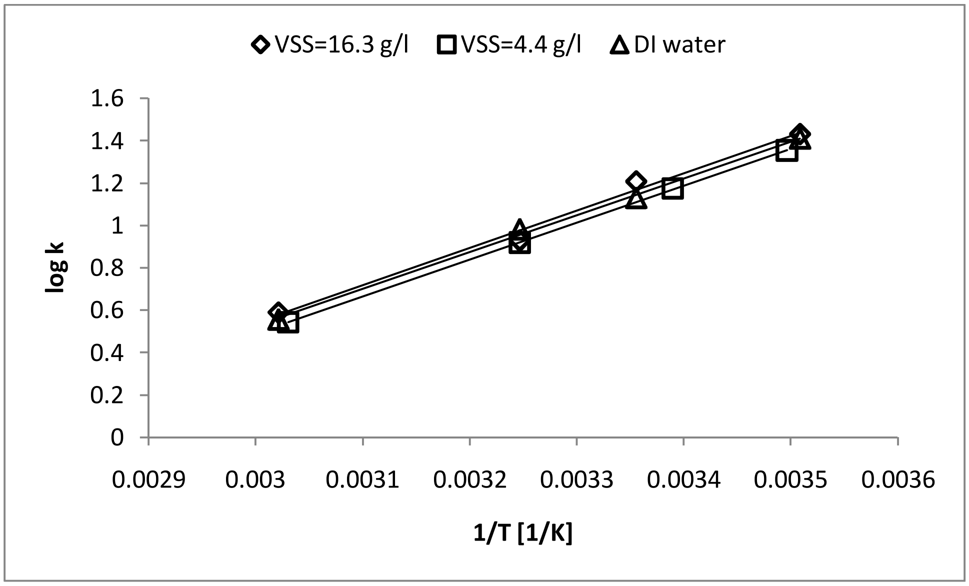

Figure 4-6 Temperature dependence of sludge-gas partitioning coefficients (DMS)

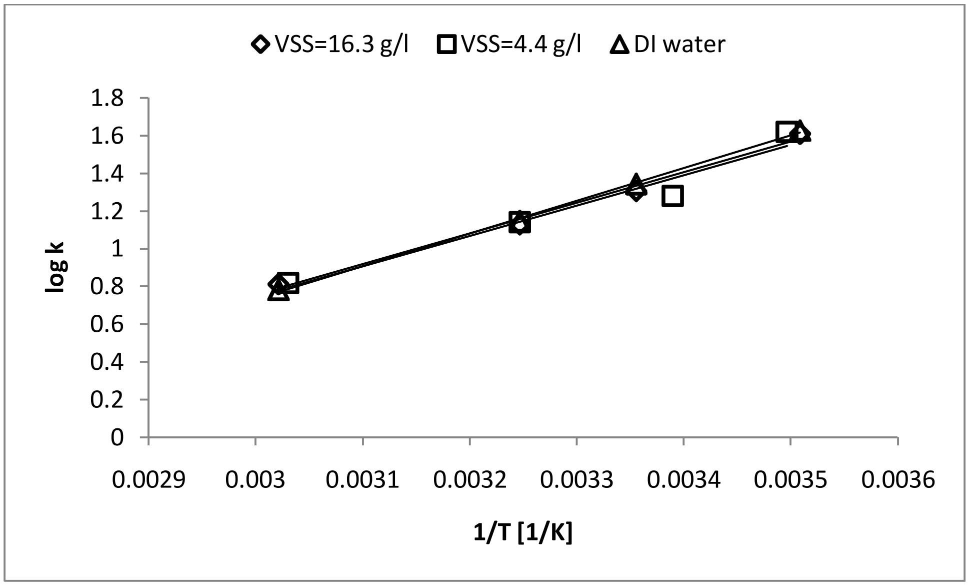

Figure 4-7 Temperature dependence of sludge-gas partitioning coefficients (DMDS)

Table 4-4 Regression parameters for DMS and DMDS portioning in the sludge

Compound	matrix	Regression parameters		
		a	b	r^2
DMS	sludge (VSS=16.3 mg/l)	1825.8	-4.9333	0.9936
	sludge (VSS=4.4 mg/l)	1582.1	-4.1574	0.9999
DMDS	sludge (VSS=16.3 mg/l)	1389.9	-3.3583	0.9054
	sludge (VSS=4.4 mg/l)	1481.7	-3.6319	0.9546

The target temperatures for the mesophilic and thermophilic digestion systems in this study were 35 and 55°C respectively. Hence the partitioning coefficients of DMS and DMDS in different liquid-gas systems at the two temperatures were calculated by the linear regression models. The calculated coefficient values are listed in table 4-5 and compared. No apparent dependence on the VSS concentration was observed. Statistical analysis could not be applied to the values calculated by linear models. Instead, average, standard deviation, and relative variation of these model-calculated-coefficients for different systems were calculated. Taking DMDS partitioning at 35 °C as an example, the relative variation of the calculated coefficients from their average was only 5.18%, smaller than the analytical error in this study. Similarly, small relative variations were also found for DMS partitioning coefficients at 55 °C and DMDS partitioning coefficients at 35 and 55 °C in the three liquid-gas systems. The results suggest that the partitioning coefficients of DMS and DMDS in the three liquid-gas systems would be same. To confirm this conclusion, further tests at the two temperatures in sludge-gas systems were conducted.

Table 4-5 Estimated DMS and DMDS partitioning coefficients by the regression equations

	Calculated k from regression models			
	DMS		DMDS	
System	35°C	55°C	35°C	55°C
DI water	8.34	4.03	15.83	7.80
VSS=16.3 g/l	9.88	4.30	14.27	7.57
VSS=4.4 g/l	9.53	4.64	15.09	7.68
AVE	9.25	4.32	15.06	7.68
std	0.81	0.31	0.78	0.12
RV (%)	8.73	7.07	5.18	1.52

To verify the conclusion that VOSC partitioning in the sludge-gas system was not different from the partitioning in the water-gas system, DMS and DMDS partitioning in sludge samples with a range of VSS concentrations were conducted at 35 and 55 °C. The estimated coefficient values are listed in table 4-6. At each temperature, partitioning coefficients for each VOSC did not show apparent dependence on the VSS concentration. The relative variations for VOSC partitioning coefficients estimated in all the sludge samples were less than 15%.

Table 4-6 Partitioning coefficients in sludge with different VSS

VSS (g/l)	k of DMS	VSS (g/l)	k of DMS	VSS (g/l)	Estimated k of DMDS	
	35 °C		55 °C		35 °C	55 °C
2.21	8.71	1.17	4.13	2.57	13.67	6.64
2.57	10.25	2.33	4.23	5.14	14.22	6.43
4.43	8.56	4.67	4.18	7.7	14.87	7.47
5.14	10.51	7.00	4.52	10.27	14.67	7.11
7.7	9.67	9.33	4.54	Ave ($\bar{x}$)	14.36	6.91
8.85	9.57			std	0.53	0.47
10.27	9.41			RV (%)	3.71	6.75
11.06	13.21					
Ave ($\bar{x}$)	9.99		4.32			
std	1.46		0.20			
RV (%)	14.66		4.57			

It was assumed that the average value ($\bar{x}$) could represent the partitioning coefficient at a specific temperature in the sludge with a VSS concentration between 2 - 12 g/l. Hypothesis tests relying on student's distribution were conducted between the average measured coefficient ($\bar{x}$) (in table 4-6) and the value calculated from the regression models (μ_0) (in table 4-5). The calculated t-test results are shown in table 4-7 and compared with the corresponding t values obtained from the t distribution chart. The t-test results (in table 4-7) for the comparison between the measured and calculated coefficients were less than the t values (t-tests for DMS: t (df=7, P=0.99)=3.499, t (df=4, P=0.99)=4.604 and t-tests for DMDS: t (df=3, P=0.99)=5.841). The results suggest that the measured partitioning coefficients in sludge with VSS concentrations between 2 - 12 g/l were not statistically different from the coefficients calculated by the regression models. The models were built from three liquid-gas systems: one system with 0 VSS, one system with VSS falling into the range of 2 ~ 12 g/l, and one system with VSS higher than 12 g/l. Hence, it was concluded that the complex background of the digested sludge did not have significant effect on VOSC partitioning.

Table 4-7 t-test values for the partitioning coefficients

	$t=\frac{\bar{x}-\mu_0}{s/\sqrt{n}}$			
	DMS (n=8)	DMS (n=5)	DMDS (n=4)	
Source of μ_0	35°C	55°C	35°C	55°C
DI water	3.180	3.294	5.521	3.810
Sludge with VSS=16.3 g/l	0.204	0.234	0.324	2.810
Sludge with VSS=4.4 g/l	0.880	3.619	2.751	3.281

4.3 Conclusion

The partitioning coefficients of MM, DMS, and DMDS were determined by the static headspace method in deionized water and deactivated digested sludge samples over the temperature range of 12 – 58 °C. With the increase of the temperature, the partitioning coefficients decreased. The measured partitioning coefficients in the water-gas system were similar to literature values.

The relationships between $logk$ and $\frac{1}{T}$ were found to be linear for VOSC partitioning in both water and sludge. Using the regression parameters of MM, DMS, and DMDS (table 4-2 and 4-5), their partitioning coefficients in the liquid-gas system (water and digested sludge) within the temperature range of 12 – 58 °C could be calculated.

The partitioning coefficients of MM could not be measured in the sludge matrix when the temperature was higher than 35 °C. The measurement was interfered by the MM release from the decay of proteineous materials in the sludge. At lower temperatures (12, 13, 22 and 25 °C), the partitioning coefficients of MM in sludge samples with VSS concentrations of 16.3 and 4.4 g/l were not significantly different from the corresponding partitioning coefficient of MM in the water.

DMS partitioning in the water and in the sludge with VSS concentrations of 16.3 and 4.4 g/l exhibited similar patterns within the temperature range of 12 -58 °C. In the tests conducted at 35 and 55 °C, the partitioning coefficients of DMS measured in the sludge with different solids concentrations had a relative variation 15% and 7% respectively. The measured values were not statistically different from the values calculated from regression models of water-gas and sludge-gas systems. The same conclusions were arrived for the partitioning of DMDS.

The VOSC partitioning coefficients did not show apparent dependence on the sludge concentration. The results suggest that VOSC liquid-solid partitioning coefficients were negligible in the digested sludge. The partitioning of VOSC in the gas and liquid phases would be the only physic-chemical process considered for VOSC quantification.

Chapter 5

Generation and Degradation of VOSCs

This chapter addresses the establishment of a model to describe the generation and degradation of VOSCs during the anaerobic digestion of the amino acid, methionine. The corresponding kinetic parameters under two different temperatures were estimated. The effect of an external carbon source on the VOSC degradation was assessed.

5.1 Introduction

In anaerobic digestion, the organic sulfur compounds that are present in the sludge undergo a two-step microbiological transformation. The first step involves fermentation processes and produces H_2S and volatile organic sulfur compounds (VOSCs) (Drotar et al., 1987) while the second step involves further degradation of the VOSCs to H_2S. Methanogens are believed to be the dominant organisms which accomplish the second step (Chen et al., 2005, Leerdam et al., 2006). Simultaneous with these microbiological processes sulfides and VOSCs can volatilize into the biogas and react chemically with heavy metals.

There is a lack of information on the impact of digester configuration, temperature, and retention time, and sludge type on VOSC emissions during anaerobic digestion. This is because of the limited information on the extent and rates of the sulfur conversion processes in anaerobic digestion and the relationship between the feed sludge sulfur content and VSC generation.

Batch tests with addition of single chemicals (an amino acid and selected VOSCs) were conducted to provide dynamic data to establish a model describing VOSC generation and degradation from incubation of simple substrates. The dominant VOSCs detected in digestion emissions, including MM, DMS, and DMDS (Anderson et al, 2004, Murthy et al, 2003, Wu, 2004) were individually employed in anaerobic incubations to estimate their degradation kinetics. Incubations of the VOSCs with acetate addition were conducted to assess the effect of an external carbon source on the VOSC degradation. The amino acid, methionine, was employed in anaerobic incubations with and without methanogen inhibition to characterize VOSC formation in digestion. Incubations with methanogen inhibition were utilized to estimate VOSC generation kinetics. A model of VOSC generation and degradation from anaerobic digestion of methionine was established and the data from the incubations without methanogen inhibition were used to assess the model validity. All the experiments were conducted at two temperatures, 35 and 55 °C to assess the effect of temperature on the kinetics.

The incubation strategy and the experimental materials which were utilized in this part of the research have been described in Chapter 3. The results of VOSC dosed and methionine-dosed incubations will be discussed in sequence.

5.2 Results and Discussion

5.2.1 VOSC Degradation

Batch tests that were dosed with individual VOSCs were conducted to facilitate estimation of degradation kinetic rate constants. Different initial dosages were applied to assess the effect of the concentration of VOSCs on their degradation. The total mass of a VOSC that was present in a serum bottle at given time was calculated using the measured gas phase concentration and the associated liquid phase concentration which was estimated assuming equilibrium partitioning between the liquid and gas phases. Based on Przyjazny et al.'s study (1983), the equilibrium state of VOSC partitioning was reached 90 minutes after injection. In the present study, time for equilibrium was shorter but still needed 45 minutes. Therefore, the same dosage of VOSC was injected to serum bottles which were filled with 200 ml DI water (same volume as the sludge). The total sulfur at time 0 was plotted in the VOSC decline figures was calculated according to the measured VOSC concentration in the gas phase of serum bottles containing DI-water. Other points of sulfur concentrations were based on the measured and calculated results from the serum bottles which contained sludge. A few points whose monitored time was less than 90 minutes might not be measured under equilibrium states.

Acetate addition was found to have no effect on the rate of VOSC degradation and hence only the responses of MM, DMS, and DMDS without addition of acetate are presented in this chapter (responses in the incubation with acetate addition are in Appendix D). Figure 5-1 presents the sums of the normalized sulfur masses (mg S/g VSS) of the individual VOSCs that were present in the bottles versus incubation time at 35 and 55 °C, respectively. The decline of the total quantities of VOSCs was attributed to biodegradation.

At 35 °C, MM and DMDS appeared to decline at a similar rate while degradation of DMS declined at a considerably slower rate. This result was determined by the time required for the degradation of VOSCs with similar initial concentrations to the level of 5 % of the original concentration (95% degradation) at 35 °C. For instance, for MM degradation with an initial concentration of 3.11 mg S/g

VSS the 95% degradation took about 55 hours (Figure 5-1a) while for DMDS degradation with an initial concentration of 2.94 mg S/g VSS the 95% degradation took about 50 hours (Figure 5-1g). For DMS degradation with an initial concentration of 2.92 mg S/g VSS, the 95% degradation however took about 120 hours (Figure 5-1d).

At 55 °C, degradation of DMDS appeared considerably faster than degradation of MM and DMS. Between the latter two compounds, the degradation of DMS was slower. This result was quantified by the time required for degradation of VOSCs with similar initial concentrations to 5 % of the original concentration at 55 °C. For instance, for DMDS degradation with an initial concentration of 1.27 mg S/g VSS the 95% degradation only took about 7.5 hours (Figure 5-1h). For MM degradation with an initial concentration of 1.84 mg S/g VSS, the 95% degradation took about 60 hours (Figure 5-1c). For DMS degradation with an initial concentration of 1.22 mg S/g VSS, the 95% degradation took about 175 hours (Figure 5-1f).

The decline patterns at 35 and 55 °C were used to estimate the effect of temperature on the VOSC degradation. Temperature was found to have different effects on the degradation of MM, DMS, and DMDS.

The rate of degradation of MM was slower at 55 °C as compared to 35 ^{o}C. At 35 °C, with an initial concentration of 1.30 mg S/g VSS, 90% of the dosed MM was degraded within 30 hours of incubation (Figure 5-1a) while at 55 °C, with initial concentrations of 0.92 and 1.84 mg S/g VSS, 90% of dosed MM was degraded within 50 and 60 hours of incubation (Figure 5-1c). In addition, the results indicate that thermophilic degradation of MM could be very slow or inhibited when the initial concentration was higher than 2 mg S/g VSS. This inhibition was not observed in mesophilic degradation. At 35 °C, with initial concentrations of 4.67 and 3.11 S/g VSS, MM degradation exhibited a similar pattern as that with an initial concentration of 1.30 mg S/g VSS (Figure 5-1a). However, at 55 °C when MM was dosed with initial concentrations of 8.53, 4.26, and 2.13 mg S/g VSS, the decline of its concentration was less than 5% after 240 hour incubation (Figure 5-1b). In summary, thermophilic temperatures resulted in a slower degradation rate for MM as compared to mesophilic temperature. Inhibited degradation was observed at 55 °C when the initial MM concentration was higher than 2 mg S/g VSS.

The rate of degradation of DMS was considerably slower at 55 °C as compared to 35 ^{o}C. At 35 °C, with initial DMS concentrations of 5.2 and 2.9 mg S/g VSS, 85-90% of the dosed DMS decayed within 120 hours of incubation (Figure 5-1d). At 55 °C, with initial concentrations of 2.13 and 1.22

mg S/g VSS (Figure 5-1f), only 55% of the dosed DMS was degraded after 120 hours of incubation, which was much less than the decline rate at 35 °C (85~90%). At the end of the 190 hours of thermophilic incubation, about 95% of the dosed DMS was degraded (Figure 5-1f). Inhibition of degradation was also observed for DMS at 55 °C when the initial concentration was higher than 3 mg S/g VSS. In the thermophilic incubation with initial dosages of 10.57, 6.60, and 3.96 mg S/g VSS, respectively, the concentration of DMS exhibited a staged and slow decline. The DMS degradation was extremely slow in the first 50 hours, with less than 10% decline in this period. In the next 25 hours, the concentrations of DMS dropped to 50% of the original dosage values. At the end of the incubation, 170 hours, less than 70% of the dosed DMS had decayed (Figure 5-1e). It would appear that when initial DMS dosages were high, thermophilic microorganisms needed a period of time to acclimate to the high concentrations.

The impact of temperature on degradation of DMDS was different from that of DMS and MM, in that the DMDS degradation rate dramatically increased at 55 °C. With an initial concentration of 1.27 mg S/g VSS, 95% degradation of the dosed DMDS took about 30 hours at 35 °C (Figure 5-1g) however, it took less than 10 hours at 55 °C to achieve this level of removal (Figure 5-1h). It will be discussed later in this section that MM was the intermediate product of DMDS degradation. The rapid decline of DMDS at 55 °C resulted in accumulation of MM. In all the VOSC dosed batch tests, the decline of DMDS was much more rapid than the decline of other VOSCs and the decline rate for DMDS was significantly affected by temperature. The results suggested that the conversion of DMDS to MM in anaerobic digestion could be abiotic.

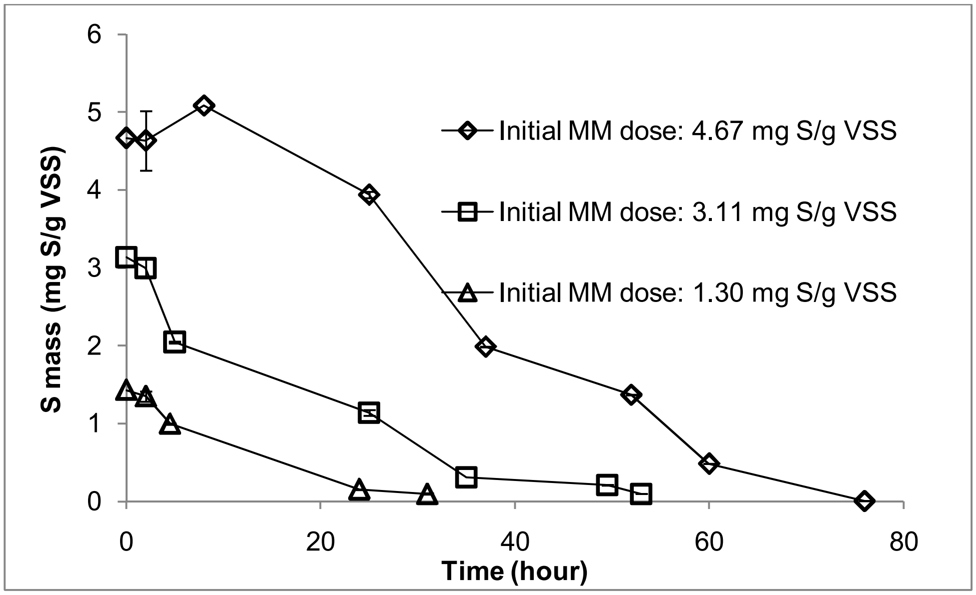

Figure 5-1a S mass decline (MM dosed, 35 °C)

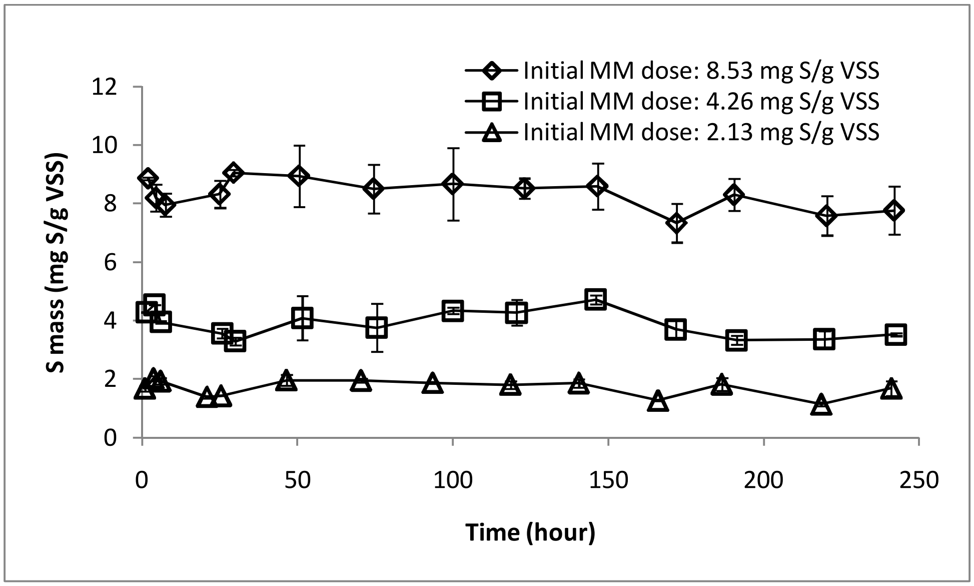

Figure 5-1b S mass decline (MM dosed > 2 mg S/g VSS, 55 °C)

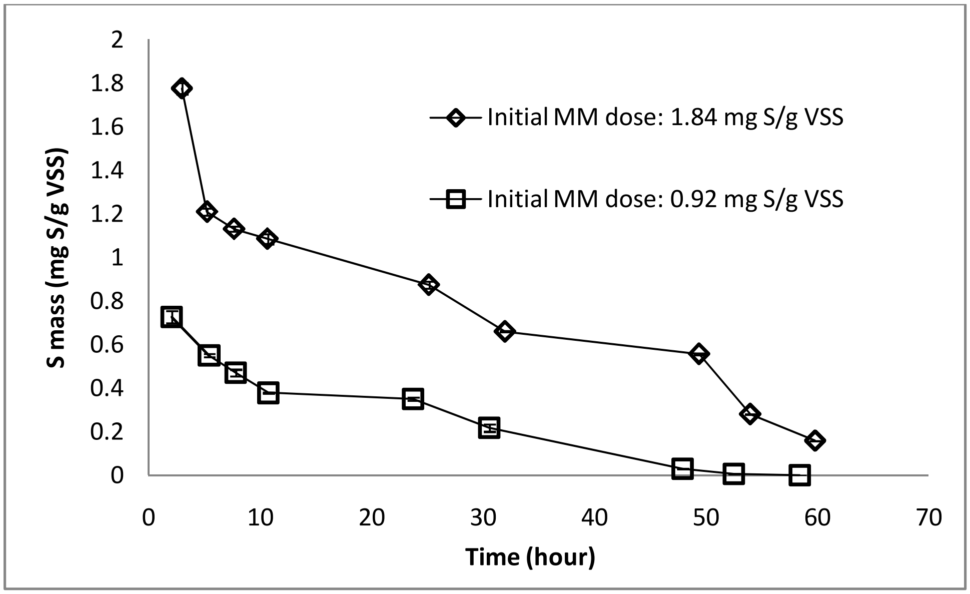

Figure 5-1c S mass decline (MM dosed < 2 mg S/g VSS, 55 °C)

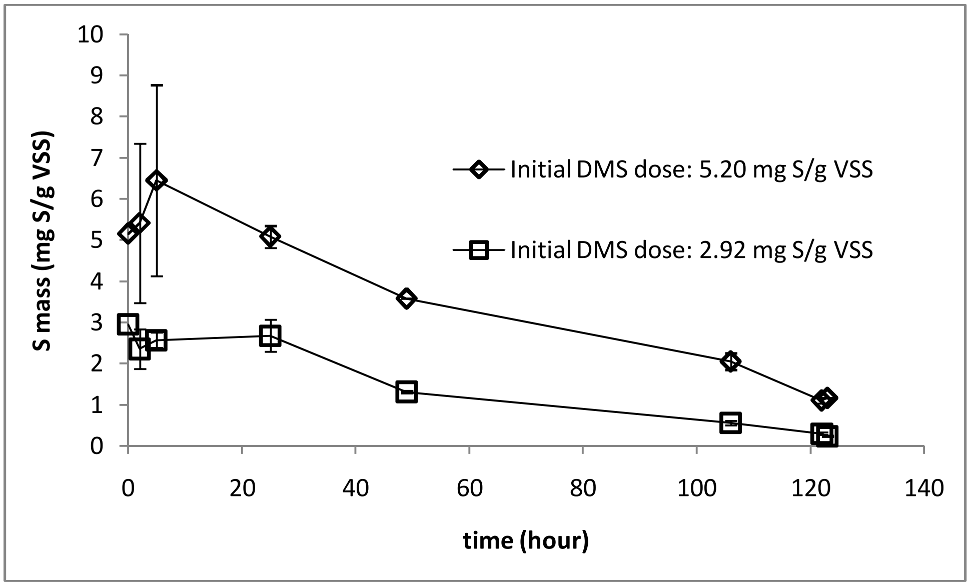

Figure 5-1d S mass decline (DMS dosed, 35 °C)

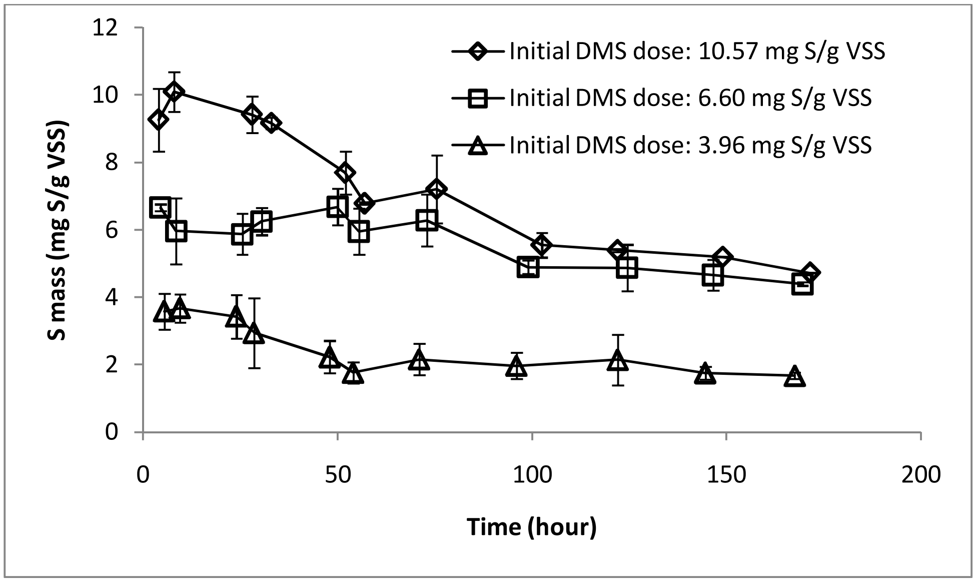

Figure 5-1e S mass decline (DMS dosed > 3 mg S/g VSS, 55 °C)

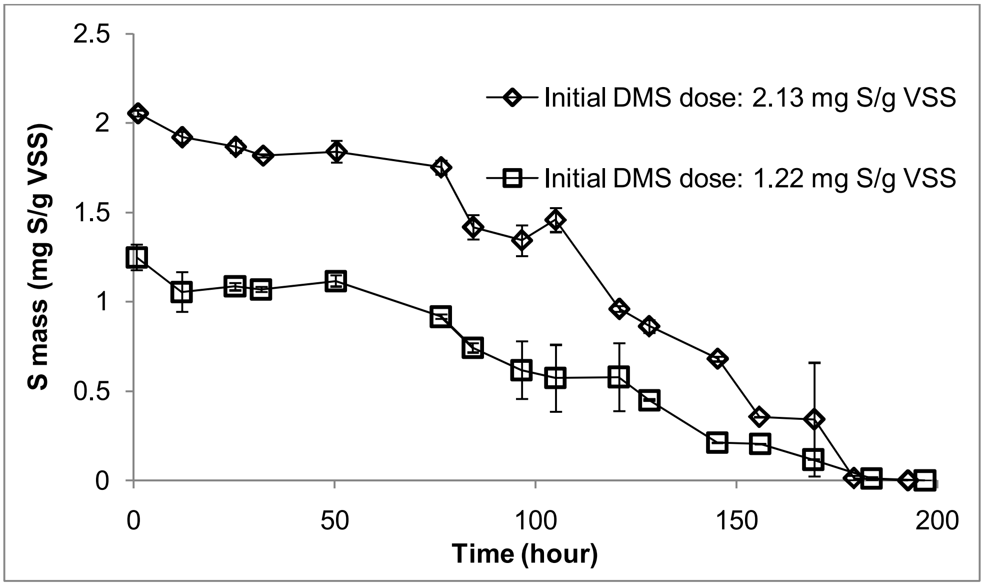

Figure 5-1f S mass decline (DMS dosed < 3 mg S/g VSS, 55 °C)

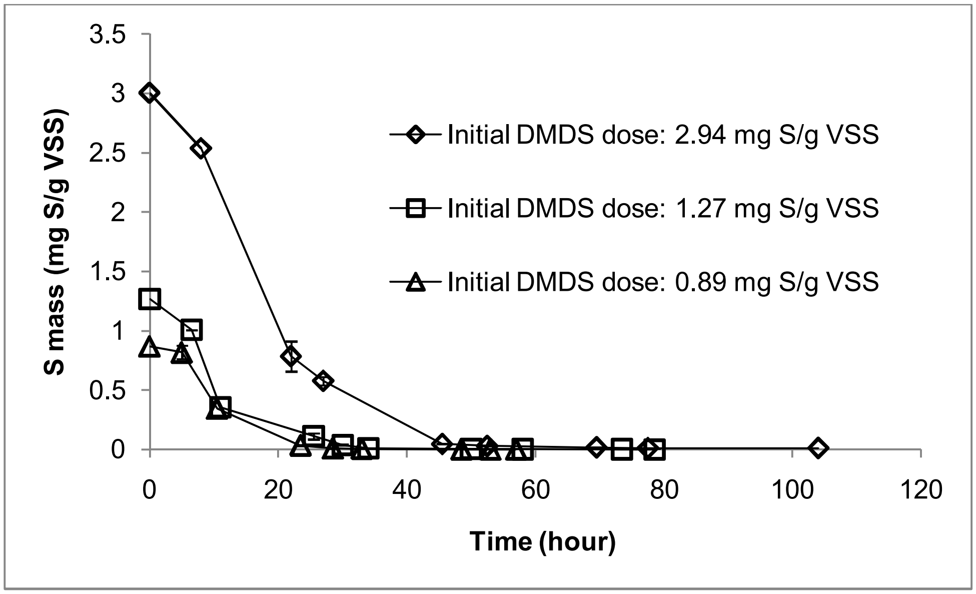

Figure 5-1g S mass decline (DMDS dosed, 35 °C)

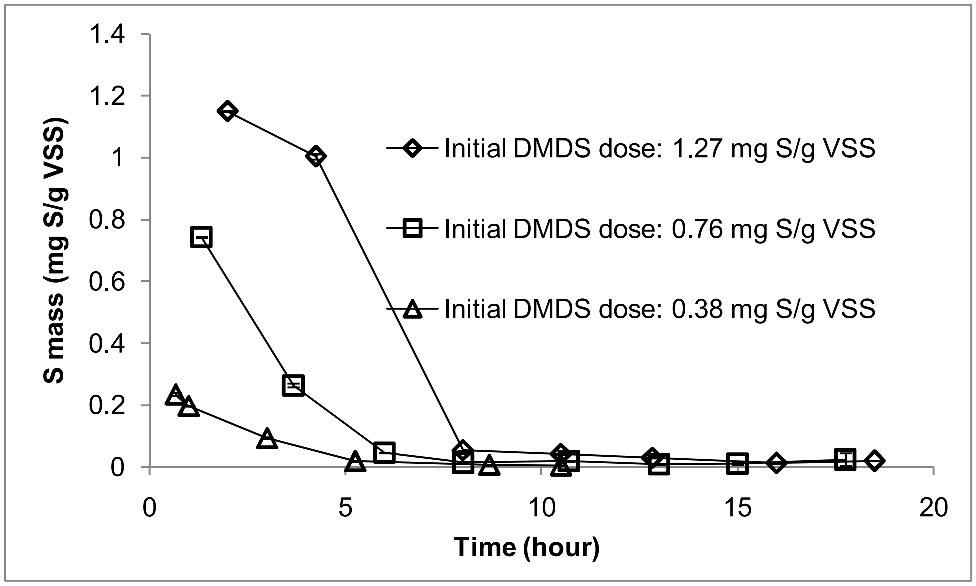

Figure 5-1h S mass decline (DMDS dosed, 55 °C)

With the degradation of MM in the dosed tests, there was a transient accumulation of DMS in the bottles. Figure 5-2a presents the DMS accumulation and degradation that was observed in the mesophilic MM degradation test with an initial dose of 3.11 mg S/g VSS and is representative of the responses observed for the other initial doses at 35 °C. From Figure 5-2a it can be seen that approximately 25 hours after the addition of MM, DMS was detected and its concentration slowly increased to a peak at around 55 hours, when MM was exhausted. The transient accumulated DMS gradually degraded below the detection limit within another 20 to 30 hours.

A similar phenomenon was observed in the thermophilic degradation of MM. Figure 5-2b shows DMS accumulation in the thermophilic MM degradation test with an initial dose of 0.92 mg S/g VSS and is also representative of the responses observed for other initial doses at 55 °C. After 5 hours of incubation DMS started to be detected and the DMS concentration slowly increased with the decrease of MM. In contrast to the mesophilic tests, the generated DMS had not started to decrease by 60 hours of incubation.

The results suggest methylation (the substitution of a methyl group for the hydrogen atom) occurred in anaerobic digestion. The continuous accumulation of DMS in the thermophilic incubation suggests a slower degradation rate of DMS at thermophilic temperatures as compared to the degradation rate at mesophilic temperatures which was consistent with the DMS dosed tests.

Methylation was not the only mechanism which resulted in the reduced concentrations of MM. In all the MM dosed tests at 35 °C, the peak concentrations of DMS were about 20% of the initial dosed MM concentrations. At 55 °C the DMS concentration slowly increased with the decrease of MM. When more than 90% of the dosed MM had degraded, the amount of the S associated with the generated DMS was less than 13% of the initial dosed S in the MM. Hence, generation of DMS could not represent the total amount of MM which declined. These results suggest that direct reduction of MM to generate H_2S was likely a competitive reaction of methylation.

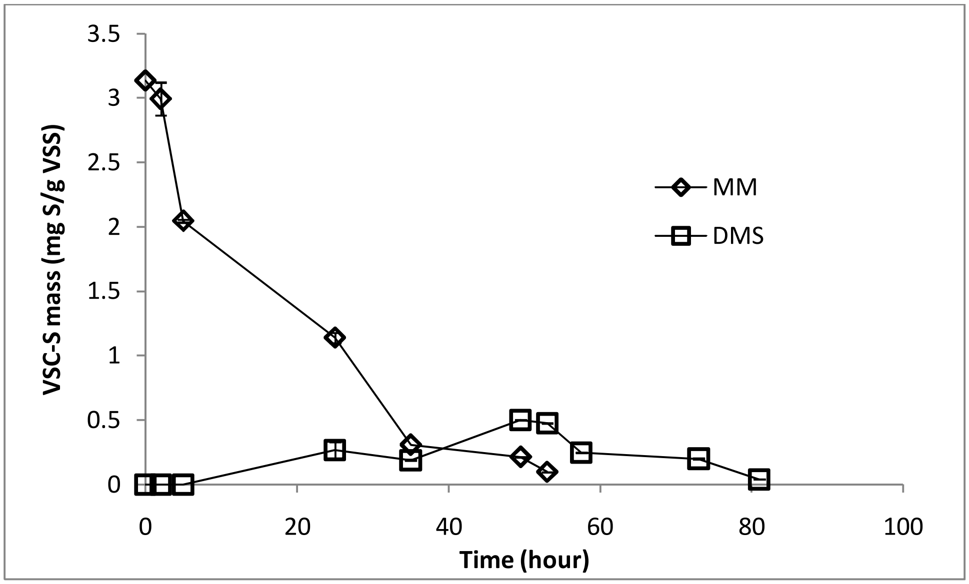

Figure 5-2a Methylation of MM to DMS (35 °C)

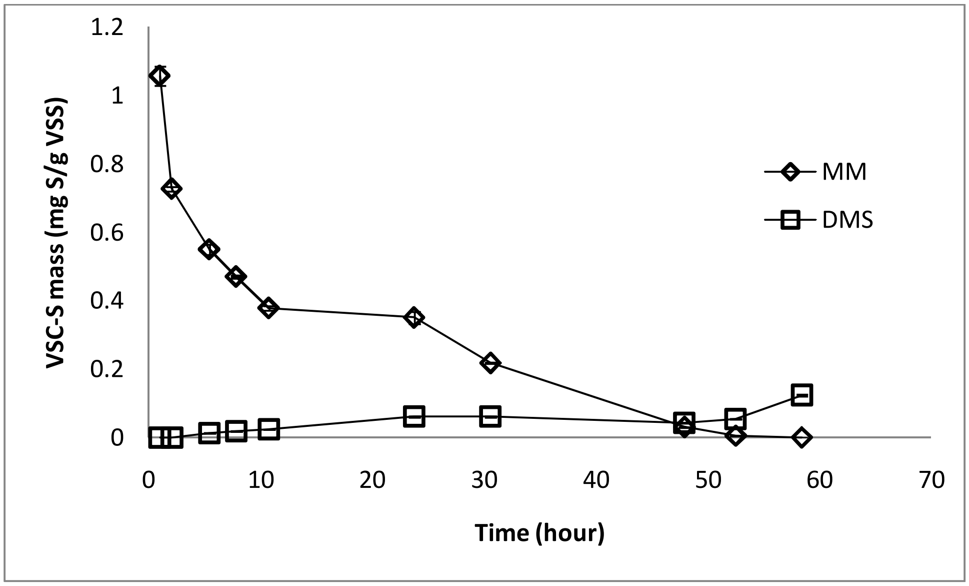

Figure 5-2b Methylation of MM to DMS (55 °C)

During the incubation of DMDS, a two-step degradation of DMDS with MM as an important intermediate product was observed. Figures 5-3a and b present the masses of MM and DMS that were observed during the degradation of DMDS at 35 °C and 55 °C respectively. From Figure 5-3a it can be seen that at 35 °C, with the initial DMDS dosage of 1.27 mg S/g VSS the mass of MM increased to a peak value within 24 hours and when the mass of the formed MM declined, DMS slightly accumulated. The degradation patterns shown in Figure 5-3a were representative of the responses observed when other DMDS dosages were employed at 35 °C.

From Figure 5-3b it can be seen that at 55 °C with the decrease in DMDS (initial dosage of 1.27 mg S/g VSS), MM was generated and accumulated. Similar degradation patterns were observed in the incubations with other initial DMDS dosages at 55 °C. Thermophilic digestion was different from mesophilic digestion in that the generated MM did not significantly decay and there was no DMS detected. These results were consistent with the results previously observed (i.e. slow degradation and methylation rates of MM under thermophilic conditions).

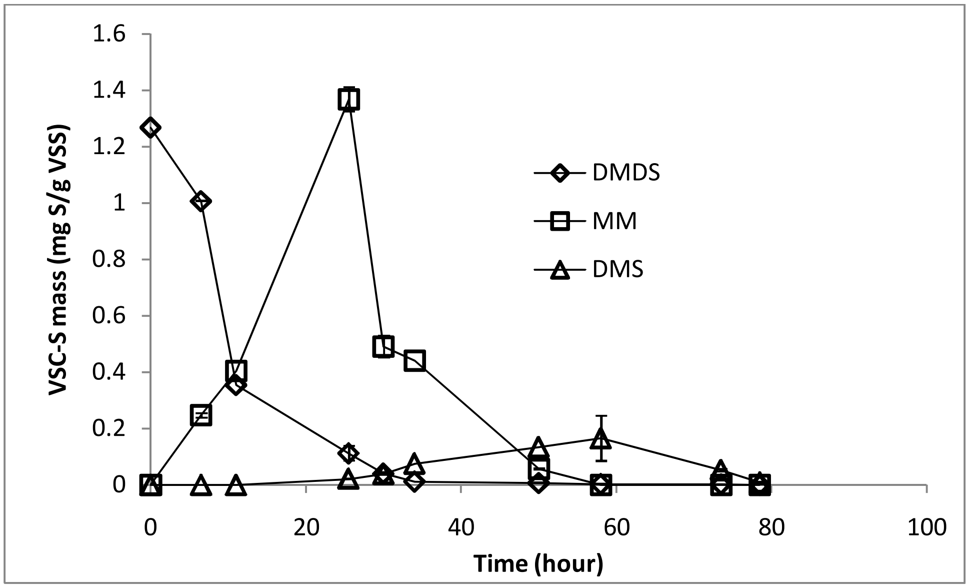

Figure 5-3a Two-step degradation of DMDS (35 °C)

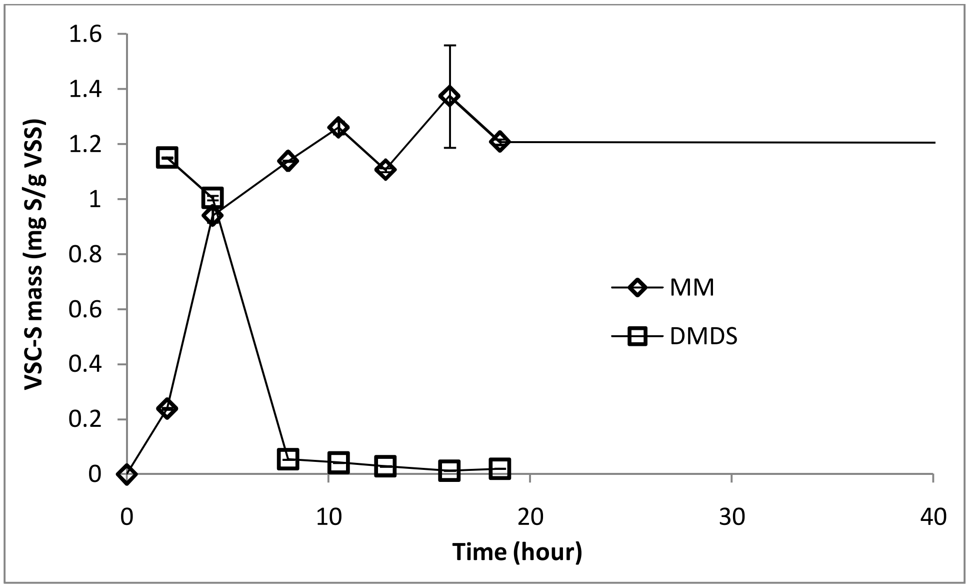

Figure 5-3b Two-step degradation of DMDS (55 °C)

It can be summarized that the two-step degradation of DMDS includes rapid DMDS decay to form MM, and subsequent MM methylation and degradation as observed in the MM degradation test. However, at thermophilic temperatures, the degradation of MM is slower than that at mesophilic temperatures. The transient accumulation of DMS as an intermediate product of decay of MM and DMDS at mesophilic temperature confirmed the earlier observations with respect to their different rates of decay. DMS accumulated since it decays at a slower rate than either MM or DMDS which appear to be parent compounds of DMS when they decay.

The ability of zero, first order, mixed-second order, and Monod kinetics to describe the relationships between the loss of mass in the serum bottles by decay and their respective concentrations was investigated. In this approach, the loss of target compounds from the headspace due to out-gassing was accounted for and decay was assumed to only occur in the liquid phase contents. It was assumed that mass transfer between the gas and liquid phases did not limit the rate of decay of the target compounds. Regression was conducted with the software AQUASIM (Reichert, 1995, Burn and Reichert, 2001).

Mixed-second order kinetics were chosen to describe the degradation of VOSC because their simulation exhibited the best agreement with the measured values at both temperatures based on having the lowest sum of squares between observed and predicted values. Mixed-second order kinetics were used to describe the relationship between the reaction rate and the concentration of the individual VOSCs as per Equation 5-1:

$$\boldsymbol{r = -K \cdot C_{liq} \cdot VSS} \tag{5-1}$$

Where r is the VOSC degradation rate ($mg \cdot l^{-1} \cdot h^{-1}$), C_{liq} is the concentration of VOSC in the sludge (liquid phase) (mg/l), K is the mixed-second order kinetic coefficient ($l \cdot g^{-1} \cdot h^{-1}$), VSS is the volatile suspended solids concentration (g/l).

Figure 5-4a presents the fit of the degradation of DMDS at 3 different initial dosages and is representative of the curve fitting for the degradation and conversion in the VOSC dosed incubations at mesophilic and thermophilic temperatures (Appendix D). The estimated values of the mixed-second order kinetic parameters are tabulated in table 5-1 for mesophilic and thermophilic VOSC degradation, respectively. The parameter values for each process estimated with different initial VOSC dosages were not statistically different (statistical tests are presented in Appendix D).

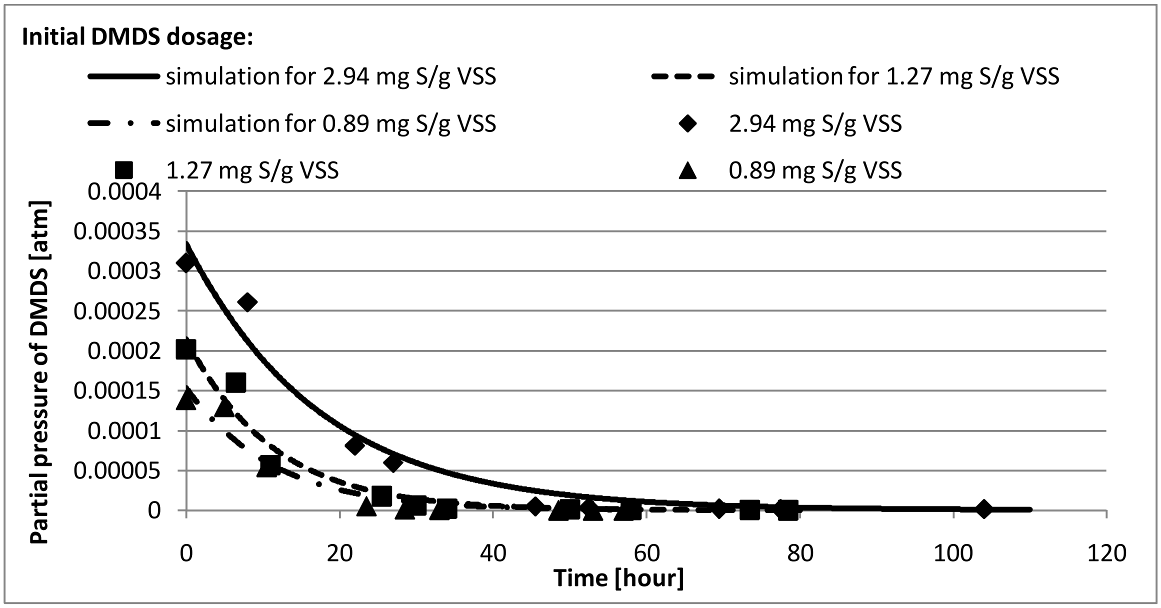

Figure 5-4a Measured partial pressure and fitted curves for DMDS (35 °C)

In the degradation of MM, decline of MM was accompanied with generation of DMS. MM methylation to DMS and MM degradation to H_2S were assumed to be two competitive reactions. Methylation of MM was also described by the mixed-second order kinetics. With the estimated DMS degradation rate constant K_DMS, the two unknown mixed-second order kinetic constants K_MM and K_{methy} for MM degradation to H_2S and MM methylation to DMS were estimated simultaneously by fitting MM decline and DMS accumulation. The fitting curves shown in Figure 5-4b was representative of the curve fitting for MM methylation and degradation with different initial MM doses at two temperatrues.

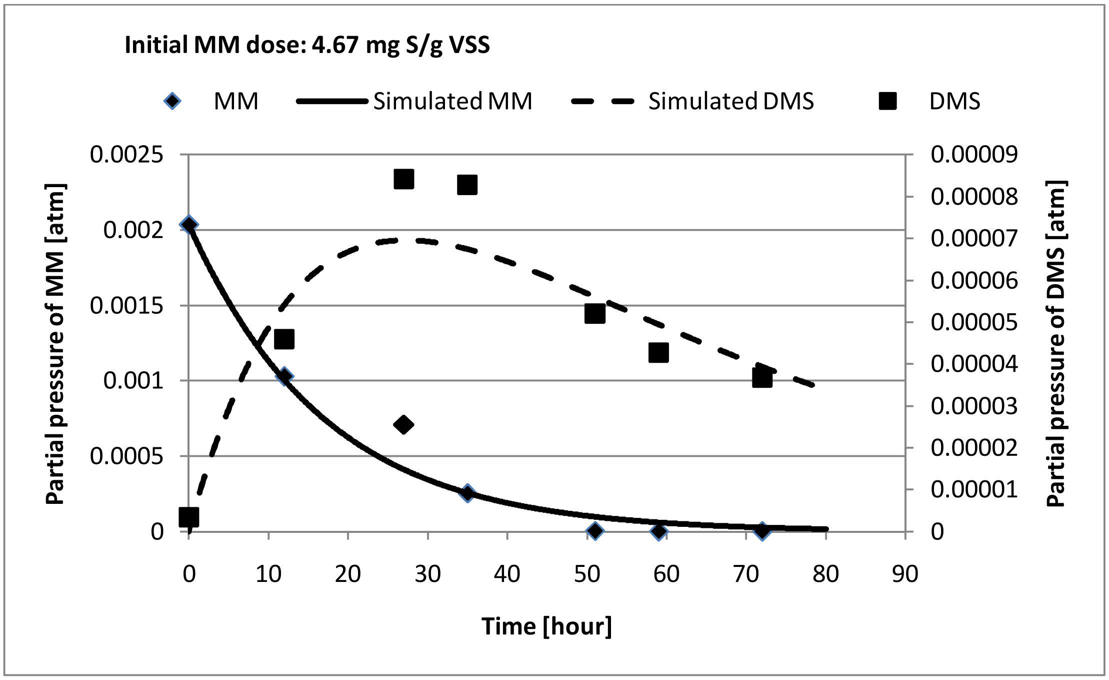

Figure 5-4b Measured partial pressure and fitted curves for MM (35 °C)

Table 5-1 Mixed-second order rate constants for mesophilic VOSC degradation (l·mg^{-1}·h^{-1})

	Mesophilic		Thermophilic	
Parameter	Mean value	Range (P=0.99)	Mean Value	Range (P=0.99)
k_DMS	0.013	0.010 ~ 0.016	0.005	0.0040 ~ 0.0057
k_MM	0.027	0.010 ~ 0.047	0.0083	0.0053 ~ 0.013
K_{methy}	0.0047	0.0038 ~ 0.0063	0.0012	0.0005 ~ 0.0041
k_DMDS	0.034	0.027 ~ 0.042	0.22	0.20 ~ 0.24

k_DMS, coefficient for degradation of DMS; k_MM, coefficient for degradation of MM to H_2S; k_{methy}, coefficient for methylation of MM; k_DMDS, coefficient for DMDS degrade to MM

Comparing the kinetic parameters estimated at 35 ºC and at 55 ºC, it can be seen that the DMDS rate constant was higher at 55 ºC while the MM and DMS rate constants were higher at 35 ºC. The values of the decay rate coefficients reflect the trends in disappearance that were previously discussed. The results suggest that thermophilic methanogens have reduced capacity for MM and

DMS degradation. In addition, the MM methylation rate constant was also greater at 35 °C as compared to its value at 55 °C. The average methylation rate constant at 35 °C was about 4 times of the constant at 55 °C. It supported the faster decline of MM at 35 °C.

The decay rate constant of DMDS was about 6.5 times higher at 55 °C as compared to 35 °C. However, the decay rate constants of MM and DMS, VOSCs which are usually more prevalent in digester emissions were about 3.4 times and 2.7 times higher at 35 °C as compared to 55 °C. The results suggest that with the same retention time, thermophilic anaerobic digesters may have more VOSC released in the biogas as compared to mesophilic digesters, which might cause more serious odor problems and require more advanced biogas cleaning technologies.

5.2.2 VOSC Generation from Methionine

Methionine was incubated with methanogen-inhibited inocula to investigate the formation of VOSCs from amino acids. In addition, the ammonia released from methionine was determined with and without methanogen inhibition to explore whether it could be used as a surrogate of VOSC release. There was no significant difference between the ammonia releases under methanogen-inhibited and -uninhibited conditions and hence only the ammonia releases measured with methanogen inhibition were presented.

From Figure 5-5, it can be seen that MM was the major sulfur product of methionine degradation. The MM generation from methionine at 35 °C was initially slow, however, after about 120 hours of incubation, the mass of MM increased rapidly. After 400 hours of incubation, the mass of MM accumulated to a level of 65 mg S/g VSS, which represented 92.5% of the sulfur available from the dosed methionine (original sulfur dose was 70.2 mg S/g VSS). The initial slow degradation phase may have been due the time required for acclimation of the microorganisms to the high concentration of methionine.

The MM generation from methionine at 55 °C also demonstrated a significant lag with rapid accumulation of MM starting after 120 hours of incubation (Figure 5-5). After 340 hours of incubation, the MM concentration approached a constant level that was maintained until the end of the batch test. The generated MM accumulated to a level of 45 mg S/g VSS, which represented essentially all the sulfur available from the dosed methionine (original sulfur dose was 45.9 mg S/g VSS).

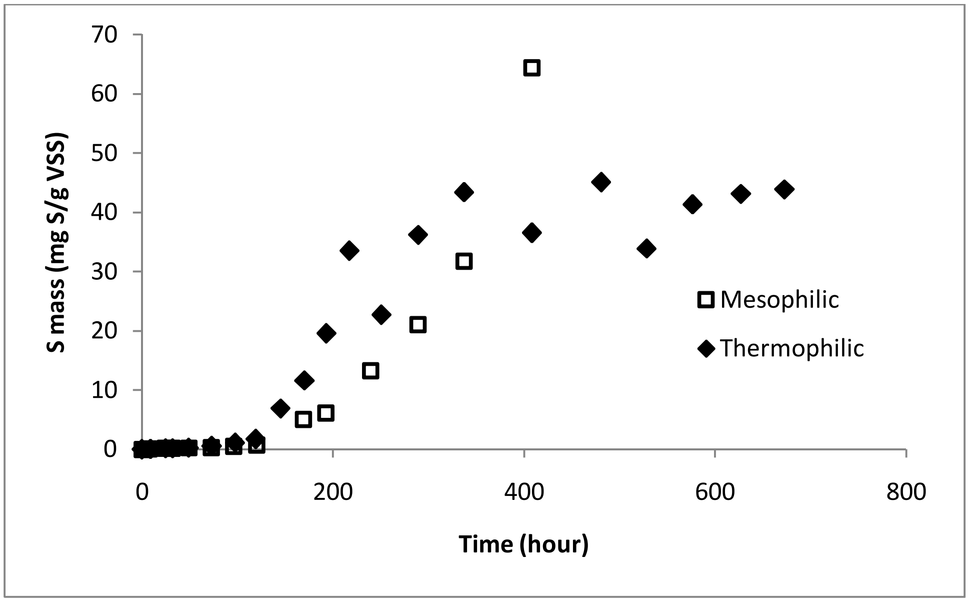

Figure 5-5 MM generation from methionine degradation

In both mesophilic and thermophilic methionine incubations with methanogen inhibition, the release of ammonia-N was monitored. Due to the structure of methionine (Figure 5-6), its degradation will release NH_3 and CH_3SH. Under anaerobic conditions, simultaneous release of NH_3 and CH_3SH is expected. If this simultaneous release were confirmed, then ammonia-N could be used as a surrogate of sulfur release from protein.

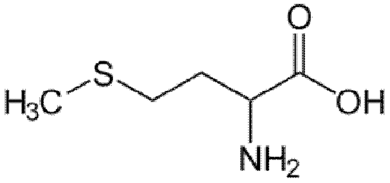

Figure 5-6 Structure of methionine

Figure 5-7 presents the percentages of VSC-S and ammonia-N that were released during mesophilic methionine incubation with methanogen-inhibition. In this case, the methionine was dosed to a concentration of 5 mmol/l, which introduced 14 mg of nitrogen and 32 mg of sulfur into the bottles. From Figure 5-7 it can be observed that the initial releases of sulfur and ammonia were slow however after 120 hours of incubation of methionine with methanogen-inhibition, VSC-S and ammonia concentrations increased rapidly. About 95.8% of the dosed methionine nitrogen was released while 92.5% of the dosed methionine sulfur was released. The releases of VSC-S and ammonia-N with time followed a similar pattern. The results suggest that sulfur and nitrogen were released simultaneously from methionine during digestion. Hence, if the substrate for anaerobic digestion is as simple as a single amino acid, ammonia can be used as surrogate for sulfur release.

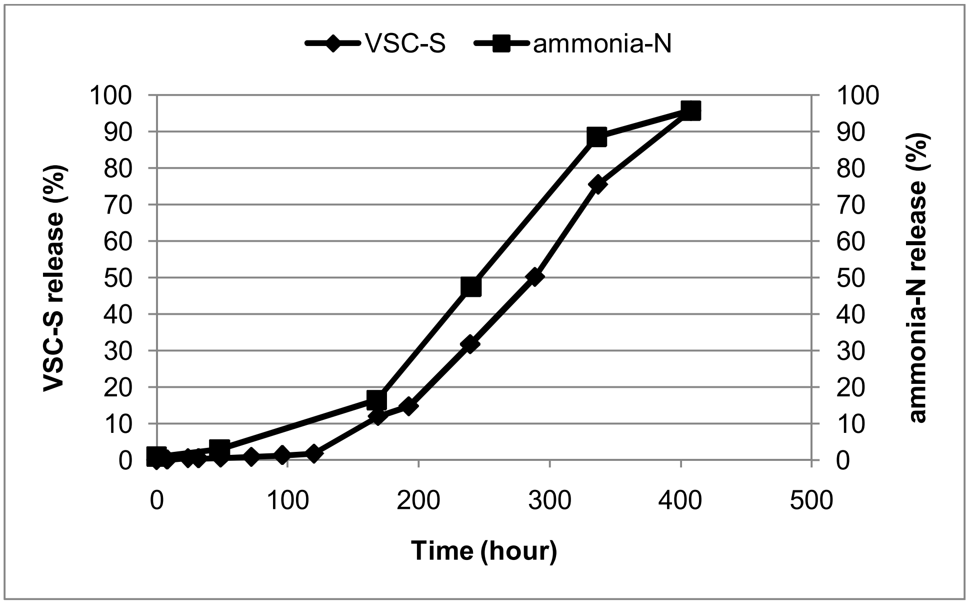

Figure 5-7 Sulfur and ammonia release from the methionine at 35 °C

Figure 5-8 presents the percentages of VSC-S and ammonia-N that were estimated to be released during thermophilic methionine incubation with methanogen-inhibition. The methionine was dosed to a concentration of 2.5 mmol/l, which introduced 7 mg of nitrogen and 16 mg of sulfur into the bottles. At the beginning of the incubation, the releases of VSC-S and ammonia-N were slow. Similar to mesophilic incubation, the ammonia-N and VSC-S release accelerated after 120 hours of incubation. At the end of the incubation, 99.4% of the dosed methionine nitrogen was released while 98% of the dosed methionine-sulfur was released. As compared to the methionine incubation at the mesophilic temperatures, more ammonia-N was released under thermophilic temperatures. However, the patterns of ammonia-N and VSC-S release in thermophilic incubation did not show significant differences from that in mesophilic incubation. Hence, ammonia-N also represented the trend of VSC-S release under thermophilic temperature.

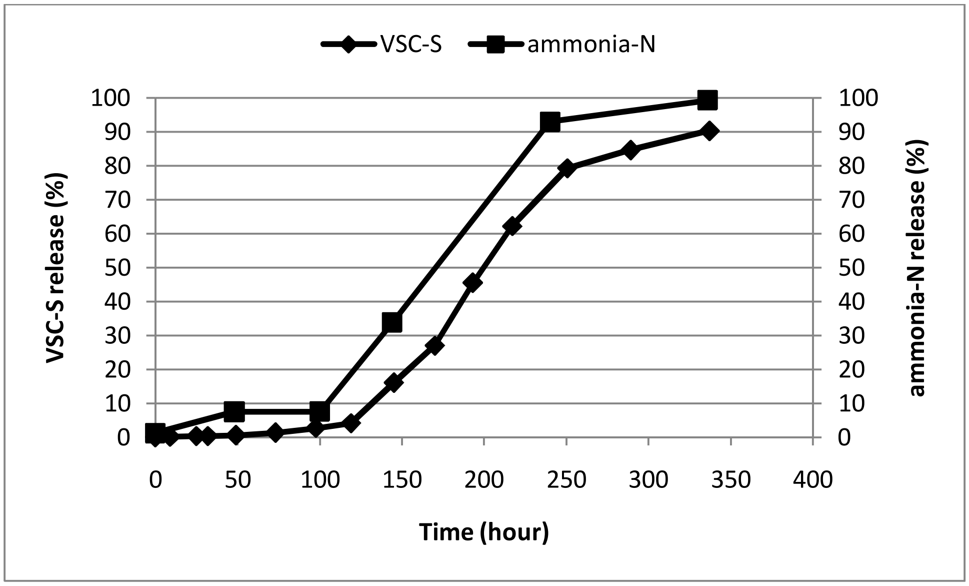

Figure 5-8 Sulfur and ammonia release from methionine at 55 °C

In both mesophilic and thermophilic incubations, MM was the dominant product from methionine degradation. Its concentration rapidly accumulated with time after the acclimation period. Mixed-second order kinetics (Equation 5-2), which are usually used to describe the hydrolysis process, were used to depict the MM release in the anaerobic methionine degradation.

$$\frac{dC_{Met-S}}{dt} = -K_{Met-S} \cdot C_{Met-S} \cdot VSS \qquad (5\text{-}2)$$

Where C_{Met-S} is the S concentration existing as methionine (mg/l), t is the time (h), K_{Met-S} is the mixed-second order kinetic parameter for the S release from methionine ($l \cdot g^{-1} \cdot h^{-1}$), and VSS is the volatile suspended solids which represents the biomass concentration (g/l).

MM was considered as the only product of methionine decay. Non-linear regression was used to fit the generated MM from methionine using the software AQUASIM and was based on the least squares method. The estimated values of K_{Met-S} for mesophilic and thermophilic methionine degradation are listed in table 5-2. The value of K_{Met-S} for thermophilic digestion was higher, which suggests that the release of sulfur from methionine was more rapid at 55 °C. Ammonia-N releases were also fitted by the mixed-second order kinetics because ammonia and MM were assumed to be released from methionine hydrolysis simultaneously. Values of k_{Met-N}, which represents the mixed-second order kinetic parameter for N release from methionine ($l \cdot g^{-1} \cdot h^{-1}$), are also listed in table 5-2. At each temperature, K_{Met-N} and K_{Met-S} were not statistically different from each other (Appendix D).

Table 5-2 Mixed-second order kinetic parameter for S release from methionine $(l\cdot g^{-1}\cdot h^{-1})$

Parameter		Mesophilic	Thermophilic
K_{Met-N}	estimate	3.05×10^{-3}	7.20×10^{-3}
	std	5.20×10^{-4}	1.22×10^{-3}
K_{Met-S}	estimate	3.18×10^{-3}	6.92×10^{-3}
	std	2.45×10^{-4}	5.25×10^{-4}

5.2.3 Model Validation

Through the tests conducted in this study, the processes of VOSC generation, degradation, and conversion have been identified and the kinetic parameters for these reactions have been estimated. A conceptual model, which was based on these identified conversion processes, has been framed for the anaerobic degradation of methionine when methanogens are not inhibited and is presented in Figure 5-9. The degradation of methionine and the conversion processes of VOSC have been tabulated in table 5-3. DMDS was not observed during methionine degradation and hence was not included in the methionine degradation model.

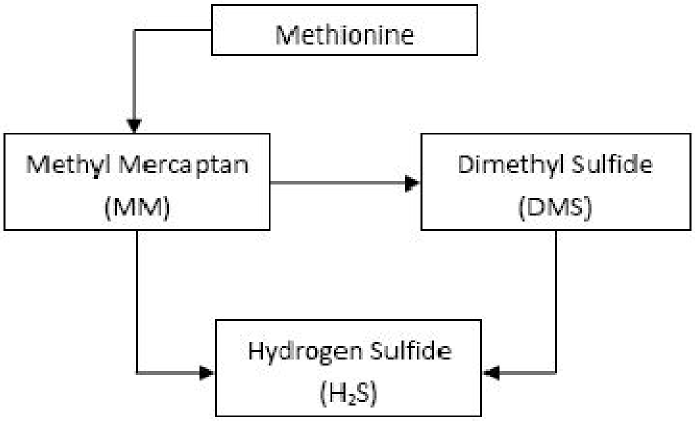

Figure 5-9 VSC conversion in methionine degradation

Table 5-3 VSC degradation and conversion processes in methionine degradation

Process	C_met	C_MM	C_DMS	C_H2S	Rate
Degradation of methionine	-1	1			k_{met}·C_met·VSS
MM degradation		-1		1	k_MM1·C_MMliq·VSS
MM methylationto DMS		-1	1		k_MM2·C_MMliq·VSS
DMS degradation			-1	1	k_DMS·C_DMSliq·VSS

The VOSC releases during the methionine dosed batch tests without methanogen inhibition were simulated and compared with the monitored data in Figures 5-10 and 11. The initial condition and the kinetic parameter values used in the simulation are listed in the table 5-4. The values for the kinetic parameters were chosen from the range listed in table 5-1 and provided the best fit of the observed data.

From Figures 5-10 and 5-11 it can be seen that the simulated values exhibited a good agreement with the monitored data and hence validated the model structure and the values of the kinetic coefficients. At 35 °C, with an initial methionine dosage of 70.2 mg S/g VSS, MM rapidly increased to 1200 ppm after 25 hours of incubation and gradually declined to less than 100 ppm after 300 hours of incubation. The concentration of DMS increased with MM, peaked at 180 ppm after 100 hours of incubation, and decreased to less than 20 ppm after 200 hours of incubation. Similarly at 55 °C, with an initial concentration of 6.9 mg S/g VSS MM rapidly increased and maintained at a high concentration of 4000 ppm for the first 20 hours of incubation and gradually declined to 54 ppm after 78 hours. The concentrations were higher than those at 35 °C because of the high volatility of MM and the slower degradation rate at 55 °C. The high concentration of MM sustained for less than 30 hours and MM disappeared after 80 hours of incubation due to the low initial methionine dosage. The DMS concentration increased to more than 600 ppm after 50 hours of incubation. The DMS concentration maintained a higher value than MM after 70 hours of incubation due to the considerably slower degradation rate of DMS at 55 °C.

Table 5-4 parameters used in simulation

Kinetic parameter	unit	mesophilic	thermophilic
k_met	l/g/h	0.0034	0.0065
k_MM1	l/g/h	0.069	0.017
k_MM2	l/g/h	0.006	0.003
k_DMS	l/g/h	0.02	0.004
Initial condition	unit	mesophilic	thermophilic
Dosed methionine	S mg/l	160	64
VSS	g/l	2.28	9.30

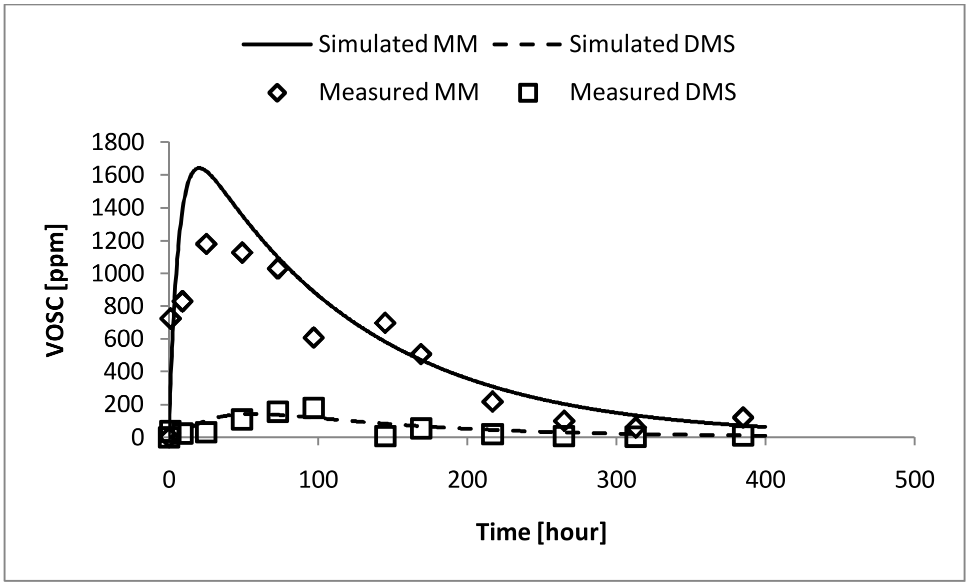

Figure 5-10 Simulated and monitored VOSCs in methionine dosed mesophilic digestion

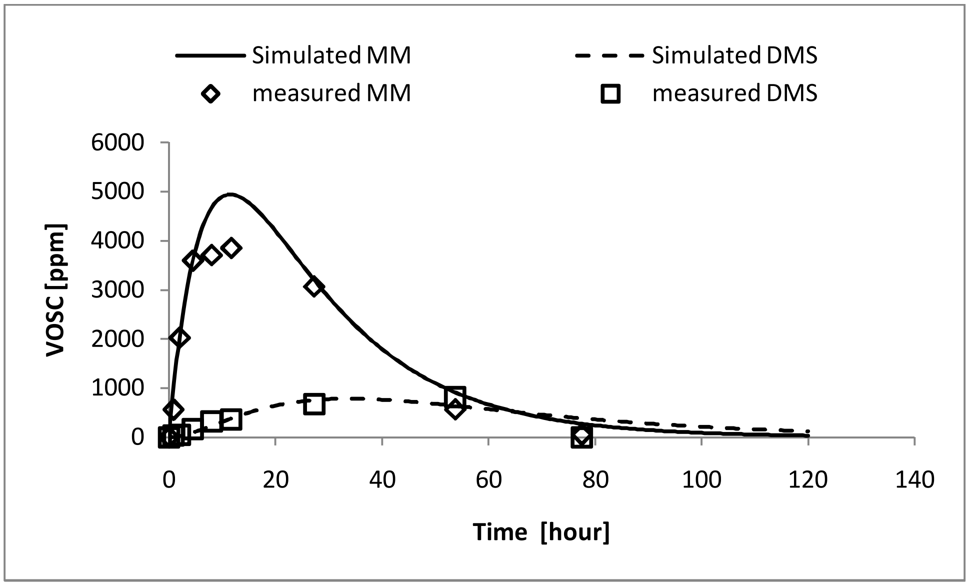

Figure 5-11 Simulated and monitored VOSCs in methionine dosed thermophilic digestion

5.3 Conclusion

The processes of VOSC generation from the amino acid methionine and their degradation have been identified, kinetic parameters have been estimated, and the effect of temperature on the conversion processes has been evaluated. The following specific conclusions were arrived at

1) Conversions of VSCs in anaerobic digestion include the degradation of MM and DMS to H_2S, methylation of MM to DMS, and the degradation of DMDS with MM as the intermediate product.

2) External carbon sources such as acetate did not affect the degradation of MM, DMS, and DMDS at both temperatures.

3) VOSC degradation and conversion processes can be well described by mixed-second order kinetics.

4) DMDS degradation is considerably more rapid at 55 ℃ as compared with its degradation at 35 ℃ while the degradation of MM and DMS had slower rates in digestion at 55 ℃. Inhibited degradation of MM and DMS was observed at 55 °C when their initial concentrations were higher than 2 and 3 mg S/g VSS.

5) Methionine decay generates MM as its major sulfur containing product in anaerobic digestion.

6) The formation of MM from methionine followed mixed-second order kinetics

7) The release of ammonia can be used as a surrogate to predict the trend of VOSC release when simple substrates, such as amino acids, are used as the digestion substrate.

The model established in this study was able to simulate VOSC generation from methionine in mesophilic and thermophilic digestion and their subsequent degradation.

Chapter 6

Sulfur Conversions with Raw Sludge Samples

This chapter presents results from raw sludge incubation batch tests and bench-scale reactors. The model established in Chapter 5 will be extended with a hydrolysis process and then employed to simulate VOSC release from raw sludge incubation. The release of VOSCs from different sludge samples will be characterized with the results from batch incubations with methanogen inhibition. The model will be evaluated by the results of raw sludge batch incubation without methanogen inhibition. Finally, modeling of VOSC release in bench-scale reactors will be presented.

6.1 Introduction

A VOSC generation and degradation model based on methionine digestion was developed in Chapter 5. Expanding the model to make it applicable to raw sludge digestion required additional study. Different from pure amino acids, raw sludge contains 2~5% solids and diverse natural and synthetic organic compounds and hence more biochemical reactions are involved in the digestion of such a complex mixture. With the focus on the sulfur related processes, a sulfur conversion model will be established for anaerobic sludge digestion.

To convert the organic compounds to methane gas and realize solids reduction and stabilization, raw sludge undergoes processes of hydrolysis, acidogenesis, and methanogenesis in anaerobic digestion. In ADM1, a process named disintegration was included before hydrolysis (Figure 2-4). ADM1 also includes a detailed classification for the substrates and hence a substantial number of stoichiometric coefficients and kinetic parameters are needed to use ADM1. To avoid this, a digestion model which includes the most important biochemical processes and employs lumped organic materials as the substrate will be utilized in this study (Figure 6-1).

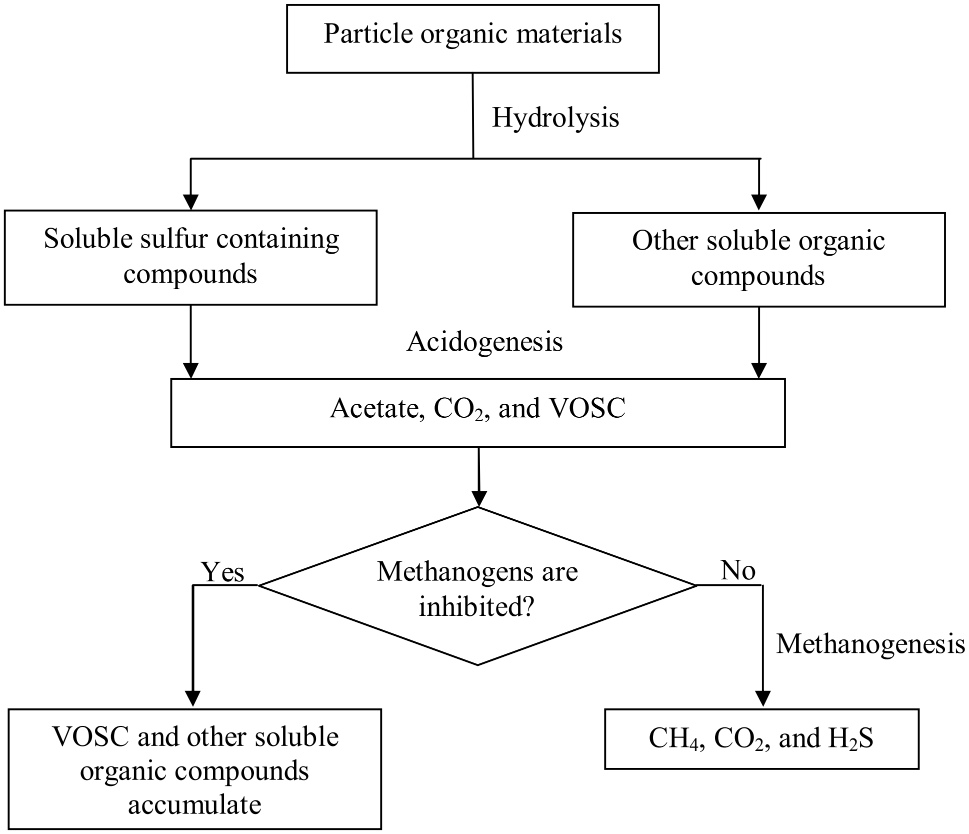

Figure 6-1 Anaerobic processes and their functions

In anaerobic digestion, the organic particulate complexes are converted to soluble compounds including carbohydrates, proteins, and lipids, and then further broken down to monosaccharides (sugars), amino acids (AA), and long chain fatty acids (LCFA). In the model shown in Figure 6-1, all these reactions are classified into one process, hydrolysis. Hydrolysis is often the rate-limiting process which would control/determine the digestion rate of wastewater treatment sludge samples (Pavlostathis and Giraldo-Gomez, 1991). Through acidogenesis, sugars, AA, and LCFA are converted to volatile fatty acids (VFA) which can be utilized by methanogens. Not only the carbon, but also the nitrogen and sulfur will be converted through these processes. For instance, organic nitrogen in the amino acids will be mineralized to inorganic nitrogen (ammonia). In the study conducted in Chapter 5, VOSC were found to be released simultaneously with ammonia in the methionine degradation.

In the model shown in Figure 6-1, the soluble organic compounds are divided into sulfur containing compounds and other organic compounds. The sulfur containing compounds are separated from the lumped organic compounds because only specific compounds participate in the sulfur conversion. In acidogenesis, the conversion of sugars, AA, and LCFA will generate VFAs, degradation of AA will release ammonia, but only degradation of sulfur containing amino acids is able to generate VSC.

To estimate the fraction of sulfur in the sludge which would degrade and release VOSC in mesophilic and thermophilic anaerobic digestions, understand sulfur conversion processes, and assess the effect of temperature and sludge source on VOSC release patterns, batch incubations with methanogen inhibition were conducted with sludge samples from four different sources at two temperatures. Hydrolysis coefficients for different components in the sludge were estimated. The model for sulfur conversion in sludge digestion was evaluated with the results obtained from the batch tests without methanogen inhibition. A modified model with consideration of the continuous biogas release was applied to the bench-scale reactors.

6.2 Results and Discussion

The results of experiments conducted with municipal sludge samples are presented in this section. Sludge samples from four different sources, including Waterloo mixed sludge (WMS), New Hamburg activated sludge from a pilot SBR (NAS), Burlington primary sludge and activated sludge from the Skyway WWTP (BPS and BAS), were employed in batch tests at two temperatures (experiment design presented in Chapter 3). Two bench-scale reactors were monitored with respect to sludge characteristics and VOSC release patterns after being fed with WMS or methionine. The monitoring and analysis related to sludge characteristics, VOSC release patterns, coefficient estimation, and model simulations are subsequently discussed.

6.2.1 Raw Sludge Used in the Batch Tests

Raw sludge samples that were obtained on two separate days were used in the batch digestions at 35 and 55 °C, respectively. At each temperature the incubations of NAS, BPS, and BAS were conducted with methanogen inhibition while the incubations of WMS were conducted with and without methanogen inhibition at the same time. The characteristics of the sludge samples before and after digestion are listed in tables 6-1 and 6-2 respectively. Both conventional and sulfur related parameters were measured. The total TKN, COD, and solids concentrations in the NAS sample were lower than those in the other sludge samples because NAS was sampled during the mixing and aeration phase from the SBR reactor.

The data in tables 6-1 and 6-2 were used to determine the initial concentrations and stoichiometric coefficients for model fitting and simulation later in this chapter. In addition, through a comparison of the composition of the sludge before and after digestion, the effect of anaerobic digestion on the organic, nitrogen, and sulfur components in the sludge could be assessed.

The raw sludge samples were obtained from different wastewater treatment facilities and different processes and hence had different characteristics. Activated sludge samples had a TSS concentration between 0.5 ~ 2%, depending on the settling time while primary and mixed sludge samples had a TSS concentration between 3 ~ 4%. The fraction of TKN out of COD (TKN/COD) in the activated sludge samples was between 5 ~ 7%. The TKN/COD fractions in the primary and mixed sludge samples were slightly lower, between 3 ~ 5%. The fraction of total S out of COD (S/COD) in the activated sludge samples was between 0.7 ~ 1.1% while the S/COD fractions in the primary and mixed sludge samples were between 0.5 ~ 0.6%. In summary, activated sludge samples had lower solids concentrations but higher N and S fractions than primary sludge. The samples that contained a mixture of activated and primary sludge had characteristics that reflected the blend of the two sludge samples: the N and S fractions were higher than primary sludge but less than activated sludge. The nitrogen and sulfur ratios can be used for comparing the content of proteineous compounds among different sludge samples. However, caution has to be employed because a portion of nitrogen and sulfur may exist as inorganic forms such as ammonia and sulfides. For instance, the fraction of precipitated sulfide out of total sulfur in the four raw sludge samples varied from 28% to 57%.

Through digestion, particulate matter was solublized and organic nitrogen was mineralized to ammonia. In the raw sludge, the VSS fraction of the TSS was between 64% and 87%. After 13 days of incubation, the fraction of VSS/TSS decreased to 55 ~ 57% for the different sludge samples. Meanwhile, the fractions of ammonia/TKN increased from less than 17% to more than 46% by mineralization of the organic-nitrogen. VSS reduction and organic-nitrogen mineralization fractions represented the portion of the organic matter in sludge samples which were biodegradable within the digestion time.

In the four sludge samples, the total sulfur concentration did not significantly decrease after digestion. The results suggest that only a small portion of the sulfur was released as VSC in the incubation. An assessment of the fraction of sulfur which is converted to VOSC will be subsequently presented. This fraction could represent the VOSC release capacity of the municipal sludge in anaerobic digestion processes.

Table 6-1 Sludge characteristics in mesophilic digestion with methanogen inhibition

Before digestion

Parameters (before digestion)		Unit	WMS	NAS	BPS	BAS
TSS		g/l	34.4	4.3	33.5	18.3
VSS		g/l	23.4	3.4	29.3	12.6
Nitrogen	TKN	mg/l	2.1×10^{3}	302.4	1.9×10^{3}	1.6×10^{3}
	soluble TKN	mg/l	511.6	38.6	58.6	180.9
	ammonia	mg/l	364.5	15.5	21.4	129.8
sulfur composition	total S (S_{total})	mg/l	273.3	64.2	314.6	183.5
	soluble S (S_{liquid})	mg/l	7.1	34.5	9.1	11.1
	sulfate S ($S_{I,liquid}$)	mg/l	1.0	10.2	0.3	3.8
	precipitated S ($S_{I,solid}$)	mg/g TSS	4.0	5.40	2.24	3.09
Initial COD	total COD	g/l	42.4	4.9	47.7	20.9
	soluble COD	mg/l	4.2×10^{3}	38.0	3.5×10^{3}	101.7

After digestion

Parameters (after digestion)		Unit	WMS with inhibition	WMS without inhibition	NAS	BPS	BAS
TSS		g/l	21.4	3.2	23.4	14.8	TSS
VSS		g/l	11.8	1.8	13.4	8.2	VSS
Nitrogen	TKN	mg/l	2.1×103	2.1×103	303.8	1.8×103	1.5×103
	soluble TKN	mg/l	1.2×103	1.4×103	144.8	986.1	731.4
	ammonia	mg/l	823.8	940.55	138.0	861.9	700.3
total S (S_{total})		mg/l	271.2	265.5	50.1	311.7	184.0
Initial COD	total COD	g/l	41.2	17.3	5.0	45.4	21.0
	soluble COD	g/l	15.4	1.6	2.6	16.2	6.8

Table 6-2 Sludge characteristics in thermophilic digestion with methanogen inhibition

Before digestion

Parameters (before digestion)		Unit	WMS	NAS	BPS	BAS
TSS		g/l	33.8	10.1	41.2	15.2
VSS		g/l	21.8	7.9	30.8	10.9
Nitrogen	TKN	mg/l	2.1×10^3	725.2	1.7×10^3	950.9
	soluble TKN	mg/l	481.5	27.7	124.1	36.0
	ammonia	mg/l	175.1	15.0	83.5	9.8
sulfur composition	total S (S_{total})	mg/l	257.5	98.2	286.7	174.0
	soluble S (S_{liquid})	mg/l	21.5	31.2	38.1	29.4
	sulfate S ($S_{I,liquid}$)	mg/l	0.7	9.8	1.1	3.9
	precipitated S ($S_{I,solid}$)	mg/g TSS	5.0	3.5	3.0	3.0
Initial COD	total COD	g/l	43.9	13.8	59.1	17.4
	soluble COD	mg/l	7.9×10^3	170.6	3.8×10^3	131.6

After digestion

Parameters (after digestion)		Unit	WMS with inhibition	WMS without inhibition	NAS	BPS	BAS
TSS		g/l	22.8	8.6	29	10.6	TSS
VSS		g/l	13	4.6	19	5.8	VSS
Nitrogen	TKN	mg/l	2.1×10^3	2.2×10^3	739.0	1.7×10^3	1.0×10^3
	soluble TKN	mg/l	1.5×10^3	1.6×10^3	447.9	1.2×10^3	712.8
	ammonia	mg/l	920.6	1084.6	298.8	831.0	463.5
total S (S_{total})		mg/l	259.6	264.0	93.2	290.0	169.7
Initial COD	total COD	g/l	43.7	22.7	14.0	57.6	15.9
	soluble COD	g/l	22.6	5.6	8.6	16.8	7.1

6.2.2 Estimation of Hydrolysis Rate Coefficients from COD and Ammonia Data

6.2.2.1 COD Hydrolysis

In anaerobic digestion particulate organic compounds are hydrolyzed to soluble organic compounds and then broken down to VFA which could be utilized by methanogens. Inert organic compounds can also be generated through these processes. Acidogenesis converts degradable soluble organic compounds to VFA and is typically not the rate-limiting step in digestion of wastewater sludge samples. Therefore, hydrolysis and acidogenesis were combined to one step and the VFA and inert soluble organic compounds were assumed to be the products of hydrolysis of the biodegradable particulate complex as shown in Figure 6-2.

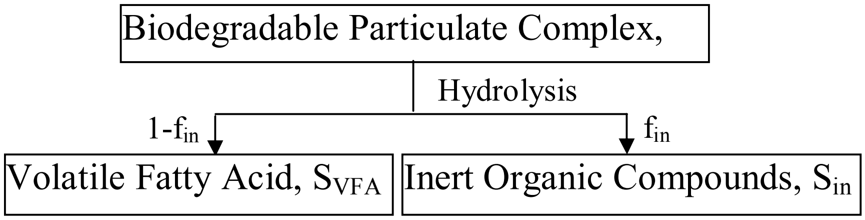

Figure 6-2 COD conversion model

In the batch anaerobic digestions of WMS with methanogenesis inhibited, COD values were measured with time. Figures 6-3a and 6-3b show COD values with time in mesophilic and thermophilic digestion of WMS with methanogen inhibition, respectively. The difference between total and soluble COD was employed to estimate the particulate organic compounds. In the incubation at 35 °C, particulate COD gradually decreased from 38 g/l to 26 g/l over 350 hours of incubation and maintained this level until the end of incubation. In the incubation at 55 °C, particulate COD decreased from 36 g/l to 21 g/l over 300 hours. With the decrease of particles, soluble COD accumulated. The kinetics of hydrolysis were of interest and were estimated from the data.

Table 6-3 Stoichiometry and kinetic expression for hydrolysis of COD

Process	X_b	S_{VFA}	S_{in}	Rate
Hydrolysis	-1	$1-f_{in}$	f_{in}	$K_{COD} \times X_b$

A first order model was employed to describe the hydrolysis of COD. Table 6-3 presents the relationships between COD components in the hydrolysis process that were employed. X_b represents the biodegradable portion of the total particulate COD (X_p). The difference between X_p and X_b was assumed to consist of inert particulate COD (X_{in}). During the hydrolysis process, it was assumed that X_b was hydrolyzed to biodegradable soluble COD (S_{VFA}) and inert soluble COD (S_{in}). The sum of S_{VFA} and S_{in} was the total soluble organic COD (S_c). The hydrolysis rate was found to be well fitted by the first order kinetics which were dependent on the concentration of X_b. K_{COD} is the first order coefficient for hydrolysis.

In the fitting of the hydrolysis model to the data, X_p was calculated as the difference of the total and soluble COD of the sludge. It was assumed that essentially all of the biodegradable particulate COD was hydrolyzed in the batch tests and hence X_{in} was assumed to equal the residual particulate COD that was measured at the end of the digestion tests. Similarly, S_{in} was assumed to equal the residual soluble COD that was measured after digestion without methanogen inhibition. For example, the X_p of WMS used in the batch test at 35 °C was 38.2 g/l (difference between 42.4 and 4.2, total and soluble COD in table 6-2). The X_{in} after the mesophilic digestion with inhibition was about 26 g COD/l, suggesting that 35% of the particulate COD was hydrolyzed in the batch test. The residual S_{in} was about 10% of the particle COD which solublized through the digestion ($f_{in} = 0.10$). Therefore, it was assumed that when particulate COD hydrolyzed at 35 °C, only 0.90 of X_b was converted to biodegradable soluble COD (S_{VFA}). The inert particulate and soluble organic matter fractions varied between sludge samples and were individually estimated.

The software AQUASIM was used to fit the hydrolysis model to the decrease of the particulate COD based on the least square method. The estimated values of K_{COD}, its standard error (STD), and the coefficient of determination (R^2) of the regression curve for WMS hydrolysis at 35 and 55 °C are listed in table 6-4. On the basis of the high R^2 values and visual examination of the curves presented in Figure 6-3 it was concluded that simulation with the estimated K_{COD} value fit the particulate COD decrease well and also well represented the soluble COD accumulation at both temperatures (Figure 6-3a and b). The K_{COD} value at 55 °C was about 1.5 times of that at 35 °C. In a review on the kinetics in anaerobic digestion, the hydrolysis coefficients obtained from mixed sludge digestion (Pfeffer, 1974, Pavlostathis and Giraldo-Gomez, 1991) varied from 0.052 to 0.2 d^{-1} (= $2.2 \sim 8.3\times10^{-3}$ h^{-1}) at 35 °C and from 0.32 to 0.62 d^{-1}(= $1.3 \sim 2.5\times10^{-2}$ h^{-1}) at 55 °C. The estimated K_{COD} were consistent with the literature values.

Table 6-4 Estimated K_{COD} values

Unit: h^{-1}	Temperature	
	35 °C	55 °C
K_{COD}	8.29×10^{-3}	1.27×10^{-2}
STD	9.43×10^{-4}	1.30×10^{-3}
R^2	0.95	0.87

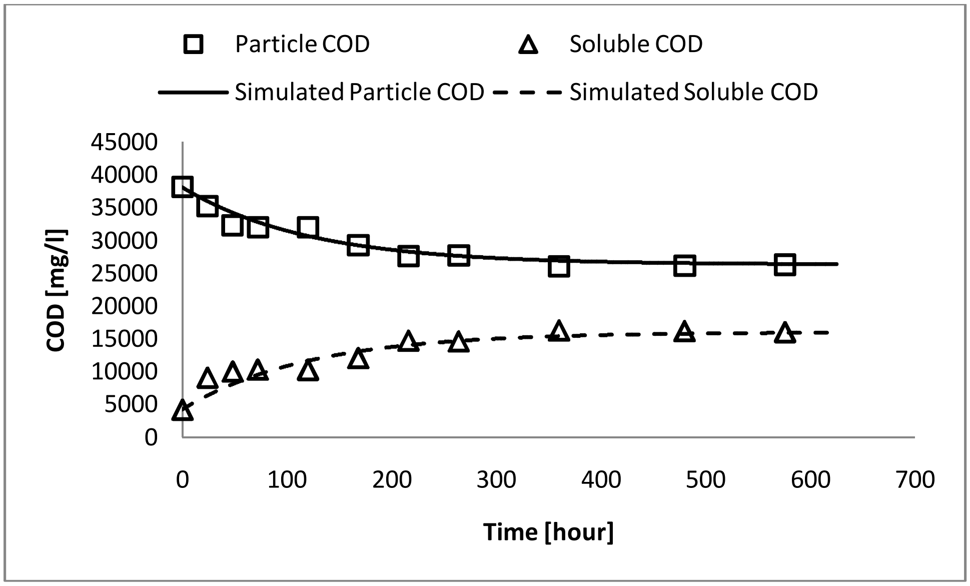

Figure 6-3a Hydrolysis of WMS at 35 °C (COD, with methanogen inhibition)

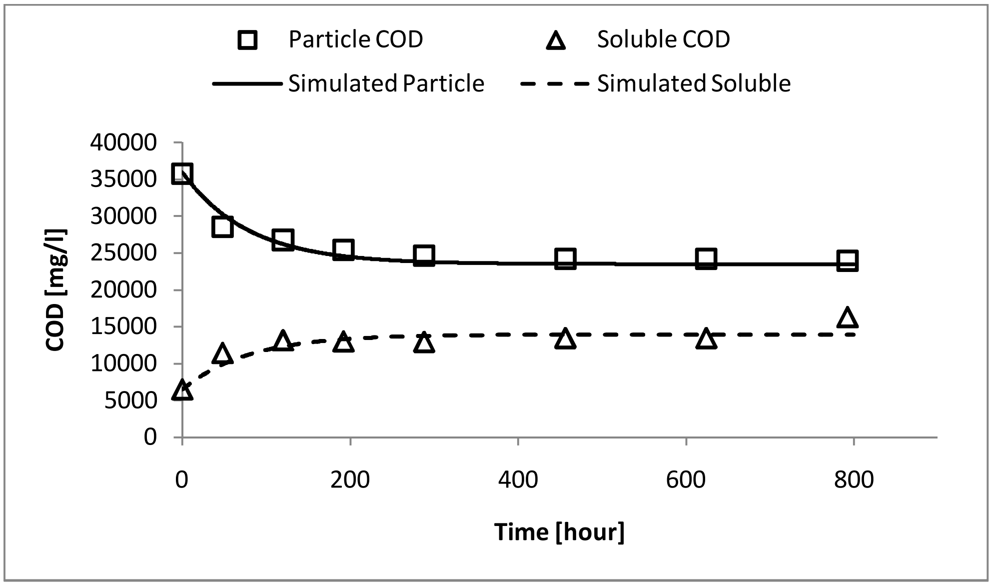

Figure 6-3b Hydrolysis of WMS at 55 °C (COD, with methanogen inhibition)

6.2.2.2 Ammonia Release

Along with solubilization of particulate compounds, organic nitrogen containing particles will be hydrolyzed to proteins and peptides, and further to amino acids. Inert soluble organic nitrogen could also be produced in this process. The rate of hydrolysis of proteinaceous compounds has been reported to be slower than the rates of carbohydrates and lipids and hence significantly affects the total hydrolysis rate of the complex (mixture of various substrates) (Pavlostathis and Giraldo-Gomez, 1991). During acidogenesis, the amino acids will be degraded to VFA and release inorganic nitrogen (in the form of ammonia). While it is possible that different amino acids could release ammonia at different rates, acidogenesis is usually fast as compared to hydrolysis and hence it was expected there would be no substantial accumulation of amino acids in the batch tests. Using a combined hydrolysis process, the model was expressed as described in Figure 6-4.

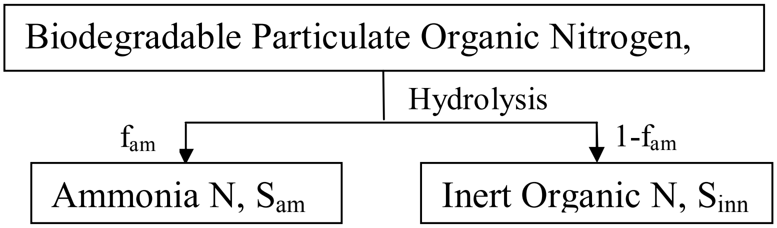

Figure 6-4 Nitrogen conversion model

In the incubation of WMS, both TKN and ammonia were measured with time while in the incubation of NAS, BPS, and BAS, ammonia alone was measured with time. The decrease of the organic-nitrogen in WMS incubation was therefore utilized to estimate the hydrolysis coefficient for biodegradable particulate organic nitrogen (K_N). The decrease of particulate TKN and the accumulation of ammonia were simulated at the same time using K_N. The values of K_N and K_{COD} of WMS were subsequently compared. In the incubation of the other sludge samples, ammonia accumulation was used to estimate the hydrolysis coefficient K_N and the K_N values of different sludge samples were subsequently compared.

1. K_N for WMS

In the incubation of WMS, the particulate TKN decreased from approximately 1600 mg/l to 900 mg/l (Figure 6-5a and b) and 600 mg/l (Figure 6-5c and d) in the incubations at 35 and 55 °C, respectively. Soluble TKN which was mainly composed of ammonia increased with the decrease of particulate TKN. On the basis of the raw data, it was concluded that inhibition of methanogens did not influence the rate of organic nitrogen hydrolysis.

Table 6-5 Nitrogen conversion (nitrogen concentration mg N/l)

Process	X_{bn}	S_{am}	S_{inn}	Rate
Hydrolysis	-1	f_{am}	$1-f_{am}$	$K_N \times X_{bn}$

Table 6-5 presents the relationships between the biodegradable particulate TKN (X_{bn}), the inert soluble organic nitrogen (S_{inn}) and ammonia (S_{am}) in the hydrolysis process. In this table X_{bn} is the biodegradable portion of the total particulate TKN (X_n). The difference between X_n and X_{bn} was assumed to be the inert particulate TKN (X_{inn}). X_{inn} was the TKN which did not solublize in the hydrolysis process during incubation. As presented in Figure 6-4, it was assumed that during hydrolysis X_{bn} was hydrolyzed to ammonia (S_{am}) and inert soluble organic nitrogen (S_{inn}). The sum of S_{am} and S_{inn} was assumed to be equally to the soluble TKN. The residual soluble organic nitrogen that was measured at the end of incubation was assumed to represent (S_{inn}). The value of f_{am} was the portion of the generated ammonia out of the particulate organic-nitrogen which degraded through the digestion. This fraction was determined for different sludge samples individually. K_N is the first order kinetic coefficient of hydrolysis.

Similar to the model calibration for COD hydrolysis, AQUASIM was used for the fitting based on the least squares method. The estimated hydrolysis coefficients, their standard errors, and the coefficients of determination are listed in table 6-6. On the basis of the R^2 values and visual examination of the simulation curves (Figure 6-5) it was concluded that the hydrolysis model could adequately represent the decrease of particulate organic nitrogen. The accumulation of ammonia as the result of mineralization of organic nitrogen was also well reflected. The K_N values estimated under the conditions with and without methanogen inhibition (Appendix E) were not significantly different. Hence, it was confirmed that the activities of methanogens did not affect the hydrolysis of organic-nitrogen.

The hydrolysis coefficients (K_{COD} in tables 6-4 and K_N in table 6-6) that were estimated from WMS incubation were found to have similar values. The coefficient values estimated at 55 °C were about 1.4 ~ 1.5 times of those estimated at 35 °C. The results suggested that in the WMS incubation hydrolyses of lumped organic particles and lumped organic-nitrogen could utilize the same coefficient value to express their kinetic rates. The ratio of their hydrolysis rates $R_{org\text{-}N}/R_{COD}$ would be equivalent to their concentration ratio X_{bn}/X_b.

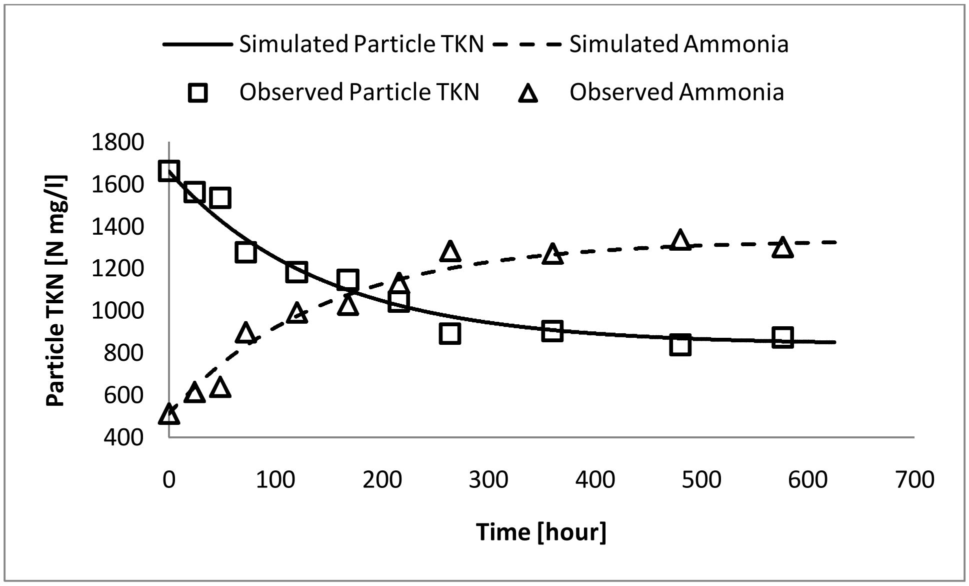

Figure 6-5a Nitrogen conversion in WMS at 35 °C (with methanogen inhibition)

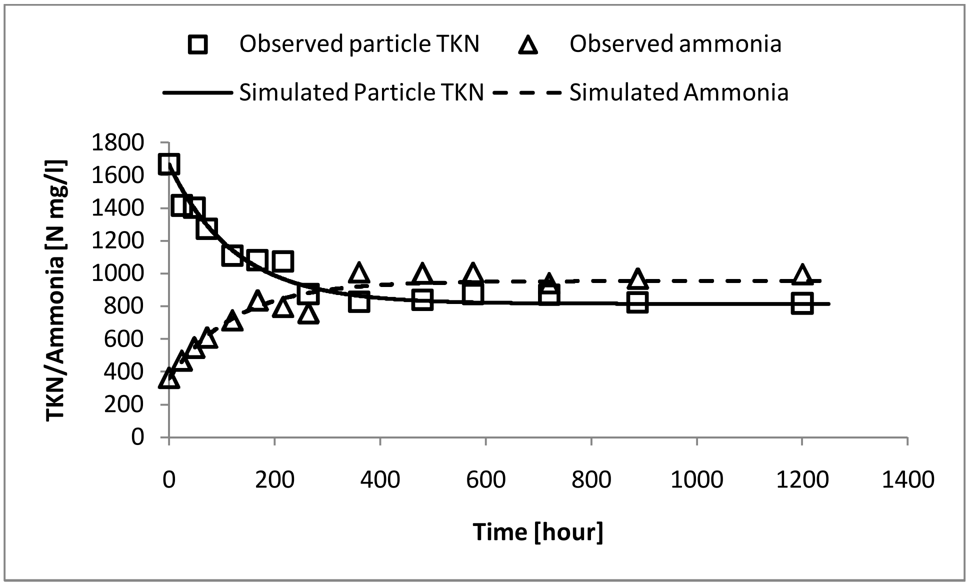

Figure 6-5b Nitrogen conversion in WMS at 35 °C (without methanogen inhibition)

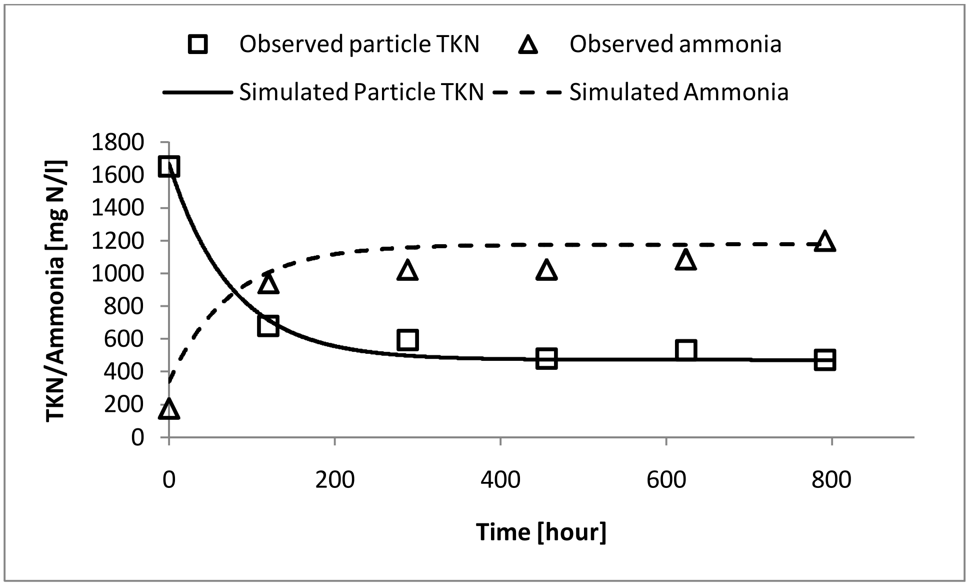

Figure 6-5c Nitrogen conversion in WMS at 55 °C (with methanogen inhibition)

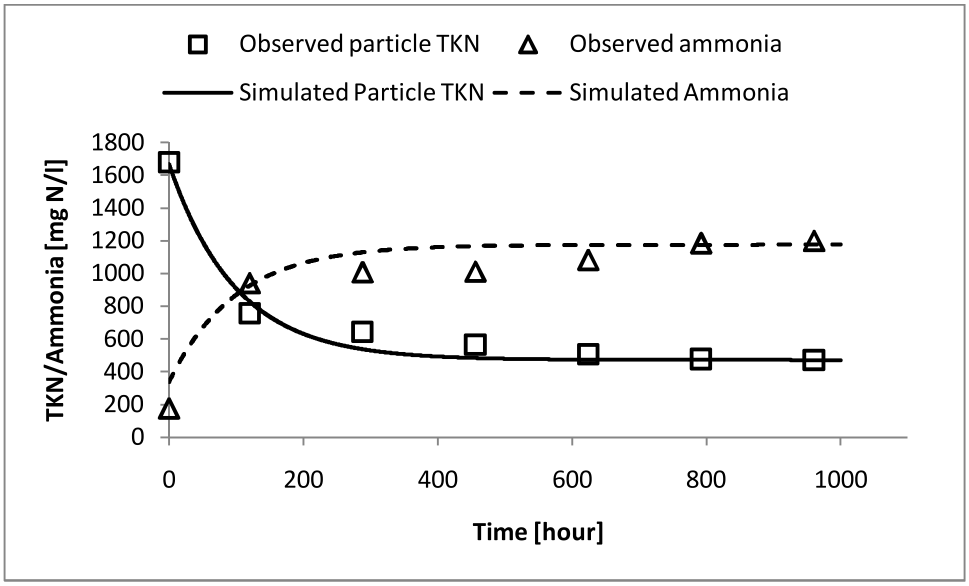

Figure 6-5d Nitrogen conversion in WMS at 55 °C (without methanogen inhibition)

2. K_N of Other Sludge Samples

In the incubation of NAS, BPS, and BAS, TKN and soluble TKN were only measured before and after digestion, however, the released ammonia was measured with time. The measured ammonia concentrations are shown in Figures 6-6a and 6-6b. At the end of incubation of the 3 sludge samples, the ammonia accumulated to a level that was 45-48% of the TKN.

Values of f_{am} were calculated for each sludge sample. AQUASIM was used for the coefficient estimation for the incubations of three different sludge samples at two temperatures, using the model described in table 6-5. The estimated K_N values, their standard errors, and the R^2 values are listed in table 6-6. The simulation curves are shown in Figure 6-6a and b. As with WMS, it was concluded that the model was able to adequately describe the accumulation of ammonia through the incubations.

Table 6-6 Estimated K_N values

			WMS		NAS	BPS	BAS
			Inhibited	Without inhibition			
	SRT (d)		(returned WAS) 4.3		15.7	N/A	9.2
K_N (h^{-1})	35 °C	Estimate	8.21×10^{-3}	7.95×10^{-3}	1.42×10^{-2}	1.23×10^{-2}	1.49×10^{-2}
		STD	3.58×10^{-4}	4.13×10^{-4}	3.23×10^{-3}	7.34×10^{-4}	2.46×10^{-3}
		R^2	0.89	0.89	0.82	0.85	0.85
	55 °C	Estimate	1.24×10^{-2}	1.28×10^{-2}	2.38×10^{-2}	1.75×10^{-2}	2.18×10^{-2}
		STD	1.21×10^{-3}	4.39×10^{-3}	2.03×10^{-3}	7.50×10^{-4}	1.54×10^{-3}
		R^2	0.92	0.91	0.96	0.92	0.87

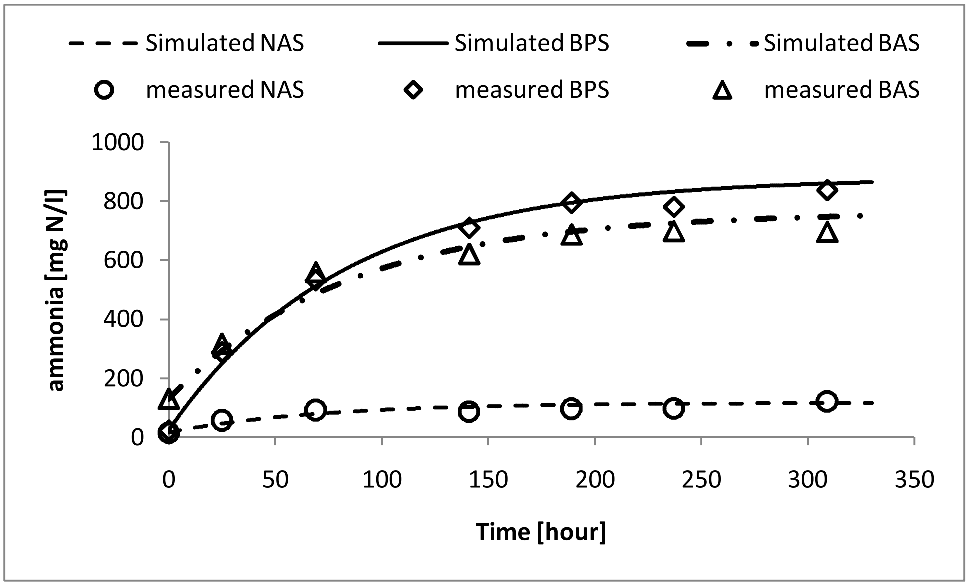

Figure 6-6a Ammonia release in sludge samples at 35 °C (with methanogen inhibition)

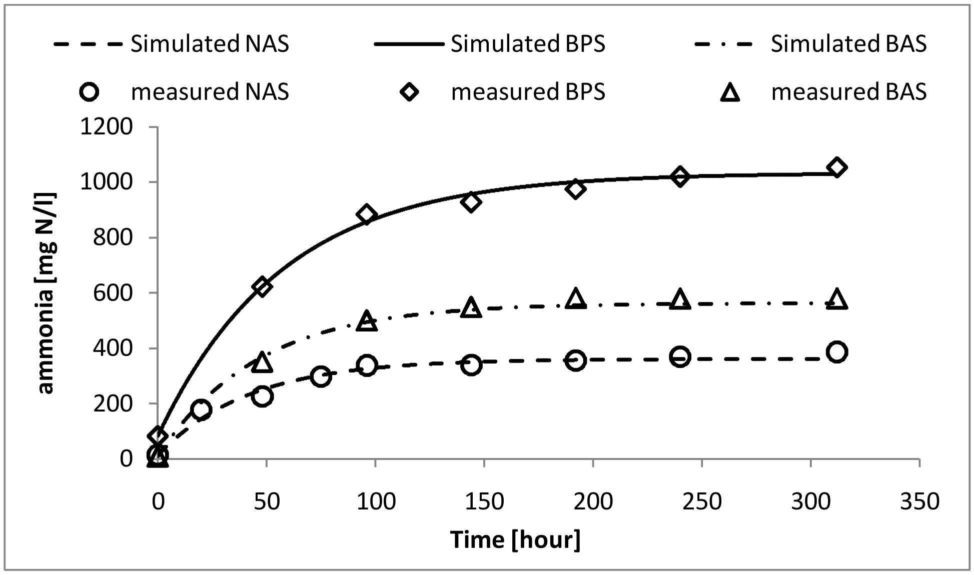

Figure 6-6b Ammonia release in sludge samples at 55 °C (with methanogen inhibition)

From table 6-6 it can be found that the value of K_N at 35°C varied from 0.008-0.015 hr^{-1}. The hydrolysis coefficient for the degradation of activated sludge was reported to be 0.007 ~ 0.025 h^{-1} (Pfeffer, 1974) while the hydrolysis coefficient for the primary sludge was reported to be 0.018 h^{-1}

(Eastman and Ferguson, 1981) at 35°C. Hence it was concluded that the estimated coefficients at 35 °C in this study were consistent with the reported values.

On the basis of the values presented in table 6-6, the effect of temperature on the hydrolysis of sludge samples could be assessed and it was concluded that temperature had similar effects on the ammonia release in the different sludge incubations. The hydrolysis coefficient of WMS at 55 °C were about 1.4 ~ 1.5 times of that at 35 °C. For the activated sludge sample samples (NAS and BAS), the hydrolysis coefficients at 55 °C were 1.7 and 1.5 times the corresponding hydrolysis coefficients at 35 °C. For primary sludge BPS, the hydrolysis coefficient at 55 °C was about 1.4 times of that at 35 °C.

In this study, the difference of the K_N between 35 and 55 °C was relatively small when compared to literature values. Pfeffer (1974) reported that the hydrolysis coefficient at 55 °C could be around 10 times that at 35 °C. However, Pfeffer (1974) also reported that the relationship between temperature and hydrolysis coefficient values was not linear. In this prior study, the hydrolysis coefficient at 50 °C was only 2 times greater the hydrolysis coefficient at 35 ºC while the coefficient at 45 °C was smaller than the coefficient at 35 °C. The variation of the hydrolysis coefficient values with temperature could vary with microorganism cultures and sludge samples (Pavlostathis and Giraldo-Gomez, 1981).

The values in table 6-6 were also employed to assess the effect of sludge source on hydrolysis. It was found that the activated sludge samples (NAS and BAS) had similar K_N values at each temperature. These two samples had K_N values that were greater than the primary sludge (BPS) (15 ~ 21% greater at 35 °C and 25 ~ 36% greater at 55 °C respectively). The K_N values of the primary sludge (BPS) were greater than the mixed sludge (WMS) (52% greater at 35 °C and 39% greater at 55 °C respectively).

To better understand the effect of sludge source on the hydrolysis coefficients, the estimated average SRT for activated sludge samples in the WWTP operation over the past years are listed in table 6-6. SRT is not applicable to BPS which is primary sludge. The average SRT for the NAS and BAS sludge samples were 15.7 and 9.2 days, respectively. WMS was a mixture of primary sludge and activated sludge that had an average SRT of 4.3 days.

It has been reported (Pavlostathis and Giraldo-Gomez, 1981) that while primary sludge was easier to hydrolyze than activated sludge, activated sludge samples with shorter SRTs were easier to hydrolyze than those with longer SRTs. The generally accepted order of the values of hydrolysis constants of different sludge samples is shown in Figure 6-7a. The value of hydrolysis constant was related to the composition of the sludge. Organic compounds in sludge sample consist of proteins, carbohydrates, and lipids. The hydrolysis of proteins was found to be slower than that of carbohydrates and lipids. Activated sludges are usually composed of 40 ~ 80% proteins while primary sludge only has less than 20% proteins. Therefore, the hydrolysis of activated sludge was believed to be slower than that of primary sludge.

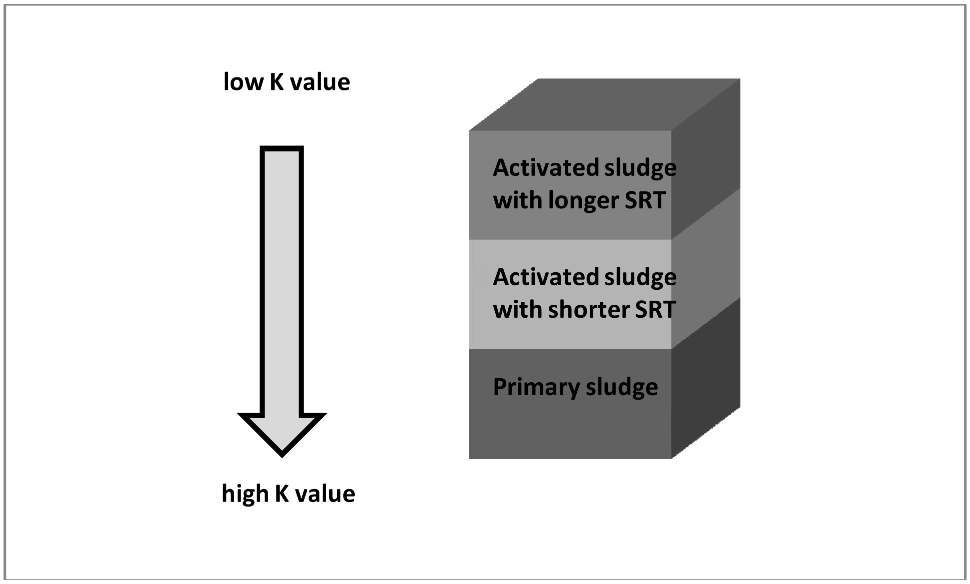

Figure 6-7a Generally accepted order of hydrolysis coefficients

In the present study, the hydrolysis of different sludge samples was compared based on the organic-N conversion to ammonia, which represented the hydrolysis of proteins. According to the estimated hydrolysis coefficient values of K_N, it was found that activated sludge samples were more susceptible to hydrolysis as compared to the primary sludge sample (Figure 6-7b). The mixed sludge contained activated sludge with a short SRT and was least susceptible to hydrolysis (Figure 6-7b). It was reported in a study on thermal hydrolysis that although the solubilization of protein was similar for primary and activated sludge samples, the protein in the extracellular materials in the activated sludge might be more accessible for degradation than the protein in primary sludge (Wilson and Novak, 2009). This might explain the ascending values of for K_N in the order of mixed sludge, primary sludge, and activated sludge in Figure 6-7b.

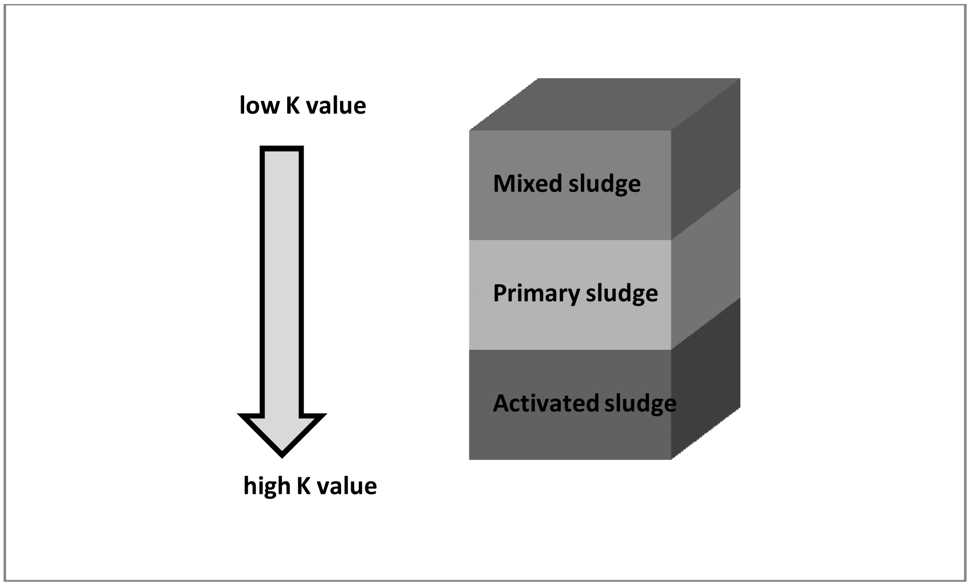

Figure 6-7b Order of hydrolysis coefficients K_N in this study

6.2.3 VOSC Release from Raw Sludge

Based on the literature review, the majority of biodegradable sulfur-containing compounds consist of proteinaceous materials. In anaerobic digestion, particulate food residues, fecal matter, and other protein-rich materials are hydrolyzed to protein and peptides which are further degraded to amino acids. Some synthetic sulfur containing compounds such as those present in detergents may not degrade in anaerobic digestion and will remain as inert organic sulfur. As previously demonstrated degradation of the protein amino acids cysteine and methionine will release H_2S and VOSC. In Chapter 5 it was observed that MM was the dominant product of methionine decay. Therefore, accumulation of MM was expected during sludge incubation with methanogen inhibition.

As previously demonstrated cysteine degradation leads to H_2S formation which is potentially susceptible to precipitation and hence this amino acid was not included in the model. H_2S can also be generated from inorganic sulfur containing compounds. The presence of various metal cations and pH will impact upon sulfide precipitation and the complexity of these mechanisms was beyond the scope of this study.

With the focus on VOSC generation, biodegradable organic-sulfur in particulate organic matter and in the soluble material which could be converted to VOSC in anaerobic digestion was of interest. Unfortunately, purification and quantitative analysis of organic sulfur containing compounds in the sludge was found to be challenging. Therefore, it was assumed in this study that methionine could be used as a surrogate for biodegradable organic-sulfur and the direct sulfur containing product of its degradation was MM. The processes of hydrolysis of particulate organic-sulfur to release free methionine-sulfur and the VOSC generation from methionine are shown in Figure 6-8.

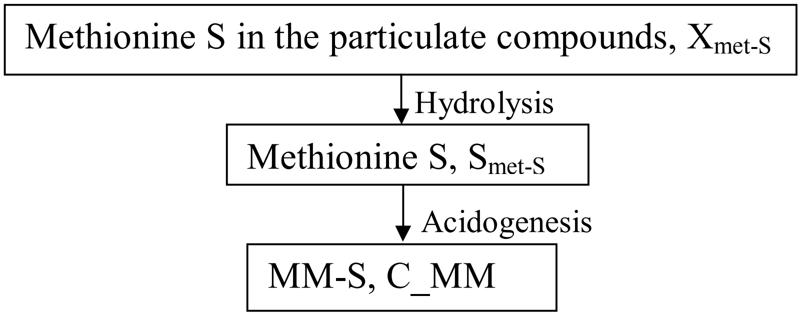

Figure 6-8 Sulfur conversion model

6.2.3.1 Raw Data Discussion

It was observed that VOSC release patterns in the sludge were more complicated than those in the pure methionine incubations. Raw data associated with sulfur conversions in sludge incubation with methanogen inhibition are discussed in this section. The hydrolysis of organic-sulfur in sludge could be studied from two aspects: sulfur sources (methionine, surrogate for organic-sulfur which was able to generate VOSC) and products (VOSC). The raw data will be discussed from these two perspectives. A summary of common characteristics and a discussion of exceptions in VOSC release will be presented.

1. Sulfur Fractions in Sludge Samples

The accumulated VOSCs were assumed to all be generated from decay of methionine in the sludge and hence the sulfur measured in the VOSC represented the methionine which decayed in the incubations with methanogen inhibition. Due to the mass balance between the degraded organic-S and released VOSC-S, total organic-S in the particulate matter and in the soluble material of the raw sludge which degraded through methionine pathway could be estimated. The results suggested that the degradable organic-S (with VOSC generation) fraction varied with sludge source and temperature.

In tables 6-7 and 6-8, the fraction of the organic sulfur in the raw sludge that was degraded via the methionine pathway was calculated. The sulfur speciation (S_{total}, S_{liquid}, $S_{I,liquid}$, and $S_{I,solid}$) of the raw sludge samples that was measured before incubation is listed in table 6-1 and 6-2. On the basis of the sulfur speciation, soluble and particulate organic sulfur ($S_{o,liquid}$and $S_{o,solid}$) in the sludge was calculated with equations 3-6 and 3-7 and their sum represented the total organic sulfur. From tables 6-7 and 6-8 it can be seen that the portion of degradable methionine-sulfur out of the organic sulfur in different raw sludge samples varied from 3.7% to 10.6%. The average fraction of the degraded methionine sulfur out of the organic sulfur was about 6.3%. Most organic sulfur remained in the raw sludge rather than being released as VOSC. The remaining organic sulfur may exist in the active enzymes and living microorganisms and part of them may be the inert organic sulfur.

In mesophilic digestion, the $\frac{met-S}{organic-S}$ fractions of activated sludge samples were slightly higher than the $\frac{met-S}{organic-S}$ fractions of primary sludge and mixed sludge samples. This supported the hypothesis that protein in the extracellular materials in activated sludge samples was more accessible to degradation.

Different from the other sludge samples, in thermophilic digestion, the $\frac{met-S}{organic-S}$ fraction of the BAS activated sludge sample was slightly lower than that in mesophilic digestion. As will be subsequently demonstrated substantial DMS was generated in the mesophilic digestion of BAS, and this may have resulted from degradation of organic-S in pathways that were different than the assumed methionine pathway. The higher degradable organic-S fraction of BAS at mesophilic temperature might represent the sum of methionine and other DMS generating organic-S. Additional discussion of the different VOSC release patterns in BAS incubation at the two temperatures will be presented later in “VOSC Release”.

The $\frac{met-S}{organic-S}$ fractions observed in thermophilic digestion of activated sludge (NAS), primary sludge (BPA), and mixed sludge (WMS), were higher than those in mesophilic digestion. In thermophilic digestion, the $\frac{met-S}{organic-S}$ fractions of the activated sludge (NAS) and the mixed sludge (WMS) were more than double of the fractions in mesophilic digestion while the $\frac{met-S}{organic-S}$ fraction of the primary sludge (BPS) was about 1.5 times of the fraction in mesophilic digestion. The results suggest that higher digestion temperature could result in more organic-S degradation in a variety of sludge samples. The fraction of degraded organic-S in sludge samples which contained activated sludge increased more at higher temperature as compared to primary sludge.

Table 6-7 Methionine degraded through mesophilic incubation

Species		WMS	NAS	BPS	BAS
organic soluble S ($S_{o,liquid}$)	mg/l	6.7 ± 0.5	24.4 ± 3.6	9.0 ± 0.7	7.3 ± 0.3
organic particle S ($S_{o,solid}$)	mg/l	128.9 ± 1.9	6.6 ± 2.1	230.3 ± 3.6	116.7 ± 4.1
organic S	mg/l	134.6 ± 1.3	31.0 ± 5.7	239.3 ± 4.3	124.0 ± 4.4
degradable methionine S	mg/l	5.0 ± 0.8	1.6 ± 0.3	9.9 ± 1.8	7.6 ± 1.6
met-S/organic S		3.7%	5.2%	4.1%	6.1%

Table 6-8 Methionine degraded through thermophilic incubation

Species		WMS	NAS	BPS	BAS
organic soluble S ($S_{o,liquid}$)	mg/l	20.8 ± 0.6	21.4 ± 3.5	37.1 ± 0.1	25.5 ± 0.4
organic particle S ($S_{o,solid}$)	mg/l	69.9 ± 4.3	32.0 ± 2.0	125.0 ± 1.4	98.8 ± 0.9
organic S	mg/l	90.7 ± 4.1	53.4 ± 1.5	162.0 ± 1.5	124.3 ± 0.5
degradable methionine S	mg/l	9.6 ± 1.5	5.4 ±1.6	10.0 ± 0.6	6.3 ± 0.5
met-S/organic S		10.6%	10.0%	6.2%	5.1%

2. VOSC Release

A preliminary review of the data from the inhibited batch tests revealed that the patterns and types of VOSCs released was affected by sludge source and temperature. As will be subsequently demonstrated the results obtained at 55°C were somewhat more reproducible than those at 35°C and hence the former will be presented first. The plots present the raw data and also curves that represent model simulations that will be subsequently described. VSS was used to normalize the sulfur quantity for comparison among sludge samples. The VSS value of the sample after mixing with the inocula at the beginning of incubation was employed for this purpose. This approach assumed that the measured VSS values were reflective of the quantity of anaerobic biomass that was present to mediate biotransformations in the tests..

The response obtained from incubation of primary sludge (BPS) at 55°C differed from the remaining sludge sources in that MM was the only VOSC which was detected (Figure 6-9c). A rapid increase of MM in the incubation to 0.2 mg S/g VSS within the first 20 hours was observed and this may have been due to degradation of the methionine that was already present in the raw sludge. The subsequent slow release of MM was attributed to degradation of particulate proteineous materials.

The MM concentration remained around 0.3 mg S/g VSS between 120 hours and the end of the incubation.

All of the samples that contained waste activated sludge generated DMS in addition to MM when incubated at 55°C. In the incubation of the mixed sludge (WMS) (Figure 6-9a), MM quickly accumulated in the first 120 hours, started to decline after 300 hours but remained above 0.45 mg S/g VSS until the end of incubation. At the same time, DMS slowly accumulated and then maintained a level less than 0.1 mg S/g VSS through the incubation.

In the incubation of the activated sludge samples (NAS and BAS), the accumulation of MM was accompanied with the generation of DMS (Figure 6-9b and d). As the DMS concentration increased, the MM concentration slightly decreased. In addition DMS did not continuously increase through the incubation but maintained at a constant level (less than 0.02 mg S/g VSS in the incubation of NAS while less than 0.05 mg S/g VSS in the incubation of BAS) after 120 hours to the end of sludge incubation. This pattern was similar to that observed in the incubation of WMS.

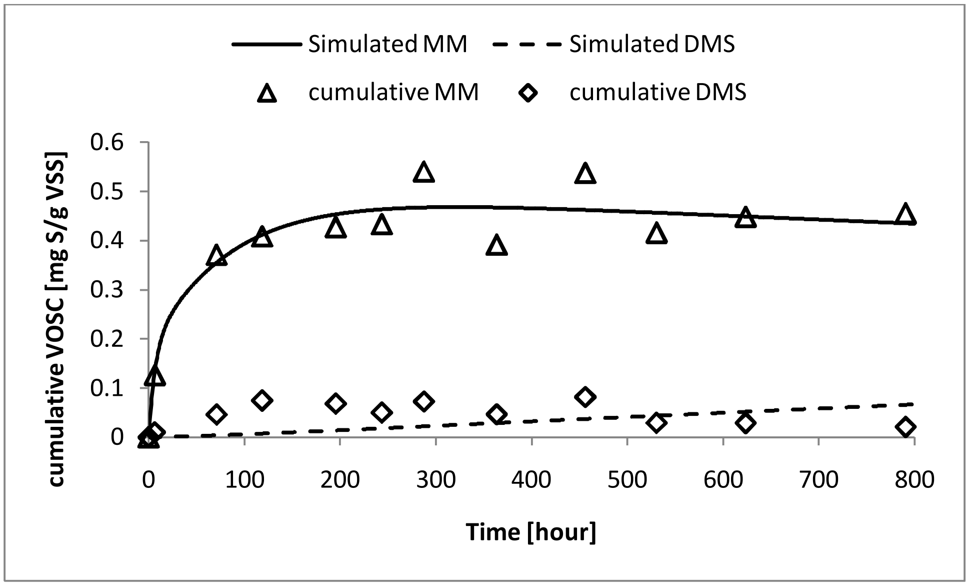

Figure 6-9a VOSC accumulation (55 °C, WMS with inhibition)

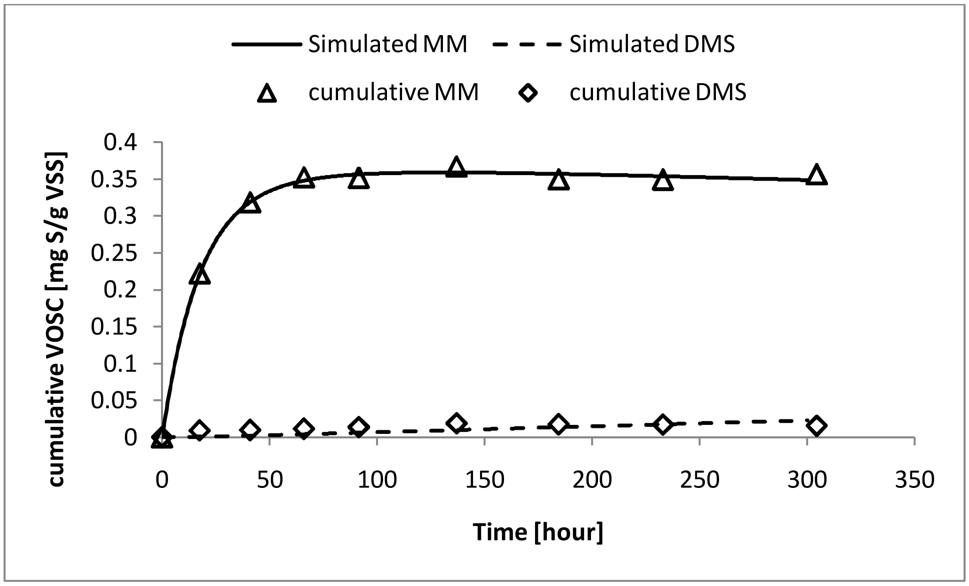

Figure 6-9b VOSC accumulation (55 °C, NAS with inhibition)

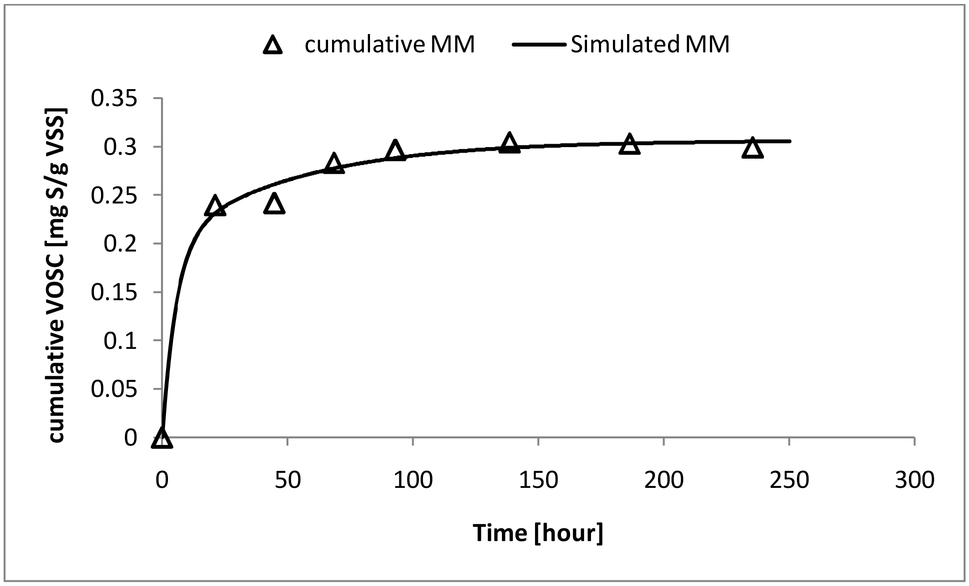

Figure 6-9c VOSC accumulation (55 °C, BPS with inhibition)

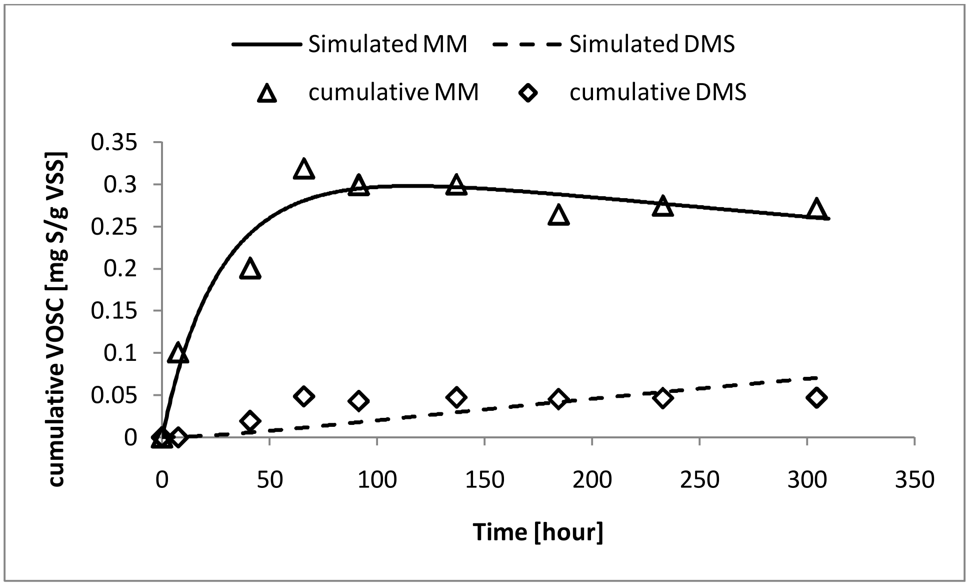

Figure 6-9d VOSC accumulation (55 °C, BAS with inhibition)

At 35 °C, the VOSC release patterns fell into two different groups. In one group, MM was the dominant VOSC with relatively low concentrations of DMS generated. In the other group, DMS accumulated to concentrations that were higher than the MM values.

The VOSCs released from the incubation of NAS and BPS with methanogen inhibition at 35 °C are shown in Figures 6-10b and c respectively. The VOSC responses observed under these conditions were similarly to the VOSC release in thermophilic incubations. MM was generated as the dominant product and accumulated to 0.30 and 0.28 mg S/g VSS in NAS and BPS, respectively. DMS was generated but at low concentrations (0.02 and 0.01 mg S/g VSS in NAS and BPS, respectively).

The incubations of WMS and BAS at 35 °C differed from all of the other incubations in that high levels of DMS accumulated in the bottles. From Figure 6-10a it can be observed that incubation of WMS resulted in DMS accumulation to a level as high as 0.32 mg S/g VSS after 120 hours of incubation and this concentration was maintained for the rest of the incubation. MM slowly accumulated to 0.08 mg S/g VSS after 250 hours of incubation and then decreased to 0.04 mg S/g VSS. Similarly, the incubation of BAS (Figure 6-10d) demonstrated a gradual increase in DMS in the first 150 hours of incubation, and this was maintained above 0.35 mg S/g VSS until the end of the incubation. The MM concentration quickly increased to 0.1 mg S/g VSS after 20 hours of incubation after which it dropped to 0.02 mg S/g VSS at 45 hours and then slowly increased back to 0.1 mg S/g VSS after 200 hours of incubation.

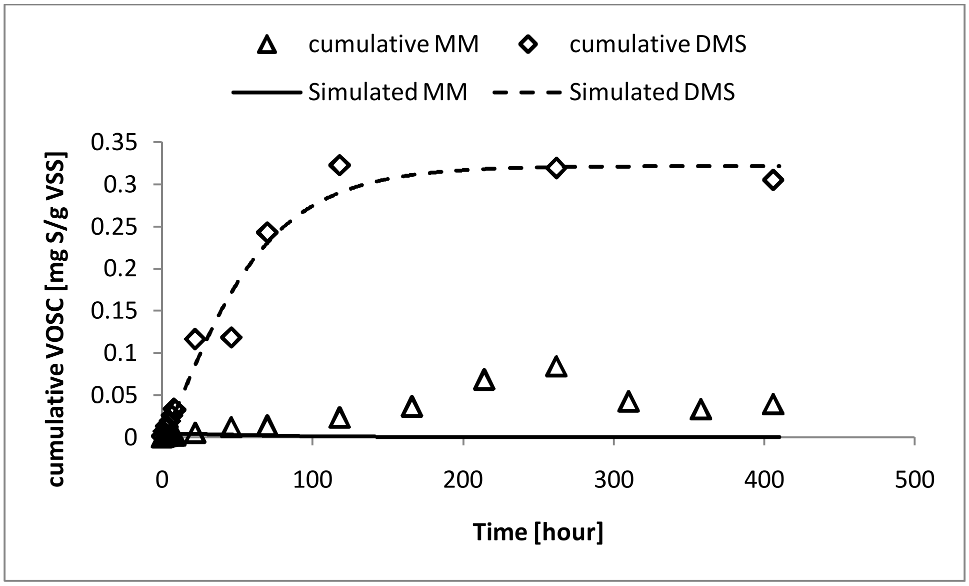

Figure 6-10a VOSC accumulation (35 °C, WMS with inhibition)

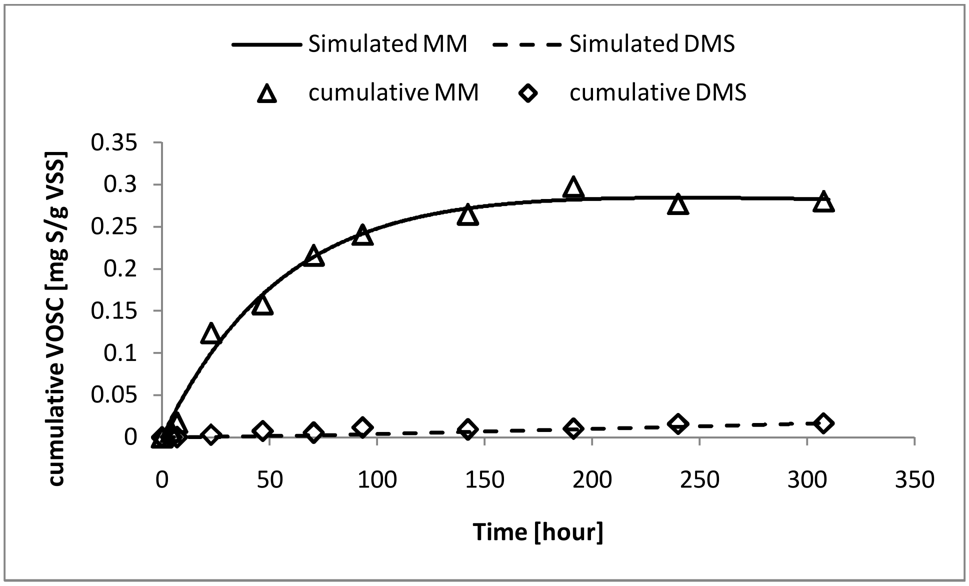

Figure 6-10b VOSC accumulation (35 °C, NAS with inhibition)

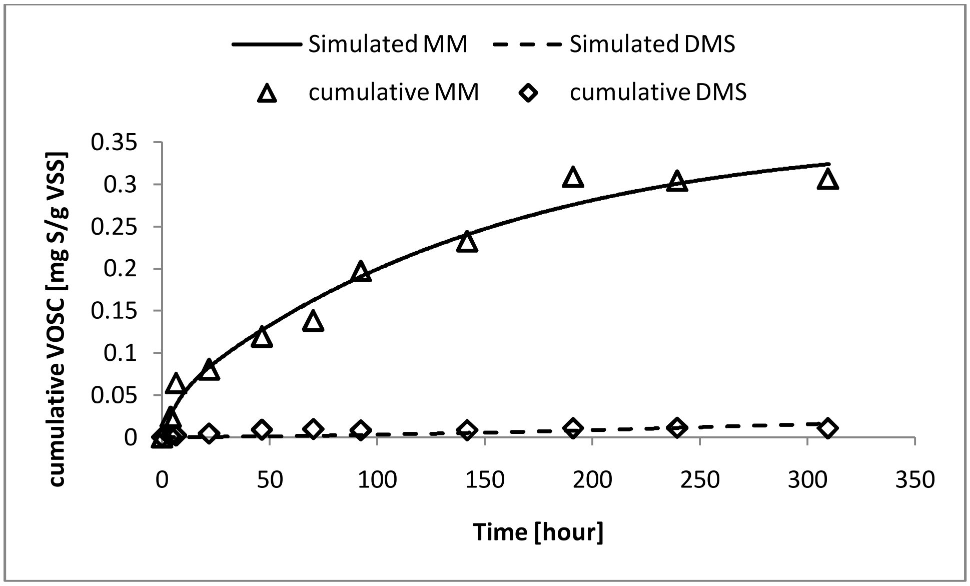

Figure 6-10c VOSC accumulation (35 °C, BPS with inhibition)

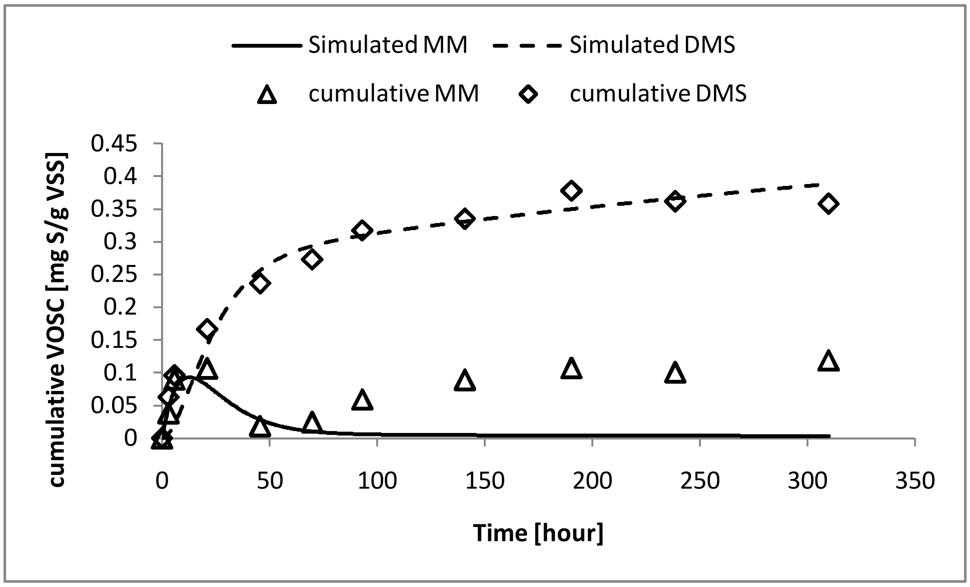

Figure 6-10d VOSC accumulation (35 °C, BAS with inhibition)

To better understand the effect of sludge source and temperature and potentially identify the cause of the different VOSC release patterns, the variety of released VOSC in the incubation of methionine and the raw sludge samples with methanogen inhibition were summarized in table 6-9. The differences in the VOSC release patterns were discussed. It was a potential effective factor in addition to sludge source and temperature that sludge samples used for the mesophilic and thermophilic incubations were obtained on two independent sampling days.

Table 6-9 VOSC variety in incubations with methanogens inhibited

VOSC	Temperature (°C)	Methionine	WMS	NAS	BPS	BAS
MM	35	Dominant	Low	Dominant	Dominant	Low
	55	Dominant	Dominant	Dominant	Dominant	Dominant
DMS	35	Not detected	Dominant	Low	Low	Dominant
	55	Not detected	Low	Low	Not detected	Low

From table 6-9, it can be observed that the VOSC release patterns were consistent at different temperatures when the substrate was either methionine, the activated sludge sample NAS and the primary sludge BPS. MM was the only VOSC detected in methionine digestion at both temperatures. Similarly, MM was the dominant VOSC product in NAS and BPS digestion at both temperatures and only low concentration of DMS was also detected. In addition, during digestion of NAS and BPS, DMS attained a low concentration after a period incubation (if it was generated) and did not continuously increase. Methylation could not explain this pattern of DMS accumulation and hence it would appear that the DMS produced in these tests came from a different and unidentified source. The reason for the lack of methylation reactions in the incubations with methanogen inhibition could not be explained.

The speciation of VOSC observed in the incubation of the activated sludge BAS and the mixed sludge WMS were similar at both temperatures. At 55 °C, MM was the dominant VOSC while DMS was generated at low concentrations. However, DMS was the dominant VOSC generated in the incubations at 35 °C. These patterns were repeatedly observed with samples of WMS that were obtained on two separate days. The same sludge source resulted in different VOSC release patterns at two temperatures. The results suggested that an unidentified mechanism was triggered only at mesophilic temperatures. This unidentified DMS generation mechanism occurred in digestion of two sludge samples which contained activated sludge but not in all the activated sludge samples (e.g. not in NAS).

To better interpret the different VOSC release patterns, iron addition to the sludge source was investigated. In the Waterloo WWTP iron was dosed in the aeration tank for phosphorus removal while in the Burlington (Skyway) WWTP iron was dosed into the primary clarifier and aeration tank. WMS was the mixture of primary and activated sludge from Waterloo WWTP. At the Waterloo and Burlington WWTPs, iron was dosed as ferric chloride. The pilot SBR at the New Hamburg WWTP did not have iron addition.

The measured iron concentration in WMS was between 30 – 39 mg Fe/g TSS. BPS had an iron concentration of 37 – 56 mg Fe/g TSS while BAS had an iron concentration of 79 – 96 mg Fe/g VSS. Without any iron addition, the iron concentration in NAS was less than 10 mg Fe/g TSS. It appears that digestion of the activated sludges which contained high iron concentrations (above 30 mg Fe/g TSS) could generate DMS as the dominant VOSC at 35 °C. The presence of iron in the primary sludge did not appear to affect VOSC release during digestion. In addition, iron concentration in the activated sludge samples did not affect the VOSC release in their digestion at 55 °C.

In summary, in the digestion of sludge more complicated VOSC release patterns were observed as compared to the VOSC generation in methionine digestion. The VOSC release varied with both sludge source and temperature. VOSC release patterns in the mesophilic incubation of BAS and WMS significantly differed from other sludge incubations. It appears that an unidentified DMS generation mechanism occurred at mesophilic temperatures in the digestion of sludge samples which contained activated sludge that was iron dosed. Iron was identified as an important growth factor of anaerobic microorganisms. Iron has been reported to chelate with cysteine and affect its microbiological uptake (Speece, 1996). In addition the presence of iron has been reported to lead to a shift in predominance from Methanosaeta (acetate-utilizing methanogens) to Methanosarcina (methylotrophic methanogens) (Speece, 1996). The abundant DMS generation with iron dosed activated sludges could be associated with the impact of iron on the bioactivities and protein degradation.

Pathways/mechanisms for direct generation of abundant DMS from non-protein sulfur sources have been reported in studies pertinent to seawaters (Chapter 2). However, studies which have illustrated DMS generation from non-protein sulfur sources in controlled digestion environments have not been reported. In addition, DMS generation pathways that are promoted by the presence of iron at mesophilic temperatures alone have not been documented.

6.2.3.2 Modeling VOSC Release

The sludge incubations with methanogen inhibition revealed that VOSC generation from sludge could not always be simply described by the conversion processes introduced in Chapter 5. The unidentified sulfur containing substrate and DMS generation pathway that were suggested in some of the batch digestions were not well described by the models developed based upon the single substrate tests (Chapter 5) and have not been reported in the literature. In the absence of an alternative conceptual model, the methylation process was used to describe DMS generation. Kinetic parameters for methylation were re-estimated in the sludge incubation to best fit the generated DMS concentrations. The methionine decay pathway introduced in Chapter 5 was employed because MM was still the dominant VOSC in most sludge incubations. A hydrolysis process was added to describe the solubilization of the particulate matter in the sludge.

1. Coefficient Estimation

A model which describes VOSC generation and accumulation in the processes of hydrolysis, methionine decay, and MM methylation is tabulated in table 6-10. In this model, hydrolysis of proteinaceous materials and release of free methionine from the particulate forms determined the VOSC release rates and the hydrolysis coefficients for different sludge samples were estimated from the experimental data. Using software AQUASIM, K_S and K_{methy} were estimated simultaneously in fitting the measured MM and DMS data based on the least squares method.

Table 6-10 Model for sludge incubation with methanogen inhibition (concentration unit: mg S/l)

Process	X_{met}	S_{met}	C_MM	C_DMS	Rate
Hydrolysis	-1	1			$K_S \times X_{met}$
Decay of methionine		-1	1		$K_{met} \times S_{met} \times VSS$
Methylation			-1	1	$K_{methy} \times C\_MMliq \times VSS$

According to visual examination and the R^2 values of the simulation curves for MM accumulation, it was found that the model in table 6-10 and the estimated parameters could well simulate the accumulation of MM in all the thermophilic incubations and in the NAS and BPS mesophilic incubations (Figure 6-9a ~ d and in Figure 6-10b and c). However, in these incubations, the simulated DMS concentrations were not well described by the simulations that were conducted with the best-fitted parameter values of K_{methy} (low R^2 values). In Figures 6-9a ~ d and in Figures 6-10b and c, it can be seen that the observed DMS concentrations initially increased quickly and then maintained a constant level for the remainder of the tests. By contrast, the simulated values continuously increased. This lack-of-fit was due to the limitation of the model which apparently did not include all the sulfur sources and biochemical pathways for DMS generation. The best fit parameter values K_S and K_{methy} were listed in table 6-11.

In Figure 6-10a and d, the simulated DMS accumulation curves by the model matched the measured DMS data on the basis of visual examination and higher R^2 values. However, when the observed MM and DMS accumulation were looked at together, their variation did not represent the pattern for the sequent reactions of MM generation and methylation. The model could not describe the VOSC generation in mesophilic digestion of WMS and BAS. The best fit parameter values are listed in table 6-11 only for completion and reference.

Table 6-11 Estimated parameters in raw sludge incubation

35 °C	WMS		NAS		BPS		BAS	
	Estimate	STD	Estimate	STD	Estimate	STD	Estimate	STD
K_S (h^{-1})	2.29×10^{-2}	1.06×10^{-3}	1.86×10^{-2}	5.96×10^{-3}	6.13×10^{-3}	1.14×10^{-3}	2.01×10^{-2}	1.06×10^{-2}
R^2	<0.5		0.98		0.98		<0.5	
K_{methy} (l/g/h)	5.24×10^{-2}	4.38×10^{-3}	4.11×10^{-5}	4.98×10^{-6}	9.28×10^{-6}	5.13×10^{-6}	7.21×10^{-3}	4.38×10^{-3}
R^2	0.99		0.57		<0.5		0.94	
55 °C	WMS		NAS		BPS		BAS	
	Estimate	STD	Estimate	STD	Estimate	STD	Estimate	STD
K_S (h^{-1})	1.15×10^{-2}	4.85×10^{-3}	2.09×10^{-2}	3.50×10^{-3}	1.89×10^{-2}	8.09×10^{-3}	2.97×10^{-2}	2.38×10^{-2}
R^2	0.92		1.00		0.99		0.95	
K_{methy} (l/g/h)	1.189×10^{-5}	4.77×10^{-6}	3.72×10^{-5}	7.33×10^{-6}	--	--	1.28×10^{-5}	1.02×10^{-5}
R^2	<0.5		<0.5		--		<0.5	

2. K_S for Hydrolysis

The effect of temperature on the K_S values was estimated for NAS and BPS sludge samples by comparing the relevant K_S values in table 6-11. The effect of temperature was not assessed for WMS and BAS because the VOSC release in the mesophilic incubations of these sludge samples was not well described by the model. For NAS and BPS, the K_S values were higher at 55 °C as compared to these at 35 °C. For the activated sludge (NAS), the K_S value at 55 °C was 1.1 times of the K_S value at

35 °C. However, for the primary sludge (BPS), the K_S value at 55 °C was 3.1 times of the K_S value at 35 °C. While the value of K_S of NAS was similar to K_S of BPS at 55 °C (0.0209 and 0.0189 h^{-1}, respectively), the K_S value of BPS was only one third of the K_S value of NAS at 35 °C. The results suggest that higher temperature will significantly stimulate the hydrolysis of sulfur containing compounds in primary sludge.

The effect of sludge source on the K_S values was assessed by comparing values in the same rows of table 6-11. At 35 °C, only the K_S values of NAS and BPS were compared and it can be observed that the value of K_S of the activated sludge NAS was about 3 times of the K_S of the primary sludge BPS. At 55 °C, the values of K_S of all the sludge samples were compared. The K_S values of the activated sludge samples NAS and BAS were higher than the K_S values of the primary sludge BPS and the mixed sludge WMS. However, the difference between the K_S of activated and primary sludge samples were not as significant as that at 35 °C. The results suggest that in general the particulate organic-sulfur in the primary sludge was less accessible /degradable than that in the activated sludge. The difference between K_S values of primary and activated sludge samples was more significant in the digestion at the mesophilic temperature as compared to that at the thermophilic temperature.

The estimated K_S value of each sludge sample was also compared with the corresponding value of K_N. It was assumed that hydrolysis of organic-N and organic-S occurred in the same process and the hydrolysis rates were proportional to their concentrations. Hypothesis tests relying on Student's distribution were conducted between K_S and K_N (Appendix E). At 55 °C, the K_S values of all the four sludge samples were not significantly different from the K_N values. At 35 °C, the estimated K_S value of NAS was statistically the same as its K_N value. However, the estimated K_S value of BPS was statistically different from its estimated K_N value.

In summary, in the thermophilic digestion, the hydrolysis of organic-N could represent the trend of the hydrolysis of organic-S in all the activated sludge, primary sludge, and mixed sludge samples. The same conclusion was also arrived for the mesophilic digestion of one activated sludge sample (NAS). In the mesophilic digestion of primary sludge (BPS), it was observed that organic-S was less accessible to hydrolysis than the organic-N. In the mesophilic digestion of mixed sludge (WMS) and activated sludge (BAS), K_S and K_N were not able to be compared due to the lack-of-fit of the sulfur conversion model.

3. K_{methy} for DMS Generation

As previously discussed, it was found that the model in table 6-10 which employed methylation as the only mechanism for DMS generation could not well describe the pattern of DMS release in sludge digestion. Except for model fitting in WMS and BAS mesophilic incubations, to fit the very low cumulative DMS concentrations in sludge incubations, low K_{methy} values at a magnitude of 10^{-5} were obtained. These values were much smaller than the kinetic parameter values estimated for methylation in the batch tests of MM degradation (Chapter 5). The estimated K_{methy} values in table 6-11 were the best fit parameter values when methylation was utilized as the solo DMS generation pathway. The unidentified DMS generation mechanism/ substrate was found to be challenging in modeling DMS release in sludge digestion.

6.2.4 Summary of Batch Incubations with Methanogen Inhibition

The batch incubations with methanogen inhibition were conducted with sludge samples from four different sources and at two temperatures. The sludge characteristics before and after digestion were analyzed and compared. The hydrolysis coefficients K_{COD}, K_N, and K_S for organic compounds, organic-nitrogen, and organic-sulfur (methionine as the surrogate) were estimated and compared. The VOSC release patterns were also discussed. According to these results, the following conclusions were summarized:

1) The fractions of total nitrogen and total sulfur out of total COD were higher in activated sludge as compared to those in primary and mixed sludge.

2) The total sulfur concentration in the sludge before and after digestion did not significantly change, which suggested the fraction of sulfur released as VOSC was small. The estimated fraction of degradable sulfur out of the organic sulfur in the sludge was 3.7 ~ 10.6% (or 6.3% as the average). The fraction of sulfur which was degradable through the VOSC pathway in activated sludge samples was slightly higher than the fractions in primary and mixed sludge samples. This fraction increased more with the increase of temperature in activated sludge samples as compared to that in primary sludge sample.

3) The hydrolysis of particulate organic matter and organic nitrogen was fit by first order kinetics. The estimated hydrolysis coefficients fell into the ranges reported in literatures. On the basis of the results of WMS incubation, the hydrolysis coefficient K_{COD}, estimated from the solubilization of lumped particulate complex (COD) was similar to the hydrolysis

coefficient K_N that was estimated from the mineralization of lumped organic-nitrogen at both temperatures.

4) The hydrolysis of organic sulfur was also fit by first order kinetics. At 55 °C, the estimated value of K_S had a similar value with K_N for all the four sludge samples. The same conclusion was also arrived for K_S and K_N values of NAS at 35 °C.
5) The hydrolysis coefficients were affected by the sludge source. It was found that primary sludge had smaller K_N and K_S values than the activated sludge. The results suggest that the proteinaceous materials in activated sludge were more accessible to degradation as compared to that in primary sludge. An increase of temperature resulted in increased K_S and K_N values. Temperature had similar effect on K_N of all the sludge samples. However, the decrease of K_S values of primary sludge was more significant than the decrease of K_S values of activated sludge when incubation temperature was changed from 55 to 35 °C.
6) The VOSC releases in the 55 °C incubation of sludge from four different sources had similar patterns: while MM was the dominant VOSC, DMS was detected at low concentrations (if it was generated). It was speculated that an unidentified sulfur source could result in the generation of DMS directly in sludge digestion. At 35 °C, VOSC release from NAS and BPS incubation also followed this pattern. However, in the incubation of WMS and BAS, DMS was the dominant VOSC instead of MM. Abundant DMS generation was only observed in the mesophilic incubation of sludge samples which contained activated sludge and the mechanism of generation was not clear.
7) The model in table 6-10 described MM accumulation in most sludge incubations except for the mesophilic incubations of WMS and BAS. Simulation of DMS generation on the basis of methylation could not describe the observed DMS generation.

6.2.5 Model Evaluation

Data that was collected when WMS was incubated without methanogen inhibition at 35 and 55 °C, respectively was employed to further evaluate the models developed in this study. VOSC concentrations were measured with incubation time and are shown in Figures 6-11a and 6-11b. At 55 °C, MM was detected as the dominant VOSC. Its concentration accumulated from 0 ppm at the beginning to 407 ppm after 21 hours of incubation and then gradually decreased below the detection limit after 70 hours of incubation. DMS slowly increased with the increase of MM, maintained a concentration above 10 ppm till 46 hours and then decreased below the detection limit after 70 hours. At 35 °C, DMS was the dominant VOSC. Its concentration increased to 30 ppm after 8 hours of incubation and maintained above 25 ppm until 21 hours of incubation. After 70 hours of incubation, the DMS concentration decreased to 1.5 ppm. MM was maintained at a low concentration through the incubation and peaked at 3 ppm after 5 hours of incubation.

A model including hydrolysis of particulate matter, methionine decay, and VOSC degradation was assembled to describe sulfur conversion and VOSC release in anaerobic sludge digestion (table 6-12). It includes the hydrolysis process and all the processes in table 5-3. The magnitudes/ ranges of the pertinent parameters were already estimated on the basis of the prior analysis described in this chapter and Chapter 5.

Table 6-12 Model for sulfur conversion in sludge

	X_{met}	S_{met}	C_MM	C_DMS	C_H2S	Rate
Hydrolysis	-1	1				$K_S \times X_{met}$
Methionine decay		-1	1			$K_{met} \times S_{met} \times VSS$
MM degradation			-1		1	$K\_MM \times C\_MM_{liq} \times VSS$
MM methylation to DMS			-1	1		$K_{methy} \times C\_MM_{liq} \times VSS$
DMS degradation				-1	1	$K\_DMS \times C\_MM_{liq} \times VSS$

The data collected in the WMS batch incubations without methanogen inhibition at 35 and 55 °C were used to verify the model. The simulation curves and the measured data are presented in Figures 6-11a and b. The utilized initial concentrations and parameter values are listed in table 6-13.

In the simulations, the VSS concentrations measured (after mixing with the inocula) at the beginning of the incubations (table 6-13) were assumed to be representative of the biomass. The estimated values of K_S and K_{met} (K_{met-s} in table 5-2) were utilized in the simulation. The hydrolysis coefficient K_S at 55 °C that was estimated from the WMS batch tests (with inhibition in table 6-11) was employed in thermophilic simulations. As previously demonstrated, the K_S estimated for WMS was not valid at 35 °C. Since it was found that the values of K_N and K_S of WMS were not significantly different from each other at 55 °C it was therefore assumed that K_N and K_S were also the same at 35 °C and the value of K_N estimated at 35 °C (table 6-6) was utilized here as K_S.

The initial particulate and soluble methionine (Initial_$X_{met\text{-}S}$ and Initial_$S_{met\text{-}S}$) concentrations could not be directly measured. However, in the incubation with methanogen inhibition, it was estimated that the total degradable organic-S through the methionine pathway was 5.2 and 13.5 mg S/g VSS in the mesophilic and thermophilic digestions respectively. Given a range of 0 ~ 5.2 (0 ~ 13.5) mg S/g VSS in the simulation of the mesophilic (thermophilic) digestions, the values of Initial_$X_{met\text{-}S}$ and Initial_$S_{met\text{-}S}$ were adjusted simultaneously with the parameters K_{methy}, K_MM and K_DMS, within the ranges listed in table 5-1 to obtain the best fit of the data. These values are summarized in table 6-13.

Table 6-13 Parameters used in the simulation of WMS batch tests

Parameter	Unit	35 °C	55 °C
Initial_$X_{met\text{-}S}$	mg/l	4.61	9.83
Initial_$S_{met\text{-}S}$	mg/l	1.77	3.02
K_S	1/h	0.0079	0.013
Kmet	l/g/h	0.0038	0.0055
K_MM	l/g/h	0.047	0.012
K_{methy}	l/g/h	0.0057	0.001
K_DMS	l/g/h	0.016	0.006
VSS	g/l	19	22.6

Considerable volumes of biogas were generated during the incubation of WMS without methanogen inhibition and in the tests biogas was released at particular points in time. The simulations that were conducted with the model in table 6-12 incorporated the gas release events. It was assumed that when VOSC was generated in the liquid phase, gas-liquid equilibrium was reached instantly. At the time of biogas release, the gas was released till the headspace reached atmosphere pressure. This led to a change of the VOSC concentration in the headspace and the change of the equilibrium concentration of VOSC in the sludge.

Figure 6-12 shows the effect of biogas release on VOSC concentrations. V_l, V_{HS}, and V were the volume of liquid phase, headspace, and released biogas, respectively. At the beginning of incubation, the initial particulate and soluble methionine sulfur concentrations were Initial_$X_{met\text{-}s}$ and Initial_$S_{met\text{-}s}$ while the concentrations of MM and DMS in the liquid and gas phases were assumed to be zero. Simulation was conducted until the first time point of biogas release. The particulate and soluble methionine were degraded to the concentrations of $X_{\text{met-S},t}$ and $S_{\text{met-S},t}$. VOSC was assumed to be generated and degraded in the liquid phase only and then partitioned in the liquid and gas phase. The total VOSC mass will be sum of VOSC masses in the liquid and gas phases, which was ($C_{VOSC,gas,t} \times V_{HS} + C_{VOSC,liq,t} \times V_l$). Biogas was generated during the incubation and was independent from VOSC conversions. However, biogas accumulation would change the pressure in the headspace (higher than 1 atm). Partitioning of VOSC under this pressure satisfied $k'_{VOSC} = C_{VOSC,gas,t}/C_{VOSC,liq,t}$, where k'_{VOSC} was the partitioning coefficient of VOSC at this pressure. During and after biogas release (at time moment t+), the pressure in the headspace would be back to 1 atm while VOSC mass would lose with V ml of biogas release. The VOSC mass in the serum bottle changed to ($C_{VOSC,gas,t+} \times V_{HS} + C_{VOSC,liq,t+} \times V_l$). Partitioning of VOSC at moment t+ satisfied $k_{VOSC} = C_{VOSC,gas,t+}/C_{VOSC,liq,t+}$. The mass balance was shown in Figure 6-12 by taking MM as an example. k'_{VOSC} was not assessed in the present study and was assumed to equal k_{VOSC} that was estimated at 1 atm. Therefore, the concentrations of $C_{VOSC,t+}$ were able to be calculated from $C_{VOSC,t}$.

After biogas release, the simulation time was reset to zero. VOSC concentrations in the headspace and liquid phase changed from $C_{VOSC,t}$ to $C_{VOSC,t+}$. $X_{\text{met-S},t}$ and $S_{\text{met-S},t}$ were the used as the initial particulate and soluble methionine sulfur concentrations at the beginning of the subsequent time step. Simultaneously, $C_{MMliq,t+}$ and $C_{MMgas,t+}$ were used as the initial MM concentrations in the liquid and gas phases while $C_{DMSliq,t+}$ and $C_{DMSgas,t+}$ were used as the initial DMS concentrations in the liquid and gas phases at time zero. Decline of methionine and conversion of VOSC would be simulated from this moment to the next time point of biogas release.

As demonstrated in Figure 6-11a the model was able to represent the VOSC release patterns at 55 °C, including the quick accumulation of MM and its subsequent decay and the generation of DMS and its disappearance with MM. The apparent discontinuities in the curves were due to the biogas release from the serum bottle which was performed at particular points in time.

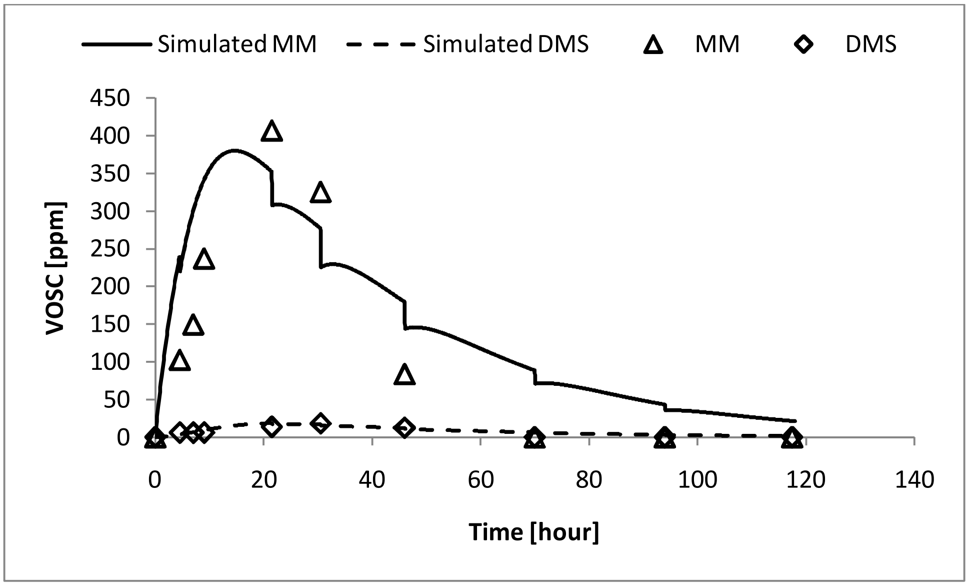

Figure 6-11a VOSC release in the WMS incubation without methanogen inhibition at 55 °C

At 35 °C, even though the parameters were re-adjusted to best fit the VOSC variation, the model in table 6-12 could not well simulate MM and DMS patterns. When the model was adjusted to fit MM and DMS release simultaneously, as shown in Figure 6-11b, the simulated MM concentrations were higher than the measured concentrations. On the basis of the model formulation, for high concentrations of DMS to be generated by methylation, the observed MM concentrations had to be as high as the level represented by the simulated MM curve. This VOSC release pattern was similar with the VOSC release observed in the WMS incubation with methanogen inhibition. The mechanism for the generation of DMS beyond the capacity of methylation at 35 °C but not at 55 °C was not clear. The present model could not well simulate the observed VOSC release pattern in WMS incubation at 35 °C.

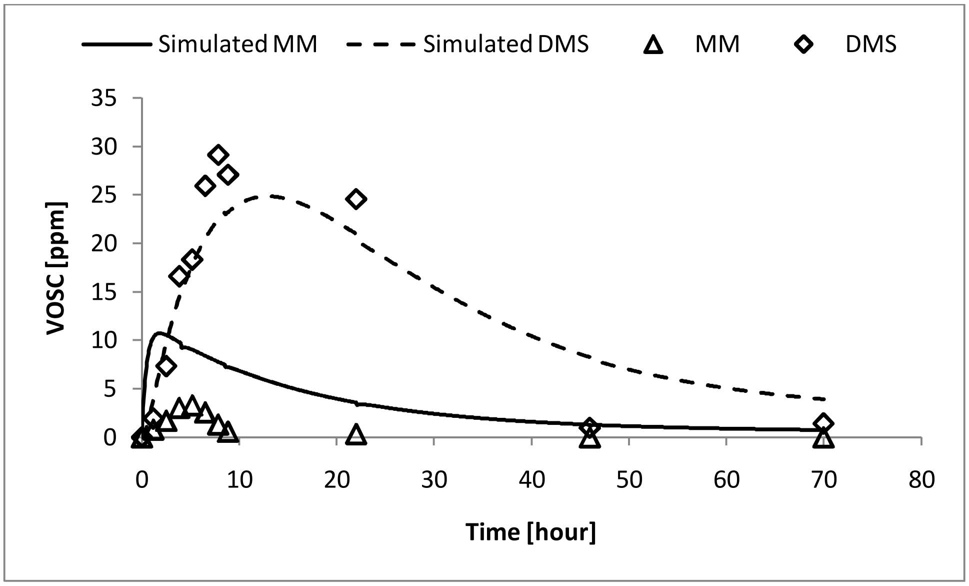

Figure 6-11b VOSC release in the WMS incubation without methanogen inhibition at 35 °C

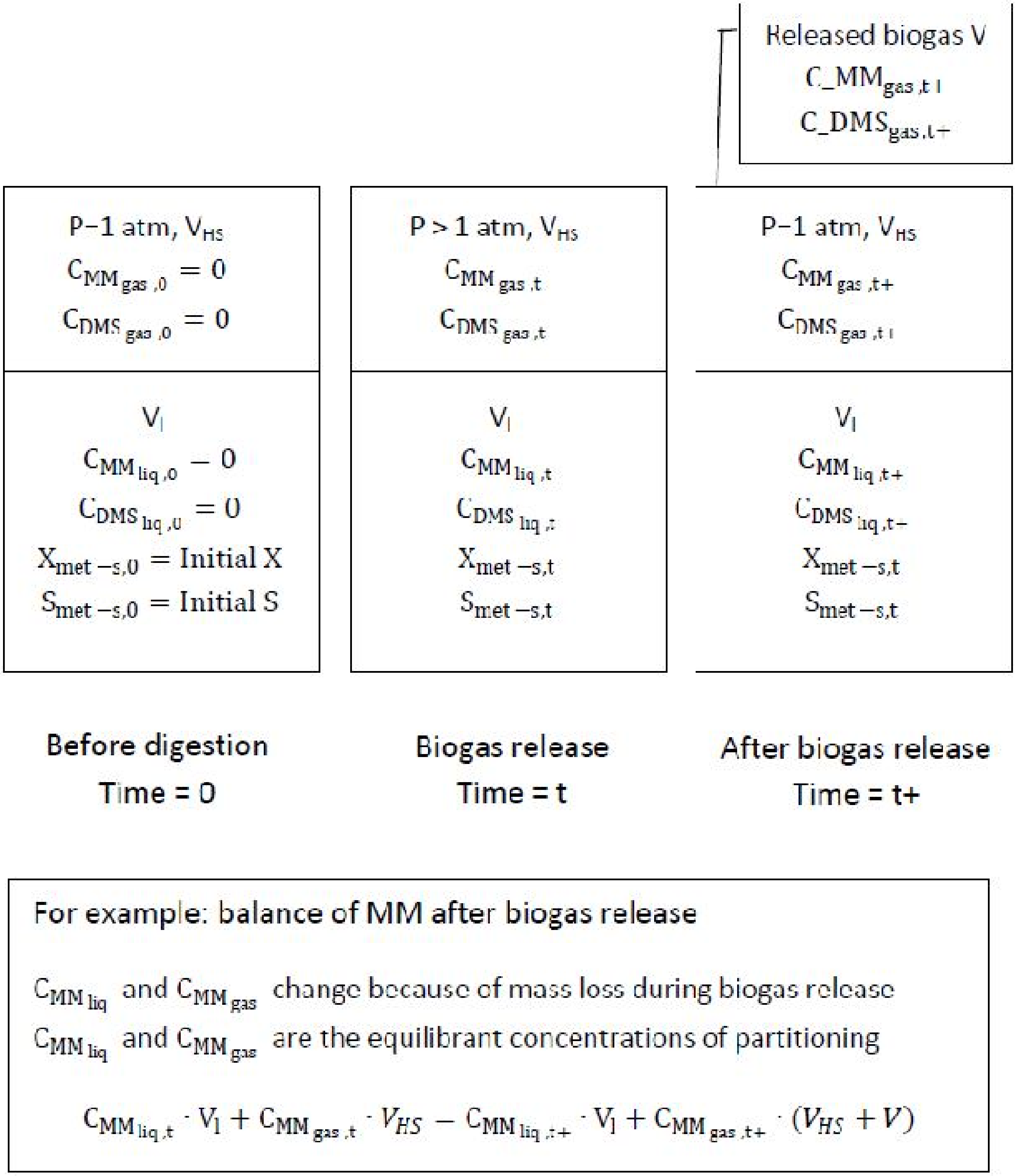

Figure 6-12 VOSC mass balance before and after biogas release

6.2.6 Sulfur Conversion in the Bench-Scale Reactors

6.2.6.1 Sludge Characteristics

The two bench-scale reactors that were operated at 35 and 55 °C in this study were fed with the mixed Waterloo sludge (WMS). Weekly monitoring was conducted to characterize the feed and the digested sludge samples. Important conventional and sulfur related parameters for the raw and digested sludge samples are listed in table 6-14.

Table 6-14 Sludge characteristics for bench-scale reactors

Sludge type	Feed (WMS)	Mesophilic digested	Thermophilic digested
VSS (g/l)	23.6 ± 7.8	11.7 ± 1.4	9.0 ± 1.1
Total COD (×10^4 mg/l)	4.6 ± 0.5	2.4 ± 0.1	2.4 ± 0.2
Total TKN (×10^3 mg/l)	2.1 ± 0.3	2.2 ± 0.2	2.1 ± 0.2
Ammonia (×10^2 mg/l)	1.1 ± 0.8	8.0 ± 0.6	10.0 ± 0.8
Total S (×10^2 mg/l)	2.5 ± 0.7	2.7 ± 0.2	2.7 ± 0.1
Soluble S (mg/l)	9.1 ± 2.4	27.2 ± 4.2	56.4 ± 16.2
Precipitated S (×10^2 mg/l)	1.1 ± 0.3	1.3 ± 0.1	1.2 ± 0.1
Particulate organic S (×10^2 mg/l)	1.4 ± 0.5	1.1 ± 0.2	1.0 ± 0.1

The data in table 6-14 reflected long-term average values of the parameters. They were employed to describe the nitrogen and sulfur composition in the digested sludge samples which were obtained from steady state reactors. In addition they were employed to compare the composition of the raw and digested sludge samples.

From Table 6-14 it can be seen that the VSS reduction was 51 % and 62% in the mesophilic and thermophilic reactors, respectively. The VSS values of the digested sludge were used as the biomass concentrations in the model simulation. The COD removal rate was 47% for both reactors. The results suggested that same amounts of methane were generated in two reactors with the same sludge feeding however more particulate matter dissolved in the digestion at the higher temperature. The extent of mineralization of organic nitrogen was 33% and 43% for the mesophilic and thermophilic reactor, respectively.

The total sulfur concentrations in the feed and digested sludge samples were at similar levels. This result was consistent with that observed in sludge batch tests and confirms that only a small fraction of the total sulfur would be released as VOSC in the digestion.

The sulfur species in the feed and digested sludge samples were compared (Figure 6-13a ~ c) to characterize the changes in the sulfur species through digestion. With similar total sulfur concentrations, the fraction of particulate organic sulfur in the sludge significantly decreased after digestion. Simultaneously, the fraction of soluble sulfur significantly increased. The soluble sulfur was mainly composed of organic sulfur because the iron concentration in the sludge was high (above 2×10^3 mg/l) and hence would precipitate any sulfide that was generated.

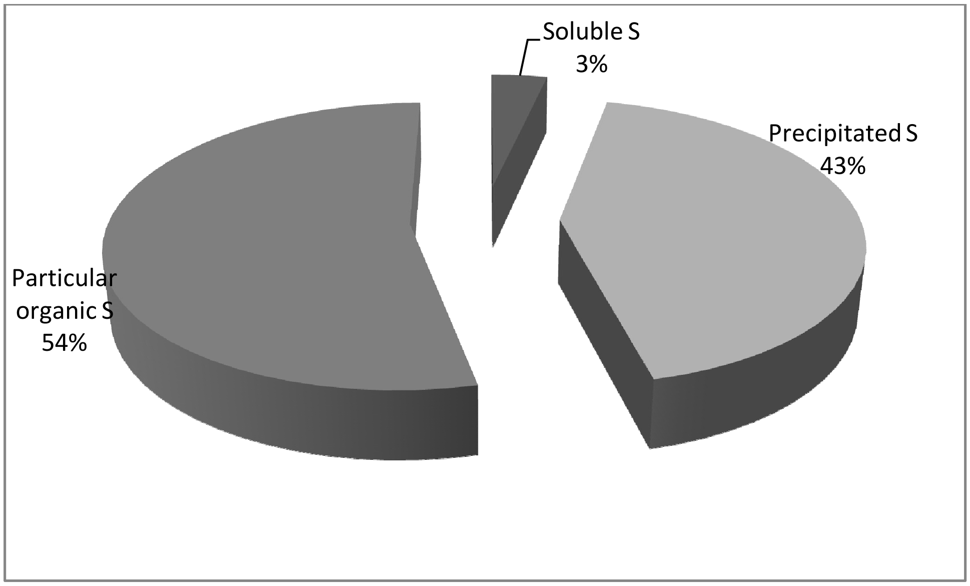

Figure 6-13a Sulfur species in the feed sludge

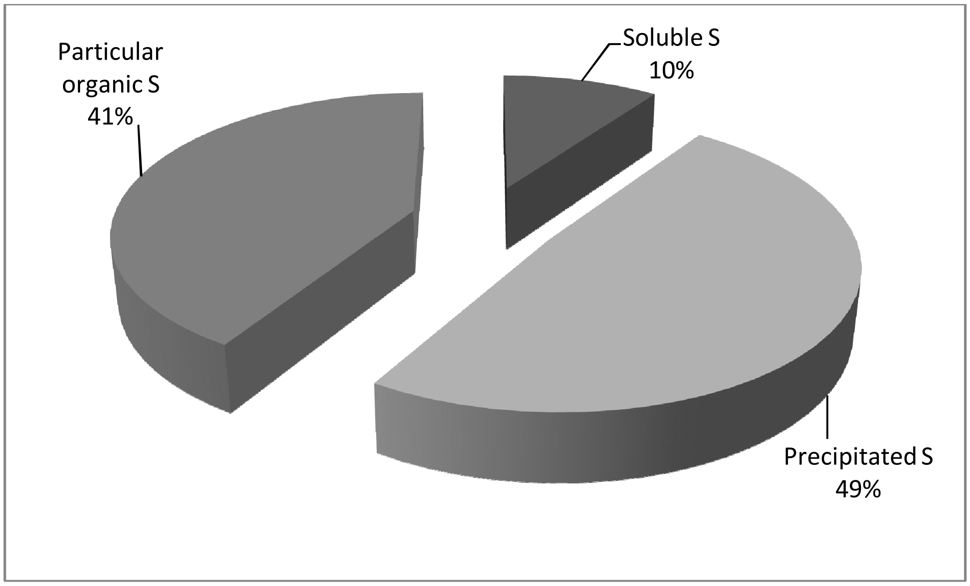

Figure 6-13b Sulfur species in the mesophilic digested sludge

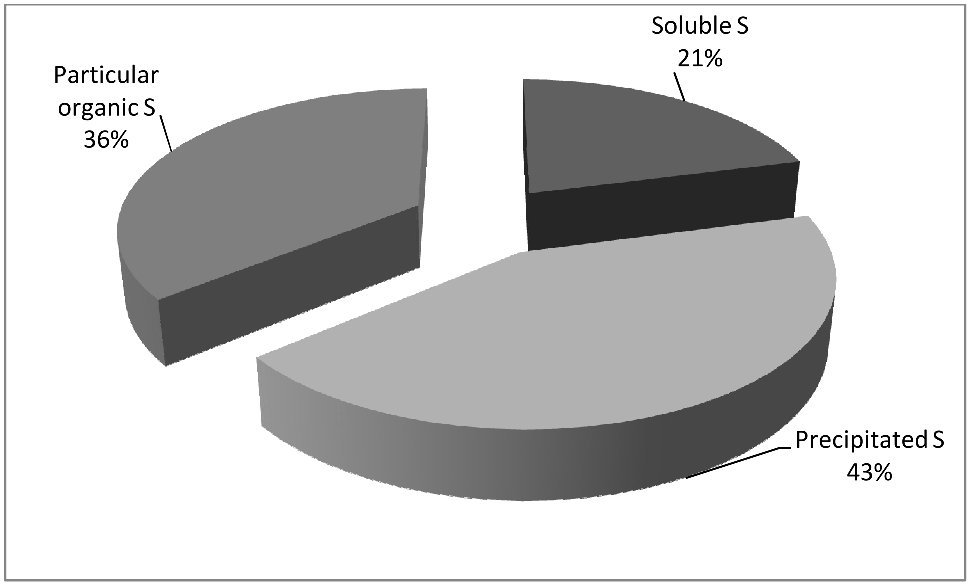

Figure 6-13c Sulfur species in the thermophilic digested sludge

6.2.6.2 VSC Release

In the long term monitoring, the VSC concentrations in the headspace of the two reactors were measured immediately before feeding. In these samples only H_2S was detected and its concentration in the mesophilic and thermophilic reactor headspaces was 30 ± 13 ppm and 26 ± 9 ppm, respectively. The results suggest that any generated VOSCs were degraded to concentrations which were lower than their detection limits within the feeding intervals.

In section 6.2.3 it was found that only 3.7% and 10.6% of the organic sulfur in the raw sludge was degraded via VOSC during the mesophilic and thermophilic digestion respectively. According to these fraction values, when 1 L raw sludge was fed, there would be approximately 4.7 and 13.1 mg of organic-sulfur degraded in mesophilic and thermophilic digesters, which would result in about 0.26 and 0.73 mg S/l after feeding and mixing the raw sludge in the reactors. To increase the initial concentration of degradable organic-S for more VOSC generation and prolonged presence of VOSC, methionine was dosed into reactors to evaluate the model with respect to prediction of VOSC release patterns.

1. Methionine Dosed Tests

The developed model (except for the hydrolysis coefficient which depended on the sludge source) and the estimated model parameters were evaluated through simulating the VOSC release when pure methionine was dosed into the reactors instead of feeding sludge. Final concentrations of methionine-sulfur of 18 and 9 mg/l were dosed into the mesophilic reactor in separate events while final concentrations of methionine-sulfur of 12 and 6 mg/l were dosed into the thermophilic reactor. Under the methionine dosed conditions, the biodegradable sulfur was much higher than that in the sludge and no significant volume of biogas was generated to dilute the headspace concentrations.

Intensive monitoring of the concentrations of VOSC in the headspace was conducted for 12 hours after methionine addition. At 35 °C (Figure 6-14a), MM accumulated to 583 ppm within 6 hours after methionine addition and maintained around 400 ppm 12 hours after methionine was added. DMS gradually accumulated to 162 ppm 12 hours after methionine addition. At 55 °C (Figure 6-14b), MM reached a peak value of 2300 ppm and remained above 1300 ppm 12 hours after methionine addition. DMS slowly accumulated to 200 ppm over the12 hours after methionine addition.

The model in table 5-1 was employed to simulate the VOSC responses after methionine addition. The VSS concentrations that were measured in the two digesters were employed in the simulations and K_{met}, K_{methy}, K_MM, and K_DMS were re-adjusted within their 99% confidence intervals to best fit the measured data. The re-adjusted values are listed in table 6-15. Figures 6-14a and 6-14b show the simulated and measured data in the mesophilic digester that was initially dosed with 18 mg/l methionine-sulfur and in the thermophilic digester that was initially dosed with 12 mg/l methionine-sulfur, respectively. On the basis of visual examination and R^2 values (0.84 and 0.83 for MM and DMS at 35 °C; 0.87 and 0.93 for MM and DMS at 55 °C), the simulation could well represent the measured MM and DMS concentrations within the 12 hours of incubation.

Table 6-15 Parameters used in the simulation of methionine dosed tests

Parameter	Unit	35 °C	55 °C
Initial_$S_{met\text{-}S}$	mg/l	18	12
Kmet	l/g/h	0.0027	0.0083
K_MM	l/g/h	0.027	0.0075
K_{methy}	l/g/h	0.0063	0.0041
K_DMS	l/g/h	0.01	0.004
VSS	g/l	14.2	10.8

VOSC concentrations in the headspaces of two reactors were also measured 24 hours after methionine was dosed. MM in the mesophilic digester was 1.0 ppm while MM in the thermophilic digester was 0.7 ppm. DMS in the mesophilic digester was 144 ppm while DMS in the thermophilic digester was 422 ppm. However, if the model simulation was extended to 24 hours, MM in the mesophilic and thermophilic digester was 190 and 700 ppm respectively while DMS in the mesophilic and thermophilic digester was 67 and 280 ppm respectively. At the time point of 24 hours after methionine addition, the measured MM concentrations in both reactors were much lower than the simulated values while the measured DMS concentrations were higher than the simulated values.

In the methionine dosed digesters, simulation described VOSC release patterns reasonably within 12 hours of incubation but not for the rest time of incubation. The kinetic constants (table 6-15) which were utilized in the simulations were estimated in the serum bottle tests. It appeared that as compared to those in batch tests MM degradation and methylation in the reactors occurred at faster rates. In addition, in the methionine dosed batch incubations, rapid increase of MM from methionine degradation started about 100 hours after methionine addition (Figure 5-5). However, MM was generated instantly in the digesters after methionine was dosed. The instant MM generation and fast MM depletion in digesters with methionine addition suggested that microorganisms in the semi-continuous reactors might better acclimate to the substrate (methionine and VOSC) rather than in the diluted inocula which was utilized in batch tests. The kinetic constants estimated in batch tests might not be able to well describe kinetic processes in digesters.

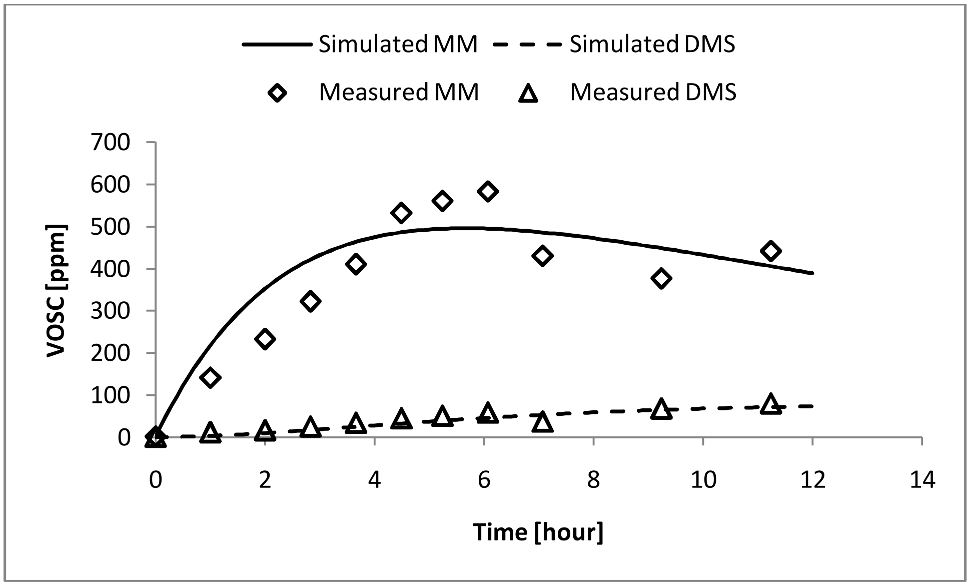

Figure 6-14a VOSC in the mesophilic digestion initially dosed with 18 mg/l methionine-sulfur

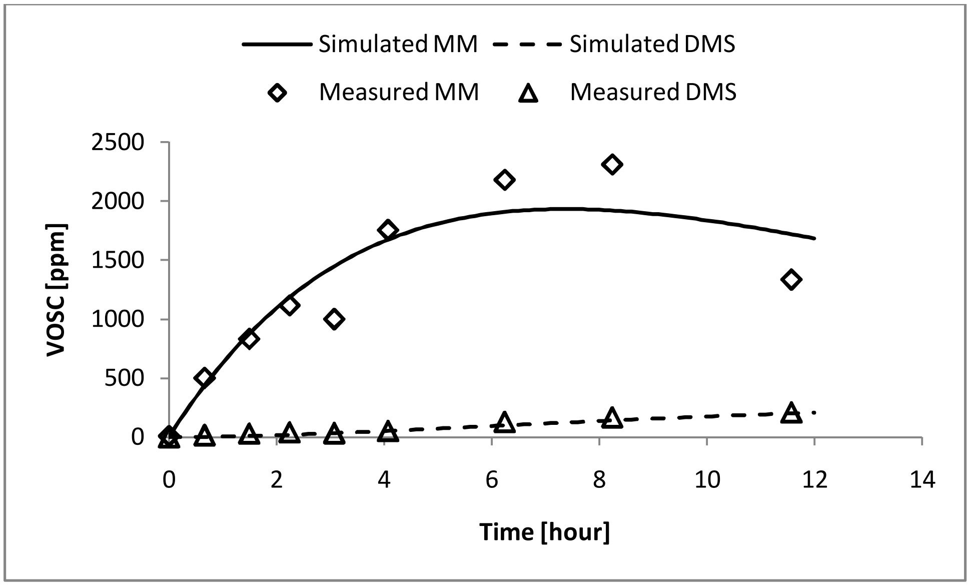

Figure 6-14b VOSC in the thermophilic digestion initially dosed with 12 mg/l methionine-sulfur

2. Raw Sludge Fed Tests

To further assess the model including the particulate organic-sulfur hydrolysis process, in selected sampling events VOSC were monitored once every hour for a period of 6-8 hours after raw sludge feeding. VOSCs were detected in the headspace of the thermophilic reactor on all the sampling days but were not detected in some of the mesophilic reactor sampling events. In these events when VOSC were detected in the headspace, their presence lasted for 5 ~ 7.5 hours after feeding and then their concentrations declined below the detection limits (0.17 ppm for DMS and 0.23 ppm for MM). In some events VOSC were only detected within the first 2 hours or even were not detected after feeding.

Figures 6-15a - 6-15d show the measured VOSC concentrations versus time after feeding sludge on 6 sampling days. The VOSC concentrations and their persistence in the headspace varied amongst the sampling days. In general VOSCs concentrations were higher in the thermophilic reactor. The highest MM concentration detected in these sampling events was 70 ppm in the thermophilic reactor and 7 ppm in the mesophilic reactor. For thermophilic reactor and most sampling events of mesophilic reactor the DMS concentrations were less than MM concentrations and reached peak values later than MM. The highest DMS concentration detected in these sampling events was 18 ppm for the thermophilic reactor and 9 ppm for the mesophilic reactor.

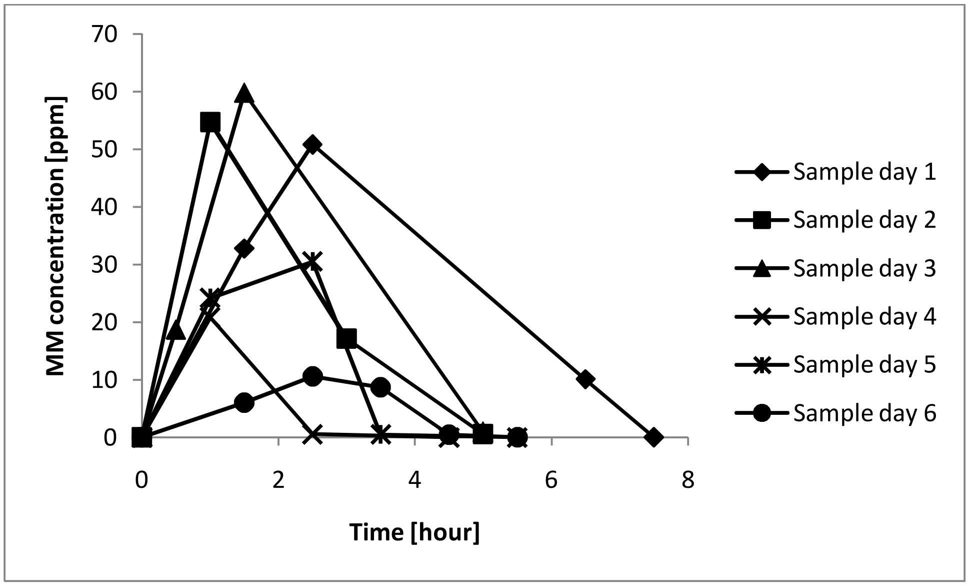

Figure 6-15a Measured MM in the thermophilic reactor headspace after feeding

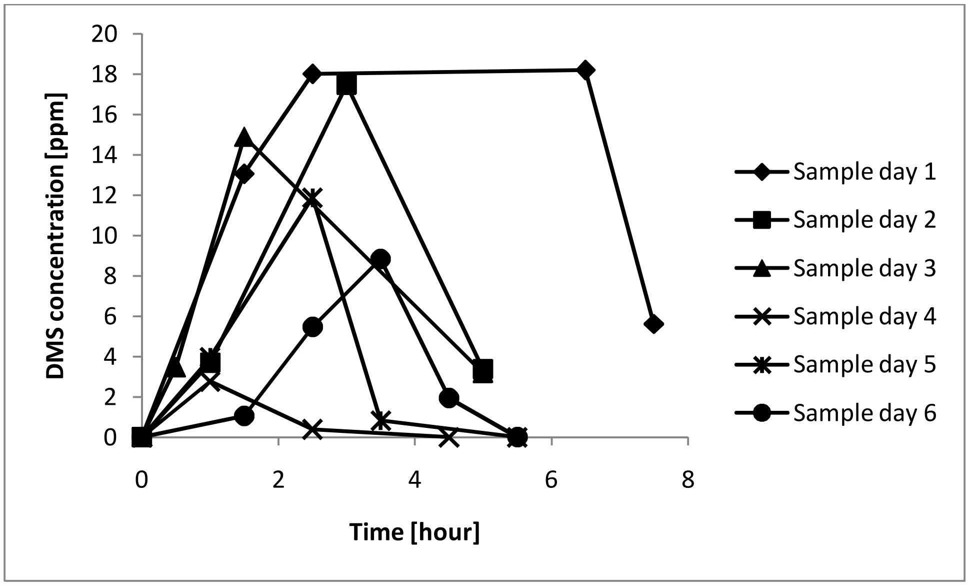

Figure 6-15b Measured DMS in the thermophilic reactor headspace after feeding

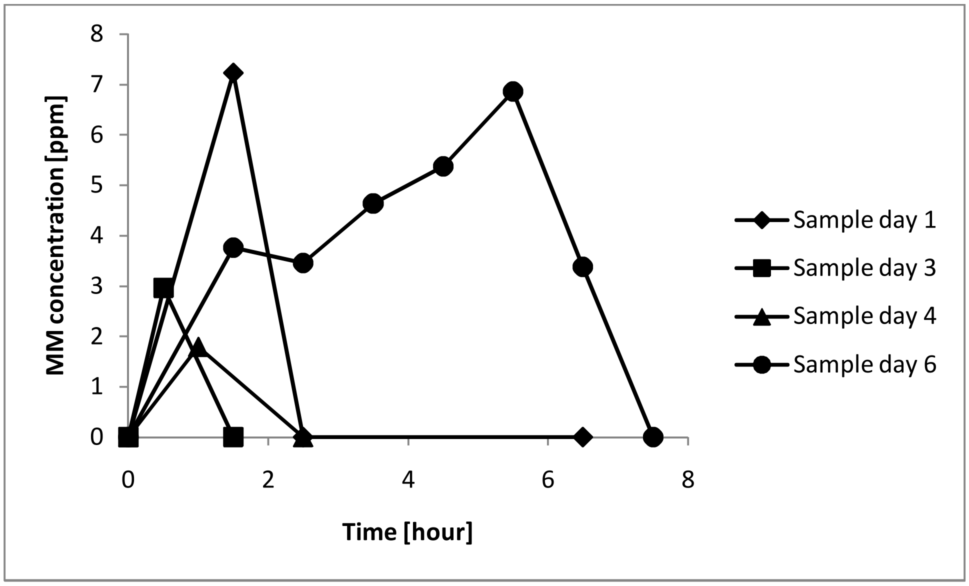

Figure 6-15c Measured MM in the mesophilic reactor headspace after feeding

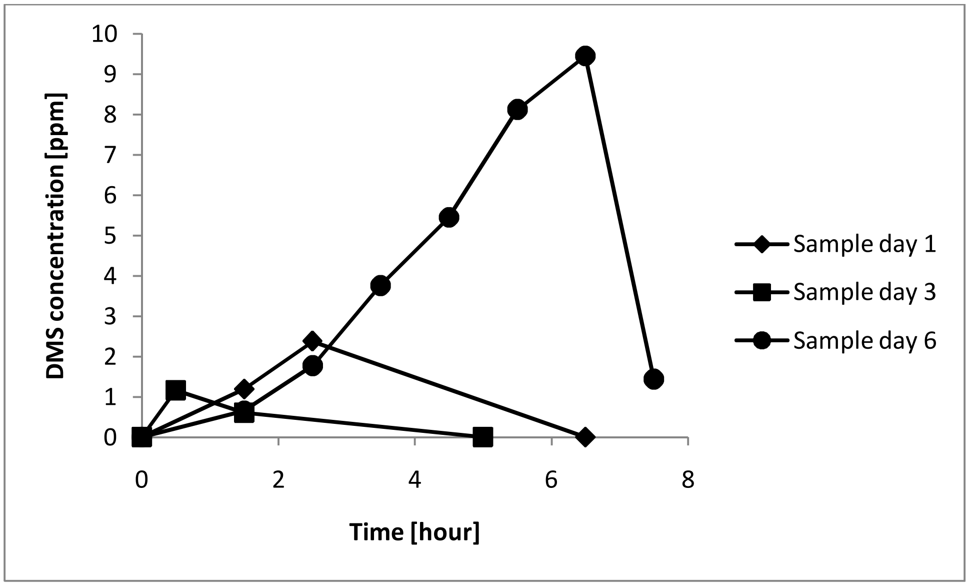

Figure 6-15d Measured DMS in the mesophilic reactor headspace after feeding

To simulate the VOSC release after raw sludge feeding, the large volume of produced biogas needed be considered. In this study, 1 L of raw sludge was pumped into the reactor over a 6 minute period each day and mixed with 18 L of digested sludge in the digester which contained abundant active anaerobic microorganisms. The daily generated biogas volume varied with the characteristics (e.g. COD concentration) of the raw sludge. The average volumes of the released biogas were 407 and 439 ml/hour from mesophilic and thermophilic digesters respectively. The portion of methane in the biogas could be as low as 45% immediately after feeding and increased to about 70% after 24 hours of incubation in both reactors. The released biogas volume increased to a level of 400 ~ 750 ml/hour (varied with days) within 0.5 hour after feeding and maintained this level for 6 ~ 8 hours (with a variation of ±100 ml/hour within the period of time when VOSC were measured). Therefore, for the purpose of modeling it was assumed that biogas was released at a constant flow rate over the duration of VOSC presence.

In the sludge batch test, it was assumed that VOSC partitioning in the sludge and headspace reached equilibrium instantly. In the semi-continuous reactors, however, the biogas was released continuously rather than at particulate time points. Therefore, for the purpose of modeling it was assumed that equilibrium was only reached instantly between the sludge and the generated biogas. The biogas then flowed into the headspace and VOSC concentrations in the headspace would change due to the mixing effect with the headspace gas.

In the raw sludge, the initial methionine concentration could not be directly measured. However, in the batch tests with sludge, it was found 3.7 and 10.6% of the total sulfur in WMS could be degraded in the mesophilic and thermophilic digestion respectively. Based on the estimated initial particulate and soluble methionine concentrations from the batch tests (table 6-13), it was estimated that 70 ~ 75% of the degradable methionine was in the particulate complex in the sludge. Taking sampling day 1 as an example, the total sulfur of the raw WMS sludge was 242.3 mg/l while the organic sulfur was 123.5 mg/l. Assuming 4% and 10% of the organic sulfur was degradable methionine sulfur, the degradable methionine sulfur in mesophilic and thermophilic digestion of 1L of sludge was estimated as 4.9 and 12.4 mg respectively. Assuming 70% of the degradable methionine sulfur was in the particulate matter, the particulate and soluble methionine quantities were 3.5 and 1.4 mg in the mesophilic digester and 8.7 and 3.7 mg in the thermophilic digester. After mixing with the digested sludge in the reactor, X_{met} and S_{met} were then estimated to have concentrations of 0.19 and 0.08 mg/l in the mesophilic digester and 0.48 and 0.21 mg/l in the thermophilic digester. The measured VSS concentrations in the thermophilic and mesophilic reactors were 13.8 and 10.4 g/l on the specific sampling days, respectively. The K_S values in table 6-13 and the values of other kinetic parameters in table 6-15 were utilized. With the assumption that the flow rate of the generated biogas was 604 and 665 ml/h for mesophilic and thermophilic reactors within the first 7.5 hours after feeding, the simulation for the VOSC concentrations in the headspace are shown in Figure 6-16a and b. The measured VOSC concentrations are shown in the same figure for comparison.

Examination of these figures reveals that the simulation did not represent the VOSC release patterns in the bench-scale digesters fed with raw sludge at either temperature. The measured MM concentrations reached their peak values (approx 50 ppm and 7.5 ppm in the thermophilic and mesophilic digesters respectively) within 1-2.5 hours after feeding and declined below the detection limit within 7.5 hours. From Figure 6-16 it can be seen that the simulated MM concentrations reached 28 ppm at 55 °C and 1.8 ppm at 35 °C. In the simulation, MM concentrations at both temperatures were still increasing 8 hours after sludge feeding. In addition the measured DMS concentrations reached their peak values (approx 18 ppm and 2.4 ppm in the thermophilic and mesophilic digesters respectively) 2.5 hours after feeding and declined below the detection limit within 7.5 hours. From Figure 6-16 it can be seen that the simulated DMS concentrations reached 5 ppm at 55 °C and 0.3 ppm at 35 °C. In the simulation, DMS concentrations at both temperatures were still increasing 8 hours after sludge feeding. Hence, the results show that at both temperatures, the simulated MM and DMS concentrations were much lower than the measured concentrations.

In the methionine dosed tests, simulations described VOSC release patterns reasonably for the first 12 hours of incubation. However, in the sludge digestion the observed VOSC peak values appeared earlier and higher than the simulated peak values and the subsequent decay was faster than that observed. The lack-of-fit may have been due to a difference between the estimated and actual initial degradable sulfur concentrations in the raw sludge. The values of the particulate and soluble methionine concentrations in the raw sludge utilized in the simulations were calculated based on the particulate and soluble methionine portions estimated in WMS batch tests. The raw sludge sampled on different days might have different amounts of degradable organic-S in particulate and soluble materials. An increase in the fraction of the materials that were readily available would result in a more rapid release of the VOSCs into solution than predicted by the model. This more rapid release would also result in a more rapid depletion of the source materials. Another cause for the lack-of-fit could be the different sludge mixing ratios in batch tests and in digesters. In sludge batch tests the ratio of the raw sludge and digested sludge was 3:1 while in digesters, the ratio was 1:17. Hydrolysis could be mediated by diverse microorganisms and their enzymes. Different mixing ratios could result in different microorganisms which mediated hydrolysis of particulate and soluble organic sulfur. Therefore, the kinetic constants estimated in batch tests were not necessarily valid in the digesters. Better fitting of MM concentrations could be achieved by adjusting kinetic constant values and initial particulate and soluble methionine-S concentrations.

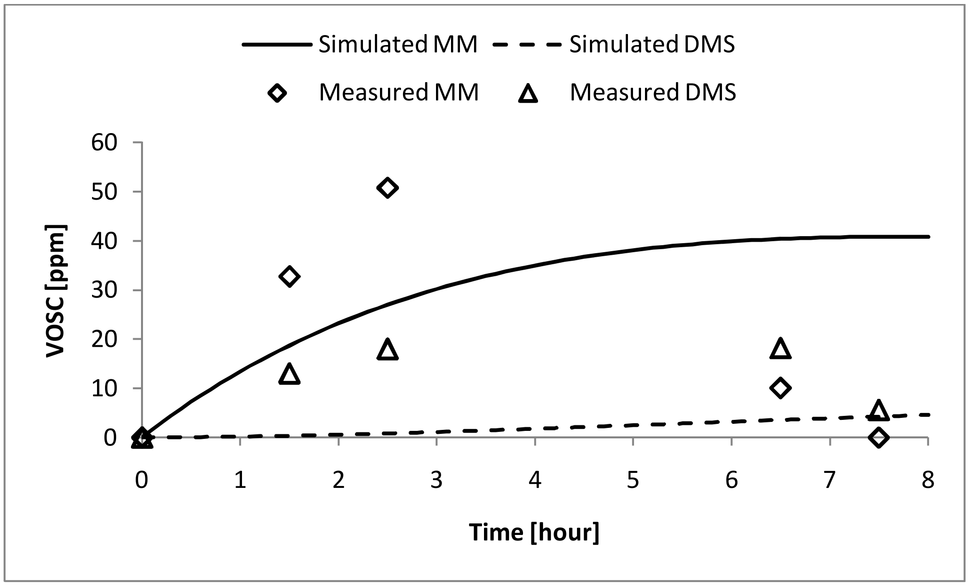

Figure 6-16a VOSC in the thermophilic reactor headspace

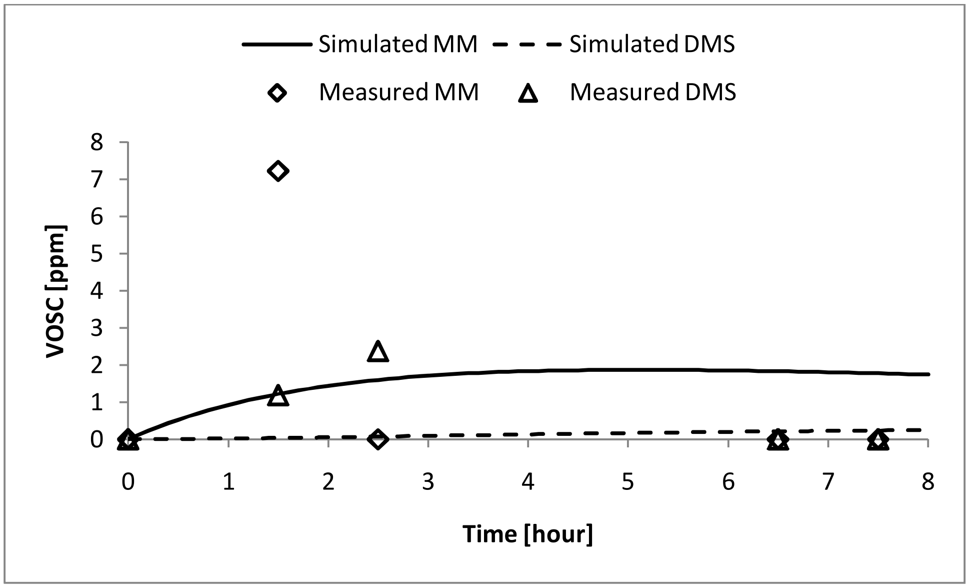

Figure 6-16b VOSC in the mesophilic reactor headspace

6.2.6.3 Summary for VSC Release in the Reactors

The feed and digested sludge samples for two bench-scale digesters were characterized. VSC concentrations in the reactor headspaces were measured under three conditions: before feeding, after feeding methionine, and after feeding raw sludge. The model was employed excluding (table 5-3) and including (table 6-12) the hydrolysis process. Based on the results of this analysis, the following conclusions were drawn:

1) In the raw sludge, the fraction of the total sulfur which could be degraded via the methionine pathway to generate VOSC was small. This conclusion was drawn from the comparison between total sulfur concentrations in raw and digestion sludge samples (table 6-14) and consistent with what was observed in batch tests.

2) With the feeding strategy employed in the semi-continuous reactors, H_2S was the dominant VSC detected 24 hours after raw sludge feeding. VOSCs were only present for a short time period (2 ~ 7 hours after feeding). In the thermophilic reactor, the concentrations of VOSCs and the frequency of their presence were higher than in the mesophilic reactor. The results suggested more serious odor and biogas quality problems would be associated with higher digestion temperatures.

3) By dosing methionine instead of feeding sludge, the availability of degradable sulfur was increased and the corresponding released VOSC concentrations were relatively high and could last for longer time (more than 12 hours). Simulations with the sulfur conversion model without the hydrolysis process were found to adequately describe the VOSC release patterns within 12 hours after methionine addition. However, according to the measured VOSC concentrations 24 hours after methionine addition, it was found that VOSC depleted faster than that predicted by the model. It appeared that microorganisms in the semi-continuous digesters could better acclimate to substrates than those in the diluted inocula utilized in batch tests.

4) The model was used to simulate VOSC release patterns after raw sludge feeding by incorporating assumptions of constant biogas flow and equilibrium between the sludge and the generated biogas. The measured DMS concentrations were higher than the simulated DMS concentrations. The measured MM concentrations peaked earlier and at higher concentrations than the simulated MM concentrations. The model could not well describe VOSC release after feeding raw sludge. The lack-of-fit of the model may have been due to imprecise estimation of the degradable sulfur in the feed sludge. In addition, in the batch tests and digester operation the ratios of the raw and digestion sludge were different. This might result in different microorganisms which mediated biotransformations and hence resulted in different kinetic constants.

6.3 Summary

VOSC conversion in digestion of raw sludge samples was studied in batch tests and bench-scale digester incubations. The observations of VOSC release patterns, pertinent parameter estimation and model simulation were elaborated in this chapter. Important conclusions for batch tests with methanogen inhibition and for monitoring of digesters were summarized in section 6.2.4 and 6.2.6.3, respectively. In this section, the overall results and modeling related to VOSC conversion in digestion of raw sludge were addressed.

1. Degradable Sulfur Fraction

The fraction of organic sulfur which was able to be degraded and converted to VOSC in the sludge was found to be small in both batch tests and digester monitoring. The quantities of total sulfur in the sludge before and after digestion were not significantly different. In the batch tests with raw sludge, the fraction of degradable sulfur (using methionine sulfur as surrogate) out of the total organic sulfur in the sludge varied from 3.7% to 10.6% depending on sludge source. Higher temperature usually resulted in higher degradable sulfur fractions.

2. VOSC Release

VOSC release pattern varied with temperature. In both batch tests and digester monitoring, the released VOSC concentrations were usually higher at thermophilic temperature. The results suggest that more serious problems would be associated with increased digestion temperature.

The pattern of VOSC releases in sludge digestion was found to be more complex than previously observed with pure sulfur-bearing substrates and varied with sludge source. Even when the same sludge sample was employed, the VOSC release pattern could differ with digestion temperature.

In the thermophilic batch incubations of all the sludge (WMS, NAS, BPS, and BAS) and mesophilic batch incubations of NAS and BPS with methanogen inhibition, MM was detected as the dominant VOSC while DMS was detected at lower concentrations. Incubations with methionine and methanogen inhibition resulted in MM as the solo VOSC generated. The difference between sludge and methionine incubations suggested that other organic sulfur sources in the sludge could be degraded and generate DMS directly.

In the mesophilic batch incubations of WMS and BAS with methanogen inhibition, DMS was generated at higher concentrations as compared to MM. It was speculated that an unidentified mechanism was triggered in the mesophilic digestion of sludge samples which contained activated sludge. Abundant DMS was generated through this unidentified pathway rather than the MM that was generated from methionine degradation.

In the mesophilic and thermophilic batch incubations of WMS without methanogen inhibition, the dominant VOSCs were consistent with that in the incubation with methanogen inhibition. DMS generation in thermophilic digestion could be well represented by methylation while DMS generated in mesophilic digestion was greater than that which could be generated by methylation. The results suggested that the VOSC generation mechanism was not affected by the methanogen inhibition. The unidentified DMS generation pathway was still associated with mesophilic digestion of this sludge sample.

In the mesophilic and thermophilic digestion of WMS in the bench-scale reactors, H_2S was the solo VSC detected immediately before sludge feeding. MM and DMS were able to be detected after sludge feeding but their presence only lasted for 2 ~ 7.5 hours. In general, the peak values of MM and DMS detected in thermophilic reactor were higher than those detected in mesophilic reactors.

3. Estimation of Kinetic Parameters

The kinetic parameters associated with methionine degradation, VOSC degradation and methylation were estimated in Chapter 5. The estimated values were evaluated with the results from methionine batch digestion without methanogen inhibition and the simulation curves could well represent the measured data.

In Chapter 6, kinetic parameter associated with the hydrolysis of the particulate matter in the sludge was estimated from solubilization of particulate organic compounds (COD), mineralization of organic nitrogen, and release of free methionine (by assuming methionine could be used as the surrogate of degradable organic sulfur). The estimated K_{COD} and K_N values fell into the ranges reported in literatures. It was found that statistically, the estimated values of K_{COD} and K_N were the same. The results suggested that the hydrolysis of lumped organic-N was representative of the hydrolysis of lumped organic particulate matter. K_N and K_S were also statistically the same with the exception of K_N and K_S that were estimated in the mesophilic incubation of a primary sludge (BPS). Therefore, in most sludge incubations the release of nitrogen from the proteineous materials was representative of the release of sulfur from the proteineous material. According to the values of the hydrolysis coefficients, it was found that the proteineous materials in the activated sludge were more accessible (higher coefficient values) to degradation as compared to primary sludge (lower coefficient values).

4. Model Simulation

The model developed in this study included hydrolysis of particulate matter, degradation of free methionine, VOSC degradation and methylation. Methionine was used as the surrogate of degradable organic-S in particulate and soluble materials. In the methionine dosed tests, the hydrolysis process was excluded. In the methanogen inhibited tests, the VOSC degradation processes were excluded.

The simulation of VOSC release in the sludge batch incubations with methanogen inhibition could well describe MM accumulation however methylation could not well represent the DMS accumulation patterns. The simulation of VOSC conversion in the sludge batch incubation without methanogen inhibition could well describe MM and DMS variation at 55 °C but not at 35 °C. The potential reason was that unidentified sulfur sources and VOSC generation pathways were not included into the model.

The simulation of VOSC release in the digester operation could well describe MM and DMS variation within 12 hours after methionine was dosed but could not predict the fast depletion of MM afterwards. The simulation conducted with the model and incorporated with the equilibrium between sludge and the continuously generated biogas could not present VOSC release in the digesters when raw sludge was fed. It is hypothesized that kinetic constants estimated in batch tests could not well describe kinetic processes in semi-continuous reactors. In addition, variations in the degradable sulfur concentrations in the raw sludge samples may be another cause for the difference between the observed and simulated values in digester tests with raw sludge feeding.

Chapter 7

Conclusions and Recommendations

7.1 Conclusions

The present study focused on modeling VOSC conversion in anaerobic sludge digestion. VOSC generation and degradation in anaerobic digestion was studied with simple substrates and wastewater treatment sludges. The inter-phase partitioning characteristics of VOSCs and the kinetics of VOSC conversion processes were estimated. A model was established based on the identified conversion processes and estimated kinetic constants. The effects of temperature and sludge source on VOSC release in sludge digestion were assessed. Application of the model to data collected from bench-scale digesters was evaluated. Important conclusions drawn from this study were summarized in this section.

The partitioning coefficients of VOSCs in DI water-gas systems that were measured in the present study were consistent with literature values. VOSC partitioning in sludge-gas systems was not significantly affected by the sludge concentration. The effect of temperature was found to be similar for partitioning of VOSC in DI water-gas and sludge-gas systems. It was concluded that adsorption of VOSC to the solid phase of the sludge was negligible.

MM, DMS, and DMDS were the most frequently observed VOSCs in anaerobic digestion. MM and DMS were found to be the major VOSCs released in sludge digestion while DMDS was occasionally detected. Individual VOSC-dosed mesophilic and thermophilic batch tests were conducted with MM, DMS, and DMDS respectively. Conversion of VOSCs in anaerobic digestion included degradation of MM and DMS, methylation of MM to DMS, and degradation of DMDS with MM as the intermediate product.

Mixed-second order kinetics were found to best fit the rates of VOSC conversion processes and kinetic parameters for VOSC conversion at 35 and 55 °C were estimated. While the concentrations of MM and DMS declined faster in mesophilic incubations, the decline of DMDS was faster at thermophilic temperatures. The corresponding kinetic constants for MM and DMS degradation at 35 °C were 3.4 and 2.7 times of those at 55 °C. The kinetic constant for MM methylation at 35 °C was about 4 times of that at 55 °C. The kinetic constant for DMDS conversion to MM at 55 °C was about 6.5 times that observed at 35 °C. MM and DMS were detected with higher concentrations and frequencies in anaerobic digestion and their degradation rates were slower at thermophilic temperatures. Hence it would be expected odor releases would be more problematic in thermophilic digestion.

In the batch digestion of methionine with methanogen inhibition, MM was identified as the direct sulfur containing byproduct. According to mass balances, more than 92% of the methionine-sulfur was converted to sulfur in MM through mesophilic and thermophilic incubations. Ammonia-nitrogen was found to be released simultaneously with MM-sulfur in anaerobic methionine degradation. Mixed-second order kinetics were found to best fit ammonia and MM accumulation from methionine hydrolysis. The kinetic parameters estimated from ammonia-nitrogen and MM-sulfur accumulation, K_{met-N} and K_{met-S}, were statistically similar at each temperature (35 and 55 °C). Both K_{met-N} and K_{met-S} values at 55 °C were about 2.2 times of those at 35 °C.

Based on the VOSC generation and degradation processes identified in the dosed batch tests, a conceptual model was established for methionine digestion, which includes MM generation from methionine degradation, MM degradation and methylation, and DMS degradation. VOSC release in methionine digestion without methanogen inhibition was monitored at 35 and 55 °C. The VOSC release in methionine digestion was simulated employing the estimated kinetic parameter values. The simulations were able to well describe the observed VOSC release at both temperatures.

Mesophilic and thermophilic batch incubations were conducted with sludge samples under a methanogen-inhibited condition. Conventional parameters such as COD degradation and ammonia generation were monitored to characterize hydrolysis of particulate matter in sludge. Hydrolysis of particulate matter was found to be well described by first order kinetics. The estimated kinetic constants fell into the range of values reported by literatures.

The hydrolysis rate constants K_{COD} and K_N were estimated for a mixed sludge from the Waterloo Wastewater Treatment Plant (WMS) by fitting the decline of particulate COD and organic nitrogen. It was found that the same kinetic constant value could be employed to describe the hydrolysis of lumped particulate materials and lumped organic nitrogen. The ratio of their hydrolysis rates R_{org-N}/R_{COD} was equivalent to their concentration ratio X_{bn}/X_b. In addition, higher temperature led to more rapid hydrolysis of particulate matter. The K_{COD} (K_N) of WMS at 55 °C was about 1.5 times of that at 35 °C.

The values of K_N for primary (BPS) and secondary (BAS, NAS) sludge samples were estimated by fitting ammonia accumulation during batch digestion tests. It was found that the hydrolysis of organic nitrogen in the activated sludge samples was more rapid than the hydrolysis of organic nitrogen in the primary and mixed sludge samples. It was hypothesized that this might be due to more ready biodegradability of the protein in the extracellular materials of activated sludges as compared to the protein in primary sludge. Temperature had similar effects on the hydrolysis of organic nitrogen in all the sludge samples. The K_N values estimated at 55 °C were about 1.4 – 1.7 times of those estimated at 35 °C.

In addition to COD and organic nitrogen, hydrolysis and further conversion of organic sulfur in the sludges was studied in the sludge incubations. It was found that the fraction of sulfur which was degraded in sludge digestion and released as VOSC was only a small portion of total sulfur in the sludge (on average, 3.7% of the total sulfur or 6.3% of the organic sulfur). The fraction of sulfur released as VOSC in thermophilic incubation (average 8.0% of organic sulfur) was slightly more than the fraction in mesophilic incubation (average 4.7% of organic sulfur). The fraction of degraded sulfur in sludge samples which contained activated sludge increased to a greater extent at the higher temperature as compared to primary sludge. The fraction of degraded sulfur in activated sludge sample increased by as much as 100% when the temperature was increased from 35 to 55 °C while it increased only 50% in the primary sludge sample.

With the hydrolysis of organic sulfur in the particulate and soluble materials, VOSCs were released in the digestion. VOSC release patterns in the sludge incubation were more complicated than that in the methionine incubation. Both temperature and sludge source was found to influence the VOSC release patterns.

In all the thermophilic sludge incubations, VOSC releases followed a similar pattern. MM was released as the dominant VOSC while DMS was released at low concentrations if it was generated. It was assumed that methionine could be used as a surrogate of degradable organic sulfur in particulate and soluble materials in the sludge. Free methionine would be released in the hydrolysis of particulate materials and further degraded to generate MM. The mechanism for the generation of low concentrations of DMS was not clear. The methylation process could not well describe the DMS accumulation in the sludge digestion. The kinetic constant, K_S, for particulate sulfur hydrolysis was estimated in each sludge incubation by fitting MM accumulation. The estimated values of K_S were statistically similar to the estimated values of K_N at 55 °C. It appeared that ammonia-nitrogen and organic-sulfur were simultaneously released in the hydrolysis of particulate proteinaceous materials at thermophilic temperatures.

In the mesophilic sludge incubations, VOSC release patterns could be divided into two groups. The first group included incubations of the activated sludge sample NAS and the primary sludge sample BPS. Similar to the thermophilic incubations, MM was the dominant VOSC while DMS was generated at low concentrations. K_S values were estimated for NAS and BPS by fitting MM accumulation through the incubations. The estimated K_S value for NAS was found to be statistically similar to the corresponding K_N value. However, for BPS, the K_S value was only one third of the corresponding K_N value, which suggested that in mesophilic incubation of primary sludge, sulfur release was slower than that of nitrogen.

The second grouping of VOSC responses during sludge incubation was observed for the activated sludge sample BAS and the mixed sludge sample WMS. In these incubations, DMS was the dominant VOSC while MM was generated at low concentrations. Both BAS and WMS contained activated sludge in which iron was dosed. It would appear that in the incubation of activated sludges with high iron concentrations (higher than 30 mg Fe/g TSS in BAS and WMS) at mesophilic temperatures, an unidentified pathway resulted in abundant DMS generation rather than MM. The model which employed methionine as a surrogate of organic sulfur and MM as the only VOSC generated from methionine degradation was not able to describe the VOSC release pattern in mesophilic incubations of BAS and WMS.

The established model for methionine degradation was extended to include hydrolysis of particulate sulfur and employed to simulate VOSC release in WMS digestion without methanogen inhibition. The model simulation was able to represent the observed VOSC release pattern at 55 °C but not at 35 °C. This was consistent with simulations of the batch incubations with methanogen inhibition in which the model did not include the mechanism for abundant DMS generation at mesophilic temperatures.

VSC monitoring was conducted for bench-scale digesters treating the WMS sludge. In the long term monitoring, the VSC concentrations in the headspace of the two reactors were measured immediately before feeding. In these samples only H_2S was detected and its concentration in the mesophilic and thermophilic reactor headspaces was 30 ± 13 ppm and 26 ± 9 ppm, respectively. The results suggested that any generated VOSCs were degraded to concentrations which were lower than their detection limits within the feeding intervals.

The VSCs in the headspaces of the two digesters were monitored immediately after feeding of either methionine or sludge into the reactors. The VOSC conversion model was employed without and with the hydrolysis process respectively to simulate VOSC releases. In the headspace monitoring after methionine addition, the model without the hydrolysis process was able to represent MM and DMS variations in the headspace of both digesters for 12 hours after methionine was dosed. However, the concentrations of MM and DMS measured 24 hours after methionine addition were much lower than the simulated values. The results suggest that depletion of VOSC occurred more rapidly in the digesters and that VOSC degradation in the batch tests may have been reduced by acclimation. In sampling events for VOSC monitoring after sludge feeding, the appearance of MM and DMS in the headspace only lasted for 2 – 7.5 hours. The highest MM concentration detected in these sampling events was 70 ppm in the thermophilic reactor and 7 ppm in the mesophilic reactor. The highest DMS concentration detected in these sampling events was 18 ppm for the thermophilic reactor and 9 ppm for the mesophilic reactor. The model was employed to simulate VOSC release patterns after raw sludge feeding by incorporating assumptions of constant biogas flow and equilibrium between the sludge and the generated biogas. The measured DMS concentrations were higher than the simulated DMS concentrations. The measured MM concentrations peaked earlier and at higher concentrations than the simulated MM concentrations. The model could not well describe VOSC release after feeding raw sludge. The lack-of-fit of the model may have been due to imprecise estimation of the degradable sulfur in the feed sludge. In addition, in the batch tests and digester operation the ratios of the raw and digested sludge were different. This might have resulted in different concentrations of the microorganisms which mediated biotransformations and hence resulted in different kinetic constants.

7.2 Recommendations

The experimental and modeling studies presented in this thesis will facilitate biogas utilization and odor control. At present, to control VSC concentrations, chemical dosing or biogas scouring facilities are employed to clean the biogas generated in anaerobic digestion. This requires investment in chemicals, construction, installation, and operation, as well as the potential risk of failure to remove VSCs to an acceptable level as required by gas turbines, engines, and fuel cells. If VSC generation and degradation in anaerobic digestion can be better understood and quantified by modeling, VSC concentrations in the biogas emissions could be predicted and hence control of VSC releases might be achieved with reduced investment in equipment and chemicals. For this purpose, further research which can provide the VSC conversion model with precision required for predictive and control applications is required. This section outlines further development which is important for the VSC conversion model to achieve better predictive capability.

1) During VOSC monitoring and simulation in sludge digestion, temperature and sludge source were found to affect VOSC release patterns. MM was identified as the dominant VOSC in most incubations. However, the dominant VOSC became DMS in the mesophilic digestion of activated sludge samples with iron dosed. Iron addition in the primary sludge did not affect the dominant VOSC. It appears that iron addition affects the VOSC generation pathways when the digestion substrate contains activated sludge however this is also dependent on the digestion temperature. Future studies to identify additional sulfur-bearing substrates in the sludge and their biodegradation pathways are recommended. A study on the role of iron in the VOSC generation at different temperatures is also recommended.

2) In the model simulations in the present study, VSS was used to represent the active biomass which mediated VOSC generation from methionine, VOSC degradation, and MM methylation processes. Development of the model to incorporate a better description of the population of each specific group of microorganisms is recommended.

3) The kinetic parameters of VOSC conversion processes were rarely reported. Kinetic estimation for VOSC generation and degradation biochemical processes by using different inocula can enrich the database of kinetic constants.

4) Methionine was used as the surrogate of sulfur containing organic compound in the sludge which was the precursor of VOSCs. However, MM generation from methionine was not able to represent all the VOSC release patterns in the sludge digestion. Other sulfur containing compounds which are present in sludge and able to be degraded to VOSC need to be identified and studied.

5) Application of the model to the one stage mesophilic and thermophilic digesters was attempted in the present study. The reasons for the lack-of-fit need to be further studied. Degradable organic sulfur in the sludge needs to be more quantitatively characterized. In addition kinetic constants should be directly estimated for semi-continuous/ continuous digesters.

6) The VOSC conversion model developed in the present study should be extended to VOSC conversions under other anaerobic conditions such as digesters with different configurations, sludge pre-treatment, and biosolids storage.

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Appendix A
Methanogen Inhibitor Assessment

In section 2.3.2.2, inhibitors which were targeted to methanogenesis and their effective mechanisms were discussed. According to the literature, the most effective inhibitors which were able to inhibit methanogenesis in complex matrices were 2-bromoethanesulphonate (BES) and iodopropane (IP). An anaerobic batch test was conducted employing cysteine as a substrate to evaluate the inhibitors. Three different types of serum bottles were prepared: cysteine dosed (type I), cysteine and BES dosed (type II), and cysteine and IP dosed (type III). All the bottles contained 200 ml of digested sludge as the inocula. The incubation was conducted for 10 days. No VOSCs were detected in the type I and type II bottles. Some unidentified sulfur containing compounds were generated in the type III bottles. In addition, more hydrogen sulfide was generated in the type III bottles as compared to type the I and type II bottles while type I and II bottles generated similar amounts of hydrogen sulfide. The results suggested that BES addition did not cause VSC release in anaerobic incubation. Figure A-1 shows the hydrogen sulfide release with incubation time in the serum bottles.

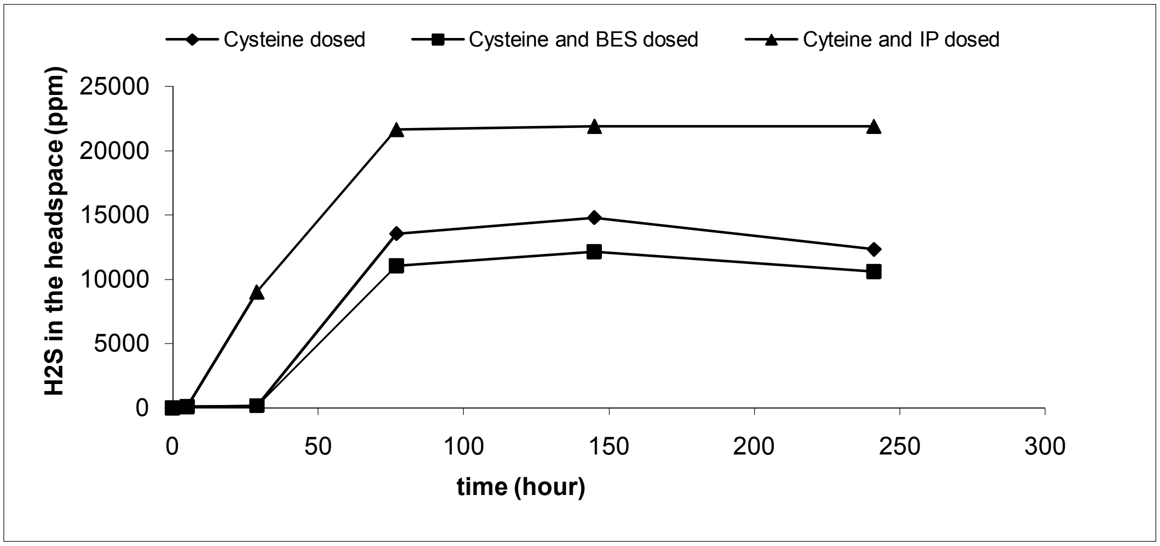


Figure A-1 Hydrogen sulfide release in the incubation with cysteine as the substrate

Appendix B

Acid Digestion and Sulfur Measurement in Wet and Dry Sludge

B.1 Introduction

When the analytical method was developed for this study, two objectives were attempted to achieve:

1) Eligibility for different sulfur species in the sludge defined by the present study.
2) Time and chemical efficiency for a large number of samples.

By employing the same analytical method for measurements of different sulfur species, analytical error will be consistent and that leads to better quantitative comparison between sulfur species. For practical application of the developed method to a large number of samples, sample processing had to be labor and time efficient with an economical chemical consumption.

When the sulfur species were classified as total, organic, and inorganic sulfur as in Figure 3-8, a group of sulfur containing compounds with similar characteristics instead of individual sulfur bearing compounds was measured. After separation of a sulfur species from the others, e.g. liquid sample separated from solids by filtration, the sample was completely digested by acids and then sulfur in the digestion solution was quantified by ICP-AES analysis.

B.2 Comparison of two acid digestion processes

To prepare samples for ICP-AES analysis, an enhanced acid digestion process was conducted with traditional heating plate and microwave respectively. The digestion processes were adapted from USEPA methods 3050B and 3051A respectively that were designed for acid digestion of sediments, sludge, and soil samples by incorporating traditional heating plate and microwave systems. Acid digestion of sludge with hot heating plate and Parr® microwave bombs (Figure 3-9) were assessed in the present study. The characteristics of the two methods were compared (table B-1).

Table B-1 Comparison between two acid digestion processes

	Traditional heating plate method	Microwave enhanced method
Sample volume	0.5, 1 or 2 g wet sample 0.5 or 1g dry sample	0.5 or 1 g wet sample 0.01 g dry sample
Reagent consumption	20 – 25 ml concentrated HNO_3 2 – 8 ml 35% H_2O_2 10 ml concentrated HCl	For wet sample: 5 ml concentrated HNO_3 For dry sample: 0.5 ml concentrated HNO_3 2.5 ml concentrated HCl
Digestion time	5 – 6 hours	1 hour for wet sample (microwave time: 35 seconds) 3 hours for dry sample (microwave time: 90 seconds)
Container and pre-treatment	Pre-treated glass tubes: 1. Soaking in glass detergent Chromerge at least for 15 min; 2. Rinsing with Millipore water followed by 1hr (minimum) soak in 10% HCl; 3. Rinsing and drying with acetone	PTFE containers: Regular wash and no pre-treatment requirement
Operation	The process needs two stages of digestion and multiple times of reagent addition: 1. Add 10 ml of 1:1 HNO_3, mix the slurry, cover with vapor recovery device. Heat to 95±5 °C and reflux for 10~15 minutes without boiling. 2. Add 5 ml concentrated HNO_3 and reflux for 30 minutes. If brown fumes are generated, repeat this step until no brown fumes are given off. Heat at 95±5 °C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all	1. Reagents are added at the start, seal the PTFE container, and microwave for 35 and 90 seconds for wet and dry sample respectively. 2. Cool down at the room temperature for about 1 and 3 hours for wet and dry sample respectively. 3. Open the container and dilute the digested samples with DI water.

	times. 3. After the sample has cooled, add 2 ml of water and 3 ml of 30% H_2O_2. Cover the vessel with the recovery device and heat for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel. 4. Continue to add 30% H_2O_2 in 1-ml aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. 5. Heat at 95±5 °C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times. 6. Add 10 ml concentrated HCl to the sample and cover with a vapor recovery device. Place the sample on/in the heating source and reflux at 95±5 °C for 15 minutes. 7. Dilute the digested samples with DI water.	

Operation of the traditional heating plate method was found to be much more complicated and required consistent attention and a longer digestion time. From the aspect of digestion effect checked by visual examination, the microwave enhanced method substantially enhanced the dissolution of the sample. Solids were totally dissolved after the microwave enhanced acid digestion. In contrast, after the traditional heating plate acid digestion, white residues were left at the bottom of the digestion tube. Microwave enhanced digestion appeared to be preferable than hot heating plate in easy operation and improved solubilization. Further discussion will mainly focus on the microwave enhance digestion method. In the discussion of analytical results, measurements by hot heating plate digestion will be referred to for comparison.

B.3 Sample Preparation

Total sulfur measurement was employed with homogenized sludge samples. Measurement of soluble sulfur was conducted with the liquid sample after solids removal. Solids removal was achieved by centrifugation (3000 rpm for 30 minutes) and sequent filtration of sludge sample through 1.2 μm glass filter. The homogenized sludge sample and liquid sample were wet samples which were able to be completely digested by nitric acid.

Sludge samples in this study had solids concentrations less than 5%. For wet samples with such a level of solid contents, 5ml of concentrated nitric acid (68 ~ 70%) was used as the digestion reagent for every 1ml of sample. After sample and acid addition, PTFE container was sealed and put into the microwave bomb. The microwave bomb was located in a microwave oven for 35 seconds heating. After removed from the oven, the microwave bomb was kept closed at room temperature for further digestion and cooling-down for 1 hour.

The inorganic sulfur that was precipitated in the solid phase was determined by digesting the ash obtained from sludge drying and combustion. The preparation of the sludge ash sample was same as that employed in the TS/VS analytical procedure. The homogenized sludge sample was added into a pre-weighted dish, dried at 105 °C overnight, and then burned at 540 °C for 1 hour. It was assumed that organic sulfur compounds would be vaporized at the high combustion temperature, 540 °C. The precipitated metal sulfide would not decompose when the temperature was less than 1171 – 1188 °C (Vaughan, 1978). The sludge residue in the dish after combustion was crushed to fine ash. The fine sludge ash would be digested as prepared for precipitated sulfur analysis. 3 ml mixed concentrated nitric (68 ~ 70%) and hydrochloride acids (36.5 ~38%) at a ratio of 1:5 (v/v) was employed as the digestion acid for every 0.01 g dry sludge ash. 90 second microwave heating to the microwave bomb was employed. The microwave bomb was held at room temperature for further digestion and cooling-down for 3 hours before being opened.

B.4 Analytical results

The repeatability and reproducibility of the two digestion processes were evaluated and compared. The results confirmed that the microwave enhanced digestion was better than the traditional hot heating plate digestion.

1. Repeatability

To determine the repeatability of the sulfur measurement incorporated with the acid digestion process, 15 replicates of one sludge sample were pre-treated and analyzed for the total sulfur quantity. In both digestion processes, 0.5 ml homogenized sludge sample was utilized. After the digestion, the digested solution was diluted to 100 ml for ICP-AES analysis. Therefore, the total sulfur value was the measured value multiplied by 200.

The analyzed results of the digested solution processed by two acid digestion methods are listed in Table A-2. The relative variation of the microwave enhanced digestion was only 1.4% which was much less than that of traditional heating plate digestion, 8.0%. It was indicated that results from analysis pre-treated with microwave enhanced digestion process was less variable.

Table B-2 Repeatability of the two methods

Unit: mg/l	Traditional heating plate digestion		Microwave enhanced digestion	
Replicate	Measured	Total sulfur	Measured	Total sulfur
1	0.97	193.92	1.50	300.16
2	1.03	206.82	1.52	303.72
3	1.04	208.09	1.49	298.92
4	1.02	204.50	1.49	298.90
5	1.09	217.56	1.53	305.76
6	1.02	203.24	1.49	297.71
7	0.99	198.27	1.51	302.05
8	0.96	192.28	1.50	300.34
9	1.12	223.78	1.51	301.37
10	1.18	236.03	1.57	314.92
11	0.99	198.93	1.53	305.54
12	1.28	256.14	1.52	303.91
13	1.06	212.38	1.52	303.80
14	1.12	223.78	1.51	302.34
15	1.07	213.69	1.51	301.45
Average	1.06	212.62	1.51	302.73
S_D	0.09	17.07	0.02	4.14
RV (%)	8.03	8.03	1.37	1.37

2. Reproducibility

The reproducibility here described the different results for the same sludge sample pre-treated and analyzed on different days. The sludge sample was stored in the fridge at 4 °C. The analytical procedure, including homogenizing for the total sulfur and drying and burning for the precipitated sulfur, acid digestion, and ICP-AES analysis, was repeated 5 times within 25 days. The results of the total and precipitated sulfur analysis incorporated with the two digestion processes are list in tables B-3 and B-4 respectively. The relative variations in tables B-3 and B-4 were comparable with the relative variation in table B-2. The results suggests that there was no significant variation introduced by the operation on different processing days and that samples were relatively stable over the storage period employed.

Table B-3 Reproducibility of methods for wet samples (mg S/l)

Unit: mg/l	Traditional heating plate digestion		Microwave enhanced digestion	
Replicate	Measured	Total sulfur	Measured	Total sulfur
day 1	1.06	212.62	1.53	305.32
day 4	1.14	227.86	1.53	306.84
day 10	1.07	214.24	1.49	298.72
day 15	0.96	192.36	1.53	306.44
day 25	0.94	188.76	1.52	303.92
Average	1.04	207.17	1.52	304.25
S_D	0.08	14.6	0.02	2.9
RV (%)	7.88	7.05	1.08	1.00

Table B-4 Reproducibility of methods for dry sample (unit: mg S/g ash)

Replicate	Traditional heating plate digestion		Microwave enhanced digestion	
	Measured	Original	Measured	Original
day 1	6.42	642.0	5.80	580.0
day 4	5.78	578.5	5.79	578.5
day 10	6.50	650.0	5.68	568.0
day 15	5.91	591.0	5.96	595.5
day 25	6.71	671.3	5.62	561.5
Average	6.26	626.0	5.77	576.8
S_D	0.40	626.47	0.26	11.61
RV (%)	6.39	6.0	2.24	2.0

3. Method Detect Limit

Based on the tests for the repeatability and reproducibility, the microwave enhanced digestion method was proven to have better precision for the sulfur analysis. The Method detect limit (MDL) of the ICP analysis incorporated with microwave enhanced digestion was assessed by repeating the whole process of 100 time dilution, digestion, and ICP analysis on 1 ml sulfate solution with 16 mg S/l. The original concentrations were calculated by multiplying measured value with 100. The results are shown in table B-5

Table B-5 MDL determination (unit: mg S/l)

Replicate #	Measured value	Original concentration
1	0.17	17.05
2	0.16	15.98
3	0.17	16.72
4	0.17	16.65
5	0.16	16.10
6	0.17	16.65
7	0.16	16.16
8	0.16	15.73
9	0.15	15.47
Standard error S		0.52

The table t (df = 8) value is 1.86 for 95% confidence and 2.90 for 99% confidence. The method detect limit could be calculated by the equation MDL = S × t (8, 95%) or S × t (8, 99%). With 95% and 99% confidence, the method detect limit is 0.97 and 2.51 respectively. According to American Public Health Association, quantification limit is usually defined as 5 times of the MDL value. Therefore quantitative measurement of sulfur in the original sample with concentrations above 4.84 (7.54) mg/l is reliable with 95% (99%) confidence.

4. Accuracy

To examine the accuracy of the microwave enhanced digestion method, methionine, cysteine, sodium sulfide, and potassium sulfate solutions were added into sludge samples to determine the recovery. The recovery for both organic and inorganic sulfur containing compounds was above 94% (Table B-6).

Table B-6 Recovery of sulfur from different compounds

Cysteine	Unit: mg/l		Measured value		
Number	without Addition	S dose	Total S	Recovered S	Recovery (%)
1	114.21	350	467.43	353.22	100.92
2	113.92	350	459.22	345.3	98.66
3	178.03	350	524.09	346.06	98.87
4	177.12	350	521.33	344.21	98.35
5	96.18	350	439.78	343.6	98.17
6	96.02	350	443.90	347.88	99.39
7	93.89	350	438.94	345.05	98.59
8	94.38	350	446.37	351.99	100.57
Average recovery (%)		99.19			

Continue Table B-6

Methionine	Unit: mg/l		Measured value		
Number	without Addition	S dose	Total S	Recovered S	Recovery (%)
1	114.21	500	599.54	485.33	97.07
2	113.92	500	594.52	480.60	96.12
3	178.03	500	662.78	484.75	96.95
4	177.12	500	642.81	465.69	93.14
5	96.18	500	599.23	503.05	100.61
6	96.02	500	591.14	495.12	99.02
7	93.89	500	592.16	498.27	99.65
8	94.38	500	595.71	501.33	100.27
Average recovery (%)		97.85			

Continue Table B-6

Na_2S	Unit: mg/l		Measured value		
Number	without Addition	S dose	Total S	Recovered S	Recovery (%)
1	114.21	500	593.69	479.48	95.90
2	113.92	500	614.23	500.31	100.06
3	178.03	500	652.99	474.96	94.99
4	177.12	500	637.51	460.39	92.08
5	96.18	500	570.37	474.19	94.84

Continue Table B-6

6	96.02	500	595.32	499.30	99.86
7	93.89	500	548.95	455.06	91.01
8	94.38	500	536.12	441.74	88.35
Average recovery (%)		94.64			

Continue Table B-6

K_2SO_4	Unit: mg/l		Measured value		
Number	without Addition	S dose	Total S	Recovered S	Recovery (%)
1	114.21	500	610.37	496.16	99.23
2	113.92	500	602.37	488.45	97.69
3	178.03	500	668.29	490.26	98.05
4	177.12	500	633.26	456.14	91.23
5	96.18	500	610.19	514.01	102.80
6	96.02	500	616.21	520.19	104.04
7	93.89	500	608.26	514.37	102.87
8	94.38	500	592.73	498.35	99.67
Average recovery (%)		99.45			

To evaluate the effect of the dosage on the recovery, sludge samples dosed with a range of methionine and cysteine concentrations were analyzed. The predicted and measured responses are shown in Table B-7. The recovery for all dosages was above 95%.

According to the recovery test, more than 95% of the sulfur in the dosed organic and inorganic compounds was recovered. The results indicate that measurement by the enhanced microwave digestion method had sufficient accuracy for the purposes of this study.

Table B-7 Recovery of varying amino acid dosages

unit: mg/l	Cysteine			Methionine		
Level	Dosed S	Recovered S	Recovery (%)	Dosed S	Recovered S	Recovery (%)
1	35	36.016	102.9	50	49.56	99.13
2	70	72.01	102.87	100	103.14	103.14
3	140	137.52	98.23	200	190.57	95.28
4	280	273.85	97.8	400	383.63	95.91

Appendix C
VOSC Partitioning

Process for calculation of partitioning coefficients and statistic tests were given in Chapter 4 by taking DMS as an example. Other calculations are accomplished and listed in the excel file "Appendix C VOSC partitioning". The contents of the excel file are tabulated in table C-1.

Table C-1 Contents of excel file for Appendix C

Name	**Format**	**Contents**
Appendix C	Excel	
VOSC Partitioning		Sheet1: Partitioning of VOSC in DI water
		Sheet2: Comparison with the literature data
		Sheet3: Partitioning of VOSC in sludge
		Sheet4: VOSC Partitioning at specific temperatures: 35 and 55 °C

Please refer to the attached CD for the excel forms or consult author by email address: wdu@uwaterloo.ca

Appendix D

VOSC and Methionine Dosed Batch Tests

One example will be taken to explain the calculation and regression processes. The rest of calculation and regression results for VOSC dosed batch tests with different initial dosages and methionine dosed batch tests at two temperatures will be shown in the excel files listed in the table at the end of Appendix D.

Example:

Part i: Raw data analysis of DMS dosed test with an initial concentration of 5.20 mg S/g VSS at 35 °C: normalized by VSS concentrations;

Part ii: Regression and simulation for part I;

Part iii: Hypothetic test based on Student's distribution for kinetic constants in tests with different DMS initial dosages at 35 °C.

i. Raw data for DMS conversion

1. Physical characteristics of DMS

Table D-1 DMS characteristics

Name	Normal phase	Molar weight	Density
DMS	Liquid	62 g/mol	0.84 g/ml
Sulfur (S)		32 g/mol	

2. Partitioning coefficient of DMS at 35 °C

$$logk_{DMS,35} = \frac{a}{T} + b = \frac{1711.25}{35 + 273} - 4.6068 = 0.95$$

$$k_{DMS,35} = 10^{0.95} = 8.89$$

3. DMS addition

   5.7 µl DMS was injected into the serum bottle. 200 ml sludge with a VSS concentration of 2.37 g/l was already loaded in the bottle.

Dosed DMS-S mass: $\frac{\frac{5.2\ \mu l}{1000\ \mu l/\ 1\ ml}\times 0.84\ g/ml}{62\ g/mol}\times\frac{32g}{mol}\times\frac{1000\ mg}{1\ g}=2.47\ mg$

Dosed DMS-S concentration: $\frac{2.47\ mg\ S}{2.37\frac{g\ VSS}{l}\times\frac{200\ ml}{1000ml/1\ l}}=5.20\ mg\ S/g\ VSS$

4. DMS decline with time

DMS concentrations declined with time during the batch tests. DMS concentration was normalized by VSS concentration of the inocula.

Table D-2 Normalized DMS concentrations (replicate bottle 1)

Replicate 1

A	B	C	D	E	F	G	H
Time	Headspace	Released	Concentration	In Gas	In Liquid	Total	Conc.
hour	ml	ml	ppm	µmol	amount µmol	amount mol	mg S/ g VSS
0	128.7	0	1012.06	5.15	71.20	76.35	5.15
2	128.7	0	993.30	5.06	69.88	74.94	2.06
5	128.7	0	691.14	3.52	48.62	52.14	1.52
25	128.7	0	657.44	3.35	46.25	49.6	2.15
49	128.7	0	363.89	1.85	25.60	27.45	1.85
106	128.7	0	223.23	1.14	15.70	16.84	1.14
122	128.7	0	117.84	0.60	8.29	8.89	0.60
123	128.7	0	119.51	0.61	8.41	9.02	0.61

Column A is time (hour)

Column B is the headspace volume (128.7 ml as constant)

Column C is the biogas volume at each time point (ml)

Column D is the measured concentration of DMS in the headspace (ppm v/v)

Column E is the mass of DMS-S in the headspace gas

$$n_{gas}=\frac{PV}{RT}$$

Where, P is the value in column D, V is the sum of column B and C, R is 0.082057, T is (35+273).

Column F is the mass of DMS-S in the liquid pahse

$$n_{liq}=n_{gas}\times k_{DMS,35}$$

Column H is the DMS-S concentration at each time point

$$C_{DMS} = \frac{(n_{gas} + n_{liq}) \times 32g/mol}{VSS \times V_{slg}}$$

Where, VSS is 2.37 g/l for this example, V_{slg} is 200 ml.

DMS-S concentrations in the replicate 2 bottle are calculated in the same way.

Table D-3 Normalized DMS concentrations (replicate bottle 2)

Replicate 2

A	B	C	D	E	F	G	H
Time	Headspace	Released	Concentration	In Gas	In Liquid	Total	Conc.
hour	ml	ml	ppm	µmol	amount µmol	amount mol	mg S/ g VSS
0	128.7	0	1012.06	5.15	71.20	76.35	5.15
2	128.7	0	715.42	3.64	50.33	53.97	0.64
5	128.7	0	1024.91	5.22	72.10	77.32	3.22
25	128.7	0	619.15	3.15	43.56	46.71	1.95
49	128.7	0	363.70	1.85	25.59	27.44	1.85
106	128.7	0	194.19	0.99	13.66	14.65	0.99
122	128.7	0	107.48	0.55	7.56	8.11	0.55
123	128.7	0	118.06	0.60	8.31	8.91	0.60

Then average value of the two replicates are calculated and plotted with time (as figures for VOSC degradation with different initial dosages at different temperatures shown in Chapter 5).

Table D-4 Average and standard error for DMS normalized concentrations

Time	Average concentration	Standard error
Hour	mg S/g VSS	mg S/g VSS
0	5.15	0.00
2	1.35	1.00
5	2.37	1.20
25	2.05	0.14
49	1.85	0.00
106	1.06	0.10
122	0.57	0.04
123	0.60	0.01

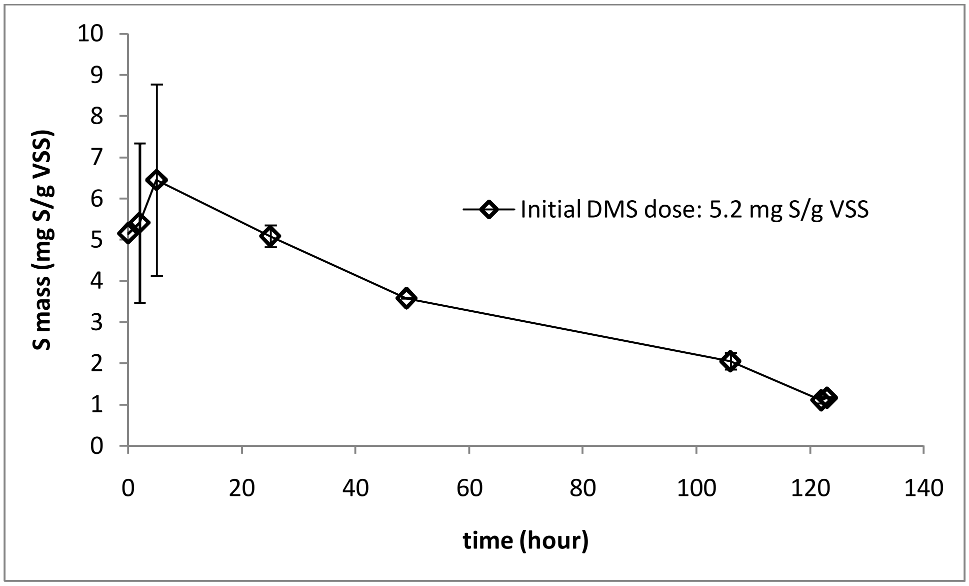


Figure D-1 Plot decline of DMS concentration with time

ii. Regression and simulation

Software AQUASIM was utilized for the regression and simulation. AQUASIM execute file

1. Variables, processes, and compartment are set respectively for regression and simulation of DMS decline with time.

Table D-5 Variable set-up in AQUASIM

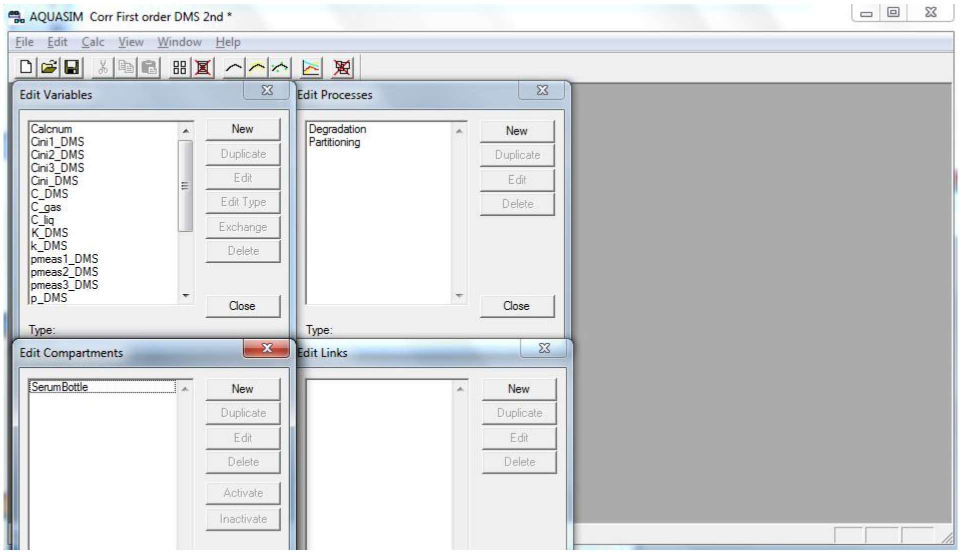
Variables	Type	Unit	Definition
Cini_DMS	Constant	mol S/l	Initial dosed concentration of DMS
C_DMS	State variable	mol S/l	Total concentration of DMS
C_DMSgas	State variable	mol S/l	Concentration of DMS in the gas
C_DMSliq	Formula variable	mol S/l	Concentration of DMS in the liquid C_DMSliq = C_DMS-C_DMSgas×0.13/0.2
k_DMS	Constant		Partitioning coefficient of DMS at 35 °C
K_DMS	Constant	$h^{-1}\cdot(g\ VSS/l)^{-1}$	Kinetic constant for DMS degradation
R	Constant	atm·l/mol/K	Ideal gas law constant, 0.0820575
T	Constant	K	Temperature, 308
VSS	Constant	g/l	VSS concentration 2.37 g/l
Pmeas_DMS	List variables	atm (10^{-6} ppm)	Measured DMS concentrations
To be continued			
P_DMS	Formula variable	atm (10^{-6} ppm)	P_DMS = C_DMSgas·R·T
r_DMS	Formula variable	mol S/l/h	r_DMS = K_DMS·C_DMSliq·VSS
t	Program variable	h	time

Table D-6 Process set-up in AQUASIM

Processes	Type	Definition
Degradation of DMS	Dynamic	Degradation of DMS to H_2S, mixed-second order kinetics
Partitioning of DMS	Equilibrium	Partitioning of DMS in the liquid-gas system C_DMSliq = C_DMSgas·k_DMS

Compartment:

Degradation is assumed to occur in the 200 ml liquid phase in the serum bottle. The listed variables and processes are included in the calculation.



Calcnum
Cini1_DMS
Cini2_DMS
Cini3_DMS
Cini_DMS
C_DMS
C_gas
C_liq
K_DMS
k_DMS
pmeas1_DMS
pmeas2_DMS
pmeas3_DMS
p_DMS

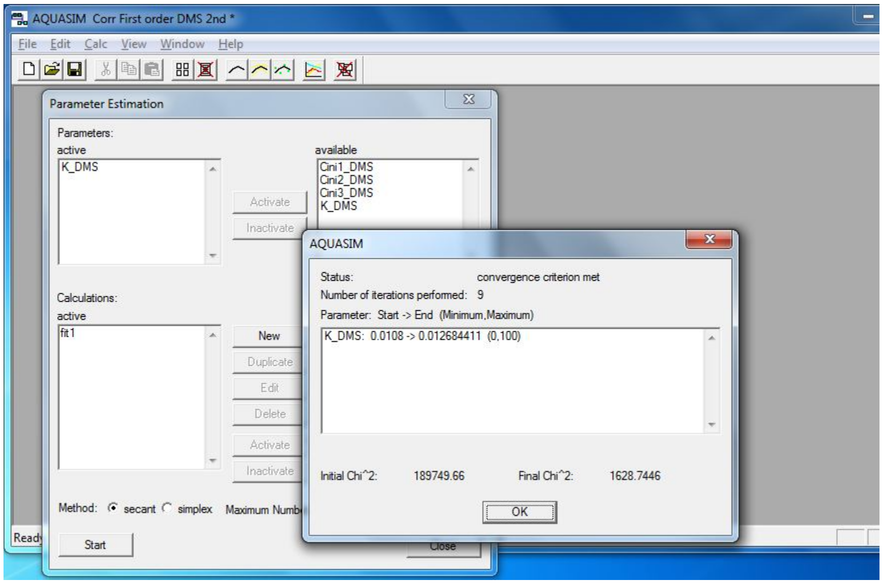
Degradation
Partitioning

SerumBottle

Figure D-2 AQUASIM interface for editing variables, process, and compartment

2. Kinetic constant estimation

With an assigned initial value, K_DMS is estimated by fitting the decline of the total DMS concentration in the serum bottle based on the least squares method.



Parameters:
active
K_DMS

available
Cini1_DMS
Cini2_DMS
Cini3_DMS
K_DMS

Calculations:
active
fit1

Method: ● secant ○ simplex

Status: convergence criterion met
Number of iterations performed: 9
Parameter: Start -> End (Minimum,Maximum)
K_DMS: 0.0108 -> 0.012684411 (0,100)

Initial Chi^2: 189749.66
Final Chi^2: 1628.7446

Figure D-3 AQUASIM interface for kinetic constant estimation

Results of the estimated kinetic constant, its standard error, and correlation coefficient were listed in a text file.

```
************************************************************************

AQUASIM Version 2.1f (win/mfc) - Parameter Estimation File

************************************************************************

Date and time of listing:  05/27/2010  10:30:47


Number of parameters  = 1
Number of data points = 8
Estimation method     = secant
```

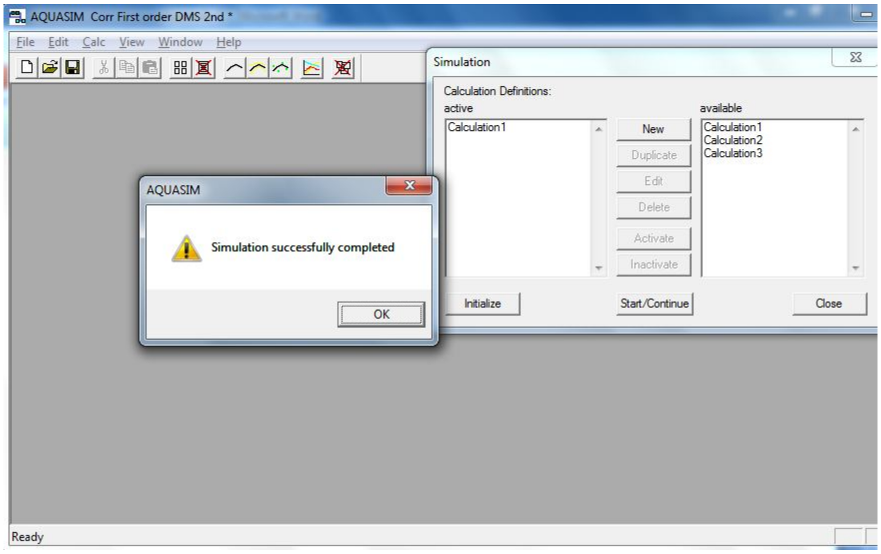
```
Parameters:

 Name          Unit          Start          Minimum        Maximum

 K_DMS          l/g/h          0.012828958     0              100



Calculations:

 K_DMS          Chi^2

 [l/g/h]


 0.014265158     1767.2408

 1.0142651       7035276.9

 0.11426516      7030592.6

 0.025265158     2140403.9

 0.015265158     43632.587

 0.014264158     2217.2172

 0.014364158     1771.7714

 0.014264158     1767.2408



Parameter estimation successfully finished (convergence criterion met)
```

```
 K_DMS

 [l/g VSS/h]



Estimated values of the parameters:


 0.014264158



Estimated standard errors of the parameters:


 1.484281e-003



Estimated correlation matrix of the parameters:


 1



Contribution of data series to Chi^2:


 Calculation:      Data Series:      Chi^2 ini:     Chi^2 end:

 fit1             pmeas1_DMS        1767.2408      1767.2408
```

```
                ------------   ------------

                1767.2408      1767.2408



Number of steps performed      = 4

Number of simulations performed = 8
```

3. Simulation with the estimated kinetic constant

Simulation was conducted with the estimated kinetic constant to generate the simulation curve with assigned simulation interval and step.



Simulation successfully completed

Figure D-4 AQUASIM interface for simulation

4. Result generation

Result of the simulation can be plotted by AQUASIM or listed as text file.

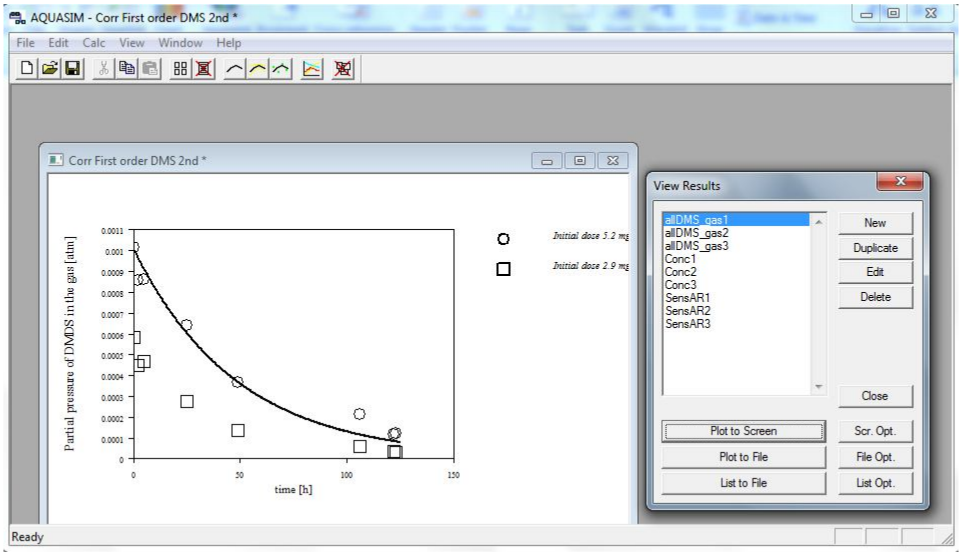


Figure D-5 AQUASIM interface for plot generation

It also could be chosen to list the simulation results in a text file. Plot can be hand-made in other software with simulation results.

```
***********************************************************************

AQUASIM Version 2.1f (win/mfc) - List File

***********************************************************************

Date and time of listing:  09/16/2009  18:39:12

Name of plot: allDMS_gas1
```

Type:	Argument	Value	Argument	Value	Argument	Value	Argument	Value
Variable:	t	pmeas1_DMS	t	pmeas2_DMS	t	p_DMS	t	p_DMS
Parameter:								
CalcNum:		0		0		1		2
Compart.:		SerumBottle		SerumBottle		SerumBottle		SerumBottle
Zone:		Bulk Volume		Bulk Volume		Bulk Volume		Bulk Volume
Time/Space:		0		0		0		0
Unit:	h	atm	h	atm	h	atm	h	atm
Legend:		Initial dose 5.2 mgS/gVSS				Initial dose 2.92 mgS/gVSS		
	0	0.001012	0	0.0005803	0	0.001007	0	0.00058
	2	0.0008544	2	0.0004427	0.1	0.001006	0.1	0.000579
	5	0.000858	5	0.0004647	0.2	0.001004	0.2	0.000578
	25	0.0006383	25	0.0002722	0.3	0.001002	0.3	0.0005771
	49	0.0003638	49	0.0001337	0.4	0.001001	0.4	0.0005761
	106	0.0002087	106	5.75E-05	0.5	0.0009992	0.5	0.0005751
	122	0.0001127	122	3.07E-05	0.6	0.0009976	0.6	0.0005741
	123	0.0001188	123	2.45E-05	0.7	0.0009959	0.7	0.0005732
					0.8	0.0009943	0.8	0.0005722
					0.9	0.0009926	0.9	0.0005713
					1	0.000991	1	0.0005703

1.1	0.0009894	1.1	0.0005694
1.2	0.0009878	1.2	0.0005684
1.3	0.0009862	1.3	0.0005674
1.4	0.0009845	1.4	0.0005665
1.5	0.0009829	1.5	0.0005655
1.6	0.0009813	1.6	0.0005646
1.7	0.0009797	1.7	0.0005636
1.8	0.0009781	1.8	0.0005627
1.9	0.0009765	1.9	0.0005618
2	0.0009749	2	0.0005608
26.5	0.0006508	26.5	0.0003709
26.6	0.0006497	26.6	0.0003703
26.7	0.0006486	26.7	0.0003697
26.8	0.0006475	26.8	0.0003691
26.9	0.0006465	26.9	0.0003684
27	0.0006454	27	0.0003678
27.1	0.0006443	27.1	0.0003672
27.2	0.0006433	27.2	0.0003666
27.3	0.0006422	27.3	0.000366
27.4	0.0006412	27.4	0.0003653
27.5	0.0006401	27.5	0.0003647
27.6	0.000639	27.6	0.0003641
27.7	0.000638	27.7	0.0003635
27.8	0.0006369	27.8	0.0003629

27.9	0.0006359	27.9	0.0003623
28	0.0006348	28	0.0003616
28.1	0.0006338	28.1	0.000361
28.2	0.0006327	28.2	0.0003604
28.3	0.0006317	28.3	0.0003598
28.4	0.0006307	28.4	0.0003592
28.5	0.0006296	28.5	0.0003586
28.6	0.0006286	28.6	0.000358
28.7	0.0006275	28.7	0.0003574
28.8	0.0006265	28.8	0.0003568
28.9	0.0006255	28.9	0.0003562
29	0.0006244	29	0.0003556
29.1	0.0006234	29.1	0.000355
29.2	0.0006224	29.2	0.0003544
29.3	0.0006214	29.3	0.0003538
29.4	0.0006203	29.4	0.0003532
29.5	0.0006193	29.5	0.0003526
29.6	0.0006183	29.6	0.000352
29.7	0.0006173	29.7	0.0003514
29.8	0.0006162	29.8	0.0003508
29.9	0.0006152	29.9	0.0003502
30	0.0006142	30	0.0003496

To be continued until time 125 hour

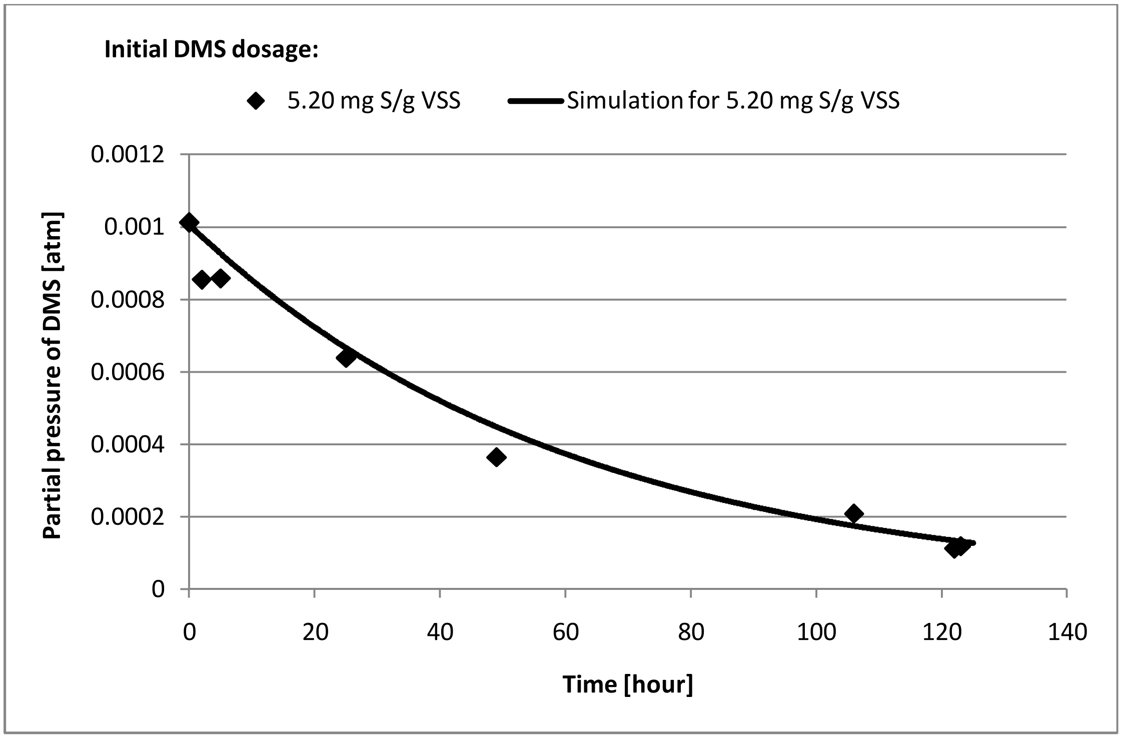


Figure D-6 Plot the results in excel

The fitting process is also conducted by assuming that degradation of DMS follows Monod kinetics or zero order kinetic. However, according to the sum of least squares and visual examination, mixed-second order kinetics could best fit the DMS decline. Therefore, only the regression and simulation based on mixed-second order kinetics are included in the Appendix D.

iii. Statistical tests

At 35 °C, kinetic constants are estimated by fitting the decline of DMS with initial concentrations of 5.20 (high) and 2.92 (low) mg S/g VSS. The estimated values and their sample deviation are as follows:

Table D-7 K_DMS values and standard errors

	High dosage	Low dosage
K_DMS	0.014264	0.012459
Std	0.001484	0.001549
n	8	8

Assumptions:

Estimate K_DMS follows normal distribution,

K_1=K_DMS estimated with high dosage, K_2=K_DMS estimated with low dosage

Different unknown variance: $S_1$and S_2, standard error with high and low dosage respectively

Null hypothesis: K_1 and K_2 are same

Alternative hypothesis: K_1 and K_2 are different

The number of point used for kinetic constant estimation with two dosages both are 8.

$$t = \frac{K_1 - K_2}{\frac{\sqrt{(n_1 - 1) \cdot S_1^2 + (n_2 - 1) \cdot S_2^2}}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}}$$

$$df = \frac{(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2})^2}{\frac{(\frac{S_1^2}{n_1})^2}{n_1 - 1} + \frac{(\frac{S_2^2}{n_2})^2}{n_2 - 1}}$$

Based on the equations, t (two tailed) was 2.38, df was 14. The t (14, 0.99) value from table was 2.97. Therefore, with a 99% confidence, fail to reject null hypothesis. K_1 and K_2 were same.

The end of example

Table D-8 Contents of excel files for Appendix D

Name	Format	Contents
Appendix D-I **Raw data for VOSC conversion**	Excel	Sheet1: Inocula and dosage information summary
		Sheet2: MM degradation at 35 °C
		Sheet3: MM degradation at 55 °C
		Sheet4: DMS degradation at 35 °C
		Sheet5: DMS degradation at 55 °C
		Sheet6: DMDS degradation at 35 °C
		Sheet7: DMDS degradation at 55 °C
		Sheet8: Methionine degradation at 35 and 55 °C
Appendix D-II **Regression and simulation for VOSC conversion**	Excel	Sheet1: DMS degradation at 35 °C
		Sheet2: DMS degradation at 55 °C
		Sheet3: MM degradation at 35 °C
		Sheet4: MM degradation at 55 °C
		Sheet5: DMDS degradation at 35 °C
		Sheet6: DMDS degradation at 55 °C
		Sheet7: Methionine digestion at 35 °C
		Sheet8: Methionine digestion at 55 °C

Please refer to the attached CD for the excel forms or consult author by email address: wdu@uwaterloo.ca

Appendix E
Experiments with Sludge

WMS incubation

Total COD, sCOD, and ffCOD concentrations were measured in WMS incubation. Particulate COD was calculated by (total COD - sCOD). Decline of the particulate COD was fitted by the first order kinetics. The measured and simulated data were listed in excel file “Appendix D-I”. Similarly, measured total TKN, sTKN, and ammonia with incubation time were tabulated. Particulate TKN was calculated by (total TKN - sTKN). Decline of the particulate was fitted by the first order kinetics. The measured and simulated data were listed in excel file “Appendix D-I”. The models for COD and nitrogen conversions including determination of stoichiometric coefficient have been explained in 6.2.2.1 and 6.2.2.2 respectively.

Concentrations of released VSCs were measured through the incubation of WMS with and without methanogen inhibition. The VSC concentrations were normalized by VSS concentration for comparison between different sludge samples. The calculation steps for normalization were same as that illustrated by the DMS example in Appendix C. The accumulated MM and DMS concentrations in the incubation with methanogen inhibition were fitted by model in table 6-10 to estimated hydrolysis constants. Then the model in table 6-12 and estimated kinetic constants were employed to simulate the concentrations of MM and DMS in the incubation without methanogen inhibition. All the VSC concentration measurements and their regression data were listed in excel file “Appendix E-I”.

NAS, BPS, and BAS incubation

The ammonia concentrations were measured with time in the incubation of three sludge samples. The accumulation of ammonia was fitted by the first order kinetics to estimate kinetic constants for mineralization of organic-nitrogen. The measured and regression data were listed in excel file “Appendix E-II”.

VSCs concentrations were measured with time in the incubation and normalized by VSS concentrations. MM and DMS accumulations in the sludge digestion were fitted by the model in table

6-10 to estimate the kinetic constants for solubilization of organic-sulfur. The VSC measurements and their regression data are listed in excel file “Appendix E-II”.

Bench-scale digesters

The COD, TKN, solids, sulfur, and iron concentrations were measured for raw and digested sludge samples. Released biogas volume, methane and carbon dioxide concentrations, and VSC concentrations were monitored for bench-scale digesters. The VOSC concentrations after feeding methionine and sludge were simulated by the model in table 6-12 without and with hydrolysis process. The measurements and regression data were listed in “Appendix E-III”.

Table E-1 Contents of excel files for Appendix E

Name	**Format**	**Contents**
Appendix E-I **Raw data and regression for incubation of WMS**	Excel	Sheet1: COD conversion
		Sheet2: Ammonia release
		Sheet3: Sulfur species in the raw sludge
		Sheet4: VSC release in the incubation with methanogen inhibition
		Sheet5: VSC release in the incubation without methanogen inhibition
		Sheet6: Statistic tests
Appendix E-II **Raw data and regression for incubation of other three sludge samples**	Excel	Sheet1: Inocula summary
		Sheet2: Sulfur species in the raw sludge
		Sheet3: Ammonia release at 35 °C

		Sheet4: VSC release at 35 °C
		Sheet5: Ammonia release at 55 °C
		Sheet6: VSC release at 55 °C
		Sheet7: Statistic tests
Appendix E-III **Raw data and simulation for reactors**	Excel	Sheet1: COD conversion
		Sheet2: Sulfur species in the raw and digested sludge
		Sheet3: Iron in the raw and digested sludge
		Sheet4: Biogas monitoring
		Sheet5: Solids concentrations in the raw and digested sludge
		Sheet6: TKN and ammonia in the raw and digested sludge
		Sheet7: VOSC release after sludge feeding
		Sheet8: VOSC release after methionine addition

Please refer to the attached CD for the excel forms or consult author by email address: wdu@uwaterloo.ca