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Mechanisms of odour production in dewatered biosolids

by

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Abstract

Odours are a common occurrence at wastewater treatment plants, biosolids processing facilities, and biosolids recycling locations. Accurate, objective measurement techniques are needed to monitor emissions, to develop new waste handling procedures and to reduce the production of the volatile gases. The odorants were collected and concentrated by solid phase microextraction (SPME) and then quantified by gas chromatography with detection by mass spectrometry. A 75- μm Carboxen-Polydimethylsiloxane coating was used for the analysis of trimethylamine, dimethyl sulfide, dimethyl disulfide and methyl mercaptan. The differences found in sensitivity between fibers, the competition for the active sites on the fiber and the production of artifacts make SPME well suited for qualitative analysis and enables quick screening for the identification of compounds with adverse organoleptic characteristics.

The objective of this research was to determine the mechanisms and pathways of volatile sulfur compounds production from biosolids in order to provide a foundation for the development of odour control. Volatile sulfur compounds were measured from the biosolids headspace. The effect of environmental conditions during the storage, the wastewater and sludge treatment processes and the protein concentrations were examined. The results from this study can be applied to understand better the volatile sulfur production from different processes.

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List of Notations

<i>Symbol</i>	<i>Brief description</i>
CO ₂	Carbon dioxide
CH ₄	Methane
CS ₂	Carbon disulfide
COS	Carbonyl sulfide
DMS	Dimethyl sulfide
DMDS	Dimethyl disulfide
DMTS	Dimethyl trisulfide
F1 to F4	Fiber 1 to 4
GC	Gas chromatograph
H ₂ S	Hydrogen sulfide
MM	Methyl mercaptan
MS	Mass spectrometer
MW	Molecular weight
ODI	Odour index
ppm	Parts per million
ppb	Parts per billion
PDMS	Polydimethylsiloxane
ROPEC	Robert O. Pickard Environmental Center
SPME	Solid phase microextraction
TMA	Trimethylamine
TS	Total solids fraction
VS	Volatile solids fraction

Chapter 1 - Introduction

Nuisance odors are a common occurrence at wastewater treatment plants, biosolids processing facilities and biosolids recycling locations. Undesirable odours can have detrimental effects on esthetics, property values and the quality of life in the communities subjected to them. An odorous biosolid product is usually perceived as unhealthy, in principal due to the origin of the solids.

In a recent study (Schiffman, 1998) it was found that sensory or respiratory irritation are caused by the exposure to an odorant at concentration above, but within an order of magnitude of the odour threshold. Continuous exposure to compounds such as ammonia lead to odour fatigue and/or tolerance and this reduced sensitivity may jeopardize health. In addition, exposure to odorous compounds at concentrations above the odour threshold, but below irritants levels may produce health symptoms. Some of the symptoms for exposure to mercaptans, thiols and sulfides include sore throat, cough, chest tightness, breathlessness, thirst, sweating, irritability and loss of libido.

The odours from biosolids are a real problem in biosolids management. Complaints and adverse publicity can culminate in local bans on land applications of biosolids, forcing the transport of treated product to more distant locations, or even in the closure of biosolids management facilities.

Odour complaints from agricultural, landfill and wastewater treatment works have increased over the last decade. Stuez et al. (2001) presented data that showed an increase in the number of complaints from agricultural practices (which included the use of biosolids as fertilizers) and industrial processes (which included sewage and sludge

treatment) for 1989/90 to 1995/96 and a slight decrease since 1995/1996. The principal cause for the increase in odour complaints (particularly towards wastewater treatment and biosolids reuse) was believed to result from a general migration of people from cities to rural areas. Thus, more people were exposed to sewage odours, which increased the number of complaints about odour pollution.

One current practice of biosolids usage is land application. Biosolids are trucked to the communities, where they are used by farmers for growing crops. One common problem with biosolids used in land application is the odour released during transportation and land application. (Jacobs et al., 2002, Ross et al., 2002). Biosolids handling practices and treatment processes are known to impact the nature and the extent to which odours are released during transportation and land application. Some of the processes that affect the odours of the final product are the stabilization process, the dewatering system, the chemical usage and the period of storage. Thus the odours from biosolids that were anaerobically digested and dewatered with high solids centrifuges have been reported to be particularly odorous (Ross et al., 2002; Jacobs et al., 2002; Murthy et al., 2003; Higgins et al., 2002)

The lack of knowledge of odour generation leads to a malodorous final biosolids product that can produce illness in the communities subjected to the biosolids. The overall objective of this study was to obtain an improved understanding of the factors leading to odour formation from biosolids. The goals of this study were defined, as follows:

- 1) To develop a method to collect and analyze volatile sulfur compounds and trimethylamine in biosolids headspace;
- 2) To evaluate the impact of storage conditions on odour formation and to identify the compounds responsible for the odours released from biosolids.
- 3) To evaluate the effect of upstream conditions on biosolids odour formation.
- 4) To analyze the proteins in biosolids as the principal precursors of odours and to develop a theory for the cause of the odours.

To satisfy all of the above goals the research was structured in phases as follows,

The first phase addressed the development of a solid phase microextraction method for odour measurements as follows,

- a) Development of calibration curves;
- b) An evaluation of the impact of the SPME needle and the presence of water vapors on the generation of artifacts;
- c) An evaluation of the impact of competitive adsorption on the SPME measurements.

The second phase was dedicated to characterization of the chemical compounds in the headspace of the biosolids matrix, as follows,

- a) Identification and quantification of chemical compounds in the odour matrix from biosolids that originated from the ROPEC wastewater treatment plant, the Kingston Ravensview wastewater treatment plant and five bench scale reactors that were operated at different temperatures;
- b) Evaluation of the redox condition in the stored biosolids;

c) Evaluation of the impact of environmental conditions (aerobic or anaerobic) on the formation/degradation of odorous compounds;

d) Evaluation of the impact of polymer on the biosolids odours;

e) Evaluation of the effect of centrifugation on the biosolids odours.

The third phase studied the protein content of biosolids during storage and included:

a) Development of a protein calibration curve;

b) Quantification of proteins from the biosolids that originated from ROPEC and the five bench scale reactors that were operated at different temperatures;

c) Development of protein and sulfur mass balances;

d) Evaluation of the impact of polymer dosing on protein concentrations;

e) Evaluation of proteins as the principal precursors of biosolids odours.

Chapter 2 - Background

The nature of air emissions from biosolids facilities is a function of a wide variety of chemical, biological and physical processes. Biosolids are an abundant source of nutrients for microorganisms including proteins, amino acids and carbohydrates. The microorganisms degrade these energy sources and odorous compounds are often formed. Organic and inorganic forms of sulfur, ammonia, amines and organic fatty acids have been identified as the most offensive odour causing compounds associated with biosolids production.

By definition, the malodorous compounds (odorants) associated with biosolids, manures and other organic material are the volatile emissions generated from the chemical and microbiological decomposition of organic nutrients. When inhaled, these odorants interact with the odour sensing apparatus (olfactory system) and the person perceives the odour. (US-EPA, 2000)

The odours from biosolids vary by the type of residual solids processed and the method of processing. Anaerobic digestion produces hydrogen sulfide (H_2S) and other sulfur containing gases, while alkaline stabilization of the solids volatilizes ammonia along with other volatile compounds. Composting odours can be caused by ammonia, amines, sulfur-based compounds, fatty acids, aromatics and hydrocarbons. Aerobically digested sludges may contain little H_2S , but have mercaptan and sulfide odours (US-EPA, 2000).

The compounds that are most often reported as responsible for odours from biosolids are presented in Table 2.1. These compounds are intense and have odour thresholds in the parts per billion-concentration range. Thus, the odours from biosolids

have bad characteristics even when the compounds in the matrix of the odours are present in very small concentrations. In the next chapters of this study a special attention will be paid to the volatile sulfur compounds and trimethylamine. These compounds have very low threshold and because of that are very difficult to quantified.

Table 2.1 – Compounds of potential concern in biosolids

Nr. crt.	Compound	Formula	MW	Odour	Odour threshold ppm
Sulfur compounds					
1	Sulfur dioxide	SO ₂	64	Pungent, irritating	0.009
2	Hydrogen sulfide	H ₂ S	34.1	Rotten eggs	0.0005
3	Carbon disulfide	CS ₂	76.1	Rotten pumpkins	0.0077
4	Dimethyl sulfide	(CH ₃) ₂ S	62.1	Rotten cabbage	0.001
5	Dimethyl disulfide	(CH ₃) ₂ S ₂	94.2	Rotten cabbage	0.0025
6	Dimethyl trisulfide	(CH ₃) ₂ S ₃	126.2	Rotten cabbage	0.01
7	Methanethiol	CH ₃ SH	48.1	Decayed cabbage	0.0005
8	Ethanethiol	CH ₃ CH ₂ SH	62.1	Sulfide, earthy	0.013
Ammonia and nitrogen containing compounds					
					ppm
1	Ammonia	NH ₃	17.1	Medicinal	17
2	Trimethylamine	(CH ₃) ₃ N	29.1	Fishy, pungent	0.0004
Volatile fatty acids					
					ppb
1	Methanoic (formic)	HCOOH	46.0	Biting	23.97
2	Ethanoic (acetic)	CH ₃ COOH	60.1	Vinegar	1019.13
3	Propanoic (propionic)	CH ₃ CH ₂ COOH	74.1	Rancid, pungent	27.77
4	Butanoic (butyric)	CH ₃ (CH ₂) ₂ COOH	88.1	Rancid butter	0.28
5	Pentanoic (valeric)	CH ₃ (CH ₂) ₃ COOH	102.1	Unpleasant	0.63
6	3-methylbutanoic (isovaleric)	CH ₃ CH ₂ CH(CH ₃)COOH	102.1	Rancid cheese	12.66
Ketones					
					µg/m ³
1	Propanone (acetone)	CH ₃ COCH ₃	58.1	Sweet	47500
2	2-Butanone (methyl ethyl ketone)	CH ₃ COCH ₂ CH ₃	72.1	Sweet, acetone	737
3	2-Pentanone (MPK)	CH ₃ COCH ₂ CH ₂ CH ₃	86.1	Sweet	28000
Other compounds					
					µg/m ³
1	Benzothiozole	C ₆ H ₄ SCHN	135.2	Penetrating	442
2	Ethanol	C ₂ H ₅ OH	44.1	Green sweet	0.2
3	Phenol	C ₆ H ₅ OH	94.1	Medicinal	178

(Williams and Miller, 1992; Hentz, 1997)

2.1 Bacterial transformation of organic sulfur compounds

The possible links between dimethyl sulfide (DMS) and climate change have stimulated research into microbial processes that produce and consume DMS. The main transformations of the sulfur cycle and interactions between organosulfur compounds and sulfur gases are presented in Figure 2.1 (Taylor, 1992)

Figure 2.1 Sulfur cycle

In Figure 2.1 dotted lines show photochemical conversions in surface ocean waters. CH_2O refers to organic matter that is oxidized anaerobically to CO_2 by sulfate reducing bacteria to form H_2S , some of which is assimilated by plants and microorganisms to make cysteine, and thus homocysteine and methionine.

2.1.1 Production and metabolism of methylated sulfur compounds

Dimethyl sulfide is formed during the biodegradation of organic sulfur compounds and by the biological methylation of sulfide and methanethiol (MM). Biomethylation is a natural phenomenon occurring in living organisms, in which a methyl group is directly bonded to one or two heavy atoms through. A considerable number of elements will undergo biomethylation and among them are As, Hg, Sn, Se, Te, Ge, Sb, P, S and Cl, all of which are environmentally important (Fatoki, 1997). In the next part of this thesis S will be considered as the element that undergo biomethylation and form dimethyl sulfide.

The precursors of DMS include methionine, s-methylmethionine, dimethylsulfoniopropionate (DMSP), dimethylsulfonioacetate (DMSA), dimethyl sulfoxide (DMSO), methionine sulfoxide and sulfone, trimethylsulfonium salts, S-methylcysteine, homocysteine, 2-mercaptoacetate. The principal reactions for DMS and MM and also some of the organisms that catalyze these reactions are presented in Figure 2.2.

Figure 2.2 – Principal routes for bacterial formations of methylated sulfur compounds from organosulfur precursors (Taylor, 1992).

Summary of DMS and MM transformation

Reaction 1 represents the methylation of homocysteine to methionine in the presence of enzymes, with some DMS production (reaction 1') (Taylor, 1992). There are two types of this enzyme: cobalamin dependent MS (*EC 2.1.1.13*) and cobalamin independent MS (*EC 2.1.1.14*) which share no homology and have different evolutionary origins. The former type is found in bacteria, protozoa, and metazoa. Bacteria and protozoa may also possess the cobalamin-independent MS, which is the only enzyme found in fungi and plants (Kocprazak et al., 2003).

Methionine has been found to primarily produce methyl mercaptan (reaction 2). However, the production of smaller quantities of dimethyl sulfide and dimethyl disulfide has also been observed. This pathway has been observed to be active under both aerobic

and anaerobic conditions. Although a majority of the literature has focused on anaerobic sediments, this reaction has also been observed in soils. Aerobic (*Pseudomonas*), anaerobic (*Clostridium*) and facultative (*Proteus vulgaris*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Serratia marcescens*) bacteria (Taylor, 1992; Pohl et al., 1984) as well as fungi (*Penicillium*, *Candida*, *Aspergillus*, *Scopulariopsis*) (Taylor et al., 1992) have been found to mediate this reaction.

Kim et al., (2001) found that sufficient enzyme activity was present in sludges to breakdown polymer and proteins (e.g. methionine), even if microorganisms were not present. The enzymes found to stimulate the production of methyl mercaptan from methionine were *methionine γ -lyase* (Taylor, 1992) and *Strains W 83* and *W 50* that were isolated from *Phyromonas gingivalis* (Yoshimura et al, 2000).

Reaction 3 represents DMS production by bacteria from S-methylcysteine. MM was found to be an intermediate product of the reaction. The enzyme *S-alkylcysteinesulfoxides* which demethylates S-methylcysteine was purified from an aerobic soil bacterium *Pseudomonas cruciviae* (Taylor et al., 1992).

Transformation of MM to DMDS (reaction 4) is a chemical and probably biochemical oxidation. The oxidation probably occurs in oxic zones and may occur abiotically. Presumably some bacteria species catalyze the oxidation of MM to DMDS as per:

Biological reduction of DMDS to MM (reaction 5) was observed with anoxic slurries from salt-marsh sediments and with cultures of *thiobacilli* that grow aerobically on DMDS as their sole source of carbon and energy as per:

In reaction 6 MM is oxidized to formaldehyde (HCHO), followed by the oxidation of formaldehyde to formate and then CO_2 . These reactions are catalyzed by pyridine nucleotide-dependent formaldehyde and formate dehydrogenases. (Wolfe et Bates, 1992, Taylor, 1992)

In reaction 7 DMS and MM are degraded to hydrogen sulfide and sometimes methane in various anoxic environments. Methanogens such as *Methanobolus siciliae* T4/M, *methanohalophilus zhilinaeae* WeN5, *methanohalophilus oregonensis* WAL and strain GS-16 were able to use MM and DMS as their catabolic substrate. The methylated sulfides were degraded to methane, sulfide and carbon dioxide according to the following reactions. (Ni et Boone, 1992, Lomans et al., 2002)

The reversible reaction DMS-MM is presented in reaction 8. Pure cultures that grow aerobically on methylated sulfides, as sole sources of carbon and energy, are either *autotrophic thiobacilli* or *methylotrophic hyphomicrobia*. Key enzymes in the catabolic sequence are *oxygenases* catalyzing the incorporation of molecular oxygen into the products. DMS monooxygenase yields MM and formaldehyde (HCHO) (Taylor, 1992). In anaerobic environments, successive methylations produce MM and DMS. These transformations may be carried out by organisms that possess *thiol S-methyltransferase* (Taylor, 1992; Taylor et al., 1992).

Reactions 9, 10 and 11 describe processes that have dimethylsulfoniopropionate as a precursor. This compound is present in algae and plants that grow in saline environments. These reactions are not considered significant in a biosolids environment.

S-methylmethionine (reaction 12) is a significant precursor of DMS in terrestrial regions. It is a sulfonium compound that is present in a variety of terrestrial plants (for example the high emissions of DMS associated with corn plants is probably due to the enzymatic breakdown of S-methylmethionine). *Corynebacterium sp.* were isolated from soil and enzymatically degraded S-methylmethionine to DMS. (Taylor et al., 1992, Taylor, 1992)

In reaction 13, DMSO is reduced chemically and biochemically to DMS. Many microorganisms, both bacteria and yeasts, reduce DMSO to DMS under anaerobic conditions. The enzymes responsible for the above phenomena were isolated from fungi and bacteria under anaerobic conditions. *Hyphomicrobium ES* and *Hypomicrobium S* were exceptions, which grew aerobically on DMSO, and carried out an aerobic reduction of DMSO to DMS catalysed by a DMSO reductase. (Taylor et al., 1992, Taylor, 1992)

Other pathways for DMS biogenesis involve its formation from inorganic sulfide via methylation reactions and its formation from methoxylated aromatic compounds and inorganic sulfide. In both of the above pathways, an important element is inorganic sulfide. The formation of H₂S arises from two sources: the reduction of sulphate and the desulphurisation of organic compounds containing sulfur in a reduced state (Gostelow et al., 2000).

Reduction of sulfate involves equation 2.3, and equation 2.4 presents the formation of hydrogen sulfide.

An example of desulphurisation of organic compounds is described in equation 2.5:

The *S-methyltransferase* enzyme catalyzes the methylation reactions (Taylor, 1992). Some examples of methylation reactions from inorganic sulfide are presented in equations 2.6 and 2.7.

Franzmann et al. (2001) reported the formation of dimethyl trisulfide through the same mechanisms. DMS can also be generated by a transmethylation reaction between sulfide and methanethiol using methoxylated aromatic compounds as methyl donors. Transmethylation processes may account for the reports of high rates of DMS production in some freshwater environments (Taylor, 2002). Bak et Finster (1992) isolated two strains (*TMBS4*, *DMS No 6591*) of strictly anaerobic rod-shaped bacteria, which used syringic acid as the substrate. The acetogenic bacteria (rod shape *Acetobacterium* species) produced DMS as the final product and MM as an intermediary as indicated by equations 2.8 and 2.9.

2.1.2 Production and metabolism of carbonyl sulfide and carbon disulfide

COS and CS₂ can be formed and degraded by both plants and microbes. Studies on the biodegradation of plant residues and animal manures in soil identified cysteine, cystine and homocysteine as the precursors for CS₂. Thiocyanate and isothiocyanates were the precursors for COS and degradation of lanthionine produced both COS and CS₂ (Taylor, 1992). All the transformations occurred in either aerobic or waterlogged soils. These pathways are presented in Figure 2.3.

Figure 2.3 – Sources of COS and CS₂ in aerobic and waterlogged soils

CS_2 and COS have been found to be degraded aerobically to CO_2 and H_2S by *Thiobacillus thio-oxidans* and *T. thioparus strain TK-m* (Taylor, 1992) as shown in equations 2.10 and 2.11.

Anaerobic digestion has nine steps, each mediated by a specific group of microorganisms and their enzyme complements. The first three steps are accomplished by hydrogen and carbon dioxide production, as follow. During the enzymatic hydrolysis of organic polymer to intermediate organic manomers 5% of the by products are H_2 and CO_2 . Second step is represented by fermentation of organic manomers to H_2 and CO_2 (10 %), bicarbonate, pyruvate, alcohols and lower fatty acids. The third step involves the oxidation of reduced organic products to H_2 and CO_2 (13%), bicarbonate and acetate (Metcalf and Eddy, 1987, Malina and Pohland, 1992). In anaerobic situations, methanogens may utilize the carbon of CS_2 and COS (Taylor, 1992), as can be seen in equations 2.12 and 2.13.

2.1.3 Discussion and conclusions

Possible links between DMS and climate change have stimulated research into microbial processes that produce and consume DMS. It was found that microorganisms can both generate and consume methylated sulfur compounds. Upon examining bacterial transformations of organic sulfur compounds in natural environments, it becomes apparent that major differences between marine and freshwater or terrestrial regions arise from the presence of dimethylsulfoniopropionate in marine plants.

The reactions described above have as products odorous volatile sulfur compounds found to be responsible for the odours inside of the wastewater treatment plant and during land application of the biosolids. The methylated sulfur products that arise from wastewater treatment processes have amino acids such as methionine, S-methylcysteine, S-methylmethionine, homocysteine, lanthionine and cysteine as precursors, with the paths of generation described in Figure 2.2 (paths 1 to 8, 12 and 13) and in Figure 2.3. Enzymes that catalyzed the cleavage of methylated sulfides from organic sulfur compounds were all isolated from different terrestrial environments (soils, forests, sediments, lakes, wastewater) (Taylor, 2002; Pohl et al., 1984).

2.2 Chemical transformation of organic sulfur compounds

Bashkova et al (2002) used activated carbon for the removal of MM from a gas stream. The results of the experiments indicated that when the pH of the surface where the reaction took place was less than the pKa for mercaptide ion, MM was oxidized by adsorbed oxygen to form DMDS, as shown below:

Where: $\text{O}\bullet$ is oxygen radical. pH is characteristic for the surface where the reaction takes place. For mercaptide anion it was found that pKa is 10.7 (US-EPA, 2001).

When the pH was higher than the pKa, MM was converted to its anion that was further oxidized by adsorbed oxygen to form DMDS.

Where: $\text{CH}_3\text{SH}_{\text{gas}}$, $\text{CH}_3\text{SH}_{\text{adsorbed-L}}$, $\text{CH}_3\text{SH}_{\text{adsorbed}}$ are concentrations of species in gas, liquid and adsorbed phases.

Chin and Lindsay (1994) found that ascorbate (vitamin C) served as a pro-oxidant that resulted in the rapid formation of dimethyl disulfide (DMDS) and dimethyltrisulfide (DMTS) from methyl mercaptan. The proposed pathways of DMTS generated under aerobic conditions were reported as:

A = dehydroascorbate

AH_2 = ascorbate

In the proposed mechanism methyl mercaptan oxidation was initiated by metal ions such as Fe(III), by accepting an electron from the thiol to form Fe(II) and a thiyl free radical (Reaction 2.20). Ascorbate could also reduce Fe(III) to Fe(II), as shown in Reaction 2.21. Hydrogen peroxide was produced from the aerobic oxidation of ascorbate in the presence of transition-metal ions (Reaction 2.22). Fe(II) may react with hydrogen peroxide to yield the hydroxyl radical (Reaction 2.23). Hydroxyl radicals are very reactive and could react rapidly with free radicals to form a sulfenic acid (Reaction 2.24). Dimethyl trisulfide would then arise from a reaction of the sulfenic acid with hydrogen sulfide (Reaction 2.25). Alternatively, two molecules of sulfenic acid may dehydrate to form methyl methanethiosulfinate (Reaction 2.26). Methyl methanethiosulfinate reacts rapidly with hydrogen sulfide to yield dimethyl trisulfide.

Hence, it is likely that functional groups on the surface of soils or other media (biosolids) or/and metal ions, such as iron ions, could catalyze the oxidation of mercaptans to polysulfides. Oxygen, vitamins and hydroxyl radicals can also be involved in the oxidation of MM. In biosolid media, under aerobic conditions, chemical oxidation catalyzed by the presence of metal ions could be an important path in the formation of polysulfides such as dimethyl disulfide and dimethyl trisulfides.

2.3 Other odorous compounds in biosolids

Ammonia is a product of decomposition under aerobic and anaerobic conditions. Its release is impacted by the rate of organic decomposition, sludge characteristics, pH and temperature (Wilber et. al., 1997). Ammonia originates from a process known as protein deamination (Hentz, 1997). Amino acids contain an amino group ($R-NH_2$). Under both, anaerobic and aerobic conditions bacteria breakdown these amino acids and ammonia is a by-product.

Amines can be produced in detectable quantities in wastewater treatment plants that employ high temperature processes, as lime stabilization. Amines result from microbial decomposition that involves decarboxylation of amino acids and degradation of proteins. The amines that are produced are easily volatilized when temperatures are elevated above $27^{\circ}C$ (EPA, 2000). In biosolids produced with polymeric flocculating agents, ambient temperatures can accentuate volatilization of amines that may be microbially split off from the core backbone of the polymer (Novak et al., 2002). Amines include methylamine, ethylamine, trimethylamine and diethylamine. Trimethylamine (C_3H_9N) is the most commonly reported amine that has been reported to be responsible for odours in biosolids.

The most common protein found to be responsible for the production of trimethylamine is trimethylamine N-oxide reductase. Karp et. al. (2002) separated more than one form of trimethylamine N-oxide reductase from *E. coli*. One possible path for trimethylamine formation is presented in equation 2.28.

Nicotinamide adenine dinucleotide (NAD) is one of the most important coenzymes in the cell, utilized alternately as an oxidizing or reducing agent in various metabolic processes. NADH is the reduced form of NAD.

Trimethylamine can be found in almost all living animals and plants, and the mechanism of generation of TMA is microbial oxidation (Seibel and Walsh, 2002). The mechanism of oxidation is presented in equation 2.29.

Where R is usually choline or carnitine.

Kim et al. (2001) generated enzyme liquor from wastewater solids. The enzyme liquor for this study was generated from wastewater, by sonicating the solids for 20 minutes and filtering the sonicate through a 0.45 μm filter to exclude microorganisms. It was found that macromolecules like proteins from biosolids and cationic polymers are degraded through enzyme hydrolysis to form TMA or its precursors from biosolids.

Ketones such as acetone (CH_3COCH_3) and 2-butanone ($\text{CH}_3\text{COCH}_2\text{CH}_3$) originate from a microbiological process known as ketosis (Hentz, 1997) that is involved in the metabolism of fat. Ketones can be found in living animals and are excreted through the urine and those that are volatile (such as acetone) can be expelled through the lungs. In addition, ketones can be formed via anaerobic decomposition of cellulose, starch, hemicellulose and pectins. *Clostridium sp.* bacteria have been identified as acetone producers and had been found in wastewater and biosolids (Rosenfeld et al., 2001).

Acetone and 2-butanone are 3 to 4 orders of magnitude less odorous than sulfur containing compounds.

As an overall conclusion, the main precursors of the odorous compounds found in biosolids are proteins and carbohydrates. These are reduced to odorous compounds through microbiological and enzyme activity.

2.4 Characterization of wastewater and solids odours

After reviewing the literature, four areas of research related to odours in biosolids have been identified. These include the chemical compounds that produce the odours, methods to measure these chemical compounds, factors that promote the formation of odours and solutions for mitigation of these odours.

2.4.1 Biosolids odours affected by the stabilization process

The generation of odours from biosolids cakes has been found to be influenced by the upstream conditions. The stabilization process was considered the most important factor in the pattern of the odour, and hence the composition of the odour will be explained for each process separately.

2.4.1.1 Lime stabilization

Dewatered biosolids from the lime stabilization process seem to be the most documented from an odour point of view. The District of Columbia Wastewater Treatment Plant at Blue Plains was the source of data for all the reports found. In all the studies, TMA was one of the most important chemical-causing odours in lime stabilized

biosolids cake (Wilber et al., 1997; Kim et al., 2001; Murthy et al., a, 2001; Murthy et al., b, 2002; Novak et al., 2002;). In all the studies the air samples were collected from the headspace of the dewatered biosolids either with tedlar bags (Wilber et. al, 1997; Murthy et al., b, 2001; Murthy et al., 2002; Novak et al., 2002) or with solid phase microextraction (Kim et al., 2001; Murthy et al., a, 2002) and analyzed by gas chromatography. The concentration of TMA in the headspace of the stabilized cake was between 170 and 370 ppb (Murthy et al., a, 2002) and seemed to increase with polymer dose.

Another important class of compounds in the composition of odour, from lime stabilized biosolids, were the volatile sulfur compounds. Because the lime raises the biosolids pH it is expected that the microbial activity to be inhibited and the DMS and MM transformations to not follow anymore the routes presented in figure 2.2. However, it was found that for the same wastewater treatment plant (the same sludge) different mixtures of volatile sulfur compounds and a range of concentrations were reported. The composition of the mixtures and the concentration of the analytes are presented in Table 2.2.

Table 2.2 – Reduced sulfur compounds in lime stabilized biosolids

	MM (ppb)	DMS (ppb)	CS ₂ (ppb)	DMDS (ppb)	Month (2000)
Kim et al. (2001)	NS	ND	ND	18	September
	NS	11	ND	19	September
	NS	3.6	3.2	14	November
Murthy et al., b, (2002)	ND	1600	NS	354	July
	4.05	1080	NS	706	August
	7.12	1070	NS	2550	September
	ND	595	NS	82	November

Where: ND – not detected, NS – not studied

From Table 2.2 can be seen that the concentration of volatile sulfur compounds varied from month to month. Also, comparing the values reported in two different studied

(Kim et al., 2001 and Murthy et al., b, 2002), high differences among concentrations were found. These differences probably appeared as a consequence of the collection method. Kim et al. (2001) used solid phase microextraction and Murthy et al., b, (2002) used tedlar bags. Higher concentrations of volatile sulfur compounds were measured when tedlar bags were used.

Trimethylamine, methyl mercaptan, dimethyl sulfide and dimethyl disulfide were the chemicals found to be responsible for odours in dewatered lime stabilized biosolids. They had a random variation during the period of storage, for different periods of the year, being a function of the collection method.

2.4.1.2 Anaerobic stabilization

Anaerobic digestion is the most practiced solids stabilization technology. Most wastewater treatment plants have mesophilic digestion, and consequently most research has addressed biosolids from this process. The principal difference that appeared between alkaline stabilization and anaerobic digestion was the fact that TMA was not reported as an odour in anaerobically digested biosolids.

The volatile sulfur compounds demonstrated a random pattern in composition and concentration. Murthy et al. (2003) measured the concentrations for DMS and MM in three different months (March, May and October 2003), for fresh dewatered biosolids and during twenty-two days of storage. For fresh biosolids, the concentration values had almost the same values for each period of study ($MM \cong 0.0$ ppm, $DMS \cong 5.0$ ppm). Over the period of storage the pattern of methyl mercaptan and dimethyl sulfide generation varied from month to month, with the higher values recorded in March ($MM \cong 950$ ppm,

DMS $\cong$ 320 ppm) and the lower values recorded in October (MM $\cong$ 450 ppm, DMS $\cong$ 200 ppm).

DMS and MM were also reported as being responsible for “the unusual scent” of Toronto biosolids (Ross et al., 2002). These odorous compounds were measured from digested sludges after 24 hours, centrifuged cake, a truck load-out facility and a storage facility. Interestingly, there was no reduced sulfur compounds detected in biosolids from the storage facilities, although odours were significant from centrifuge-dewatered cake (MM $\cong$ 30 $\mu\text{g/L}$, DMS $\cong$ 20 $\mu\text{g/L}$).

CS₂, DMDS, DMS and MM were the volatile sulfur compounds measured in the odour matrix from a dewatered biosolids (Higgins et al., 2002). The concentrations measured after 24 hours of storage (MM = 240,000 $\mu\text{g/L}$, DMS = 17,000, CS₂ = 1,300 $\mu\text{g/L}$ and DMDS = 21,000 $\mu\text{g/L}$) were much higher than the concentrations measured after 48 hours (MM = 7,100 $\mu\text{g/L}$, DMS = 230, CS₂ = 470 $\mu\text{g/L}$ and DMDS = 250 $\mu\text{g/L}$).

Volatile sulfur compounds such as methyl mercaptan, dimethyl sulfide, dimethyl disulfide and carbon disulfide dominate the odours from dewatered mesophilic digested biosolids. The variation of these odorous compounds during the period of storage would appear to be a function of both the wastewater treatment plant where they were generated and the period when the sample was taken.

There was less data available on odours from cakes that are produced by thermophilic digestion. Jacobs (2002) conducted a study at the Hyperion Treatment plant, a mesophilic digestion plant that also had a full-scale pilot testing thermophilic digestion. The analyses conducted were both qualitative and quantitative. Samples were taken from dewatering centrifuges, the storage silos and during land application for a period of three

days. The conclusions were that thermophilic cake odour was somewhat higher than the mesophilic sludge during active processing in the plant (during dewatering), but the thermophilic sludge was orders of magnitude less odorous during storage and during land application than the mesophilic sludge.

Hatfield and Burnham (1994) demonstrated that fatty acids were an important class of compounds in the odour matrix from dewatered anaerobically digested biosolids. Digested sludges consist almost completely of bacteria and fatty acids are the primary component of the membranes of bacteria, hence it appeared that fatty acids were a significant part of the organic matter in sludge. Usually fatty acids are not highly volatile, but the fatty acids present in bacteria are readily degraded to short chain fatty acids (e. g. acetic acid), that are both volatile and odorous. Other volatile fatty acids (besides acetic acid) measured from biosolids were propionic and butyric acids. The odours produced by volatile fatty acids are very intense.

2.4.2 Conditions and factors that promote the formation of odours in dewatered cake

Factors that have been found to influence odour production from dewatered cake include the protein content of the digested sludge, polymer usage, anaerobic storage and enzyme-mediated transformations. Stabilization processes that achieve greater than 45-50 % volatile solids destruction may still possess residual biological activity.

A well-digested product may consist of proteins that become labile and degrade over storage time to produce odours (Murthy et al., 2002; Murthy et al., b, 2003). It is believed that the proteins remaining after anaerobic digestion will remain unavailable as

long as the post-digestion, processing or storage does not make them available for degradation.

If available, macromolecules like cationic polymers and proteins in biosolids may be degraded by enzymes to cause odours (Kim et al., 2001; Novak et al., 2002). The extent to which polymers contribute to odour generation in biosolids has not been completely elucidated. There are conflicting reports with one concluding that biosolids odours were not affected by dewatering polymers (Rosenfeld et al., 2001) and the second stating that TMA is released from certain polymer structures (Dentel et al. 2002).

Higgins and Novak (1997) have shown that proteins can be found in the solution-phase or can be bound to the flocs. In this study, the solution-phase proteins will be considered the soluble proteins. The proteins bound to the flocs will be considered the total proteins. Only a fraction of the total proteins, the labile proteins, are considered to be precursors of the volatile sulfur compounds. The labile proteins are the bound proteins that, with applied stress, break down into amino acids and act as precursors for odorous compounds. Some of the bound proteins will become labile proteins with solids processing (polymer addition, shear force applied during the dewatering and anaerobic storage). The bioavailability of labile proteins is directly related to factors such as applied shear, period of storage, polymer concentration. Applied shear and storage act directly upon the total proteins, thereby affecting the fraction of labile proteins. Polymer addition binds much of the soluble polymer, increasing in this way the concentration of labile proteins (Murthy et al., b, 2002).

Total proteins are generally much less available to biodegradation than soluble proteins. It is hypothesized that some of these sorbed proteins can become labile with

applied shear. Shear is present in almost all bioprocesses, and high shear is associated with processes involving agitation and emulsification. Shear is common in processes such as mixing, centrifugation, filtration and even during pumping and passing through tubing. It was found that highly sheared proteins experience conformational changes and also suffer backbone clipping (Maa et Hsu, 1996).

Hence, it is expected that the dewatering process will increase the number of labile proteins in the biosolids matrix, and consequently increase the odours predecessors. Murthy et al., c (2002) found that storage of biosolids obtained from high solids centrifuges (high shear equipment) produced concentration of volatile sulfur compounds that were three orders of magnitude higher than the concentrations from biosolids obtained from a low solids centrifuge (low shear equipment). The researchers also reported that biosolids subjected to a combination of centrifugation and screw conveyance (high shear conveyance) produced concentrations of volatile sulfur compounds as much as three orders of magnitude more than concentrations from biosolids that were subject to centrifugation alone. From the above study it appeared that the high shear equipment made more proteins available for biodegradation than low shear equipment.

During long-term anaerobic storage significant changes in dewatered cake and microbial composition take place. Anaerobic storage of activated sludge was found to cause a deflocculation of the sludge (Nielsen et al., 1996). The bonds between flocs are broken by the microbiological activity and the proteins are more available for degradation. Probably the same mechanism takes place during anaerobic storage of the dewatered biosolids.

It has been assumed that cationic polymers used to enhance the dewaterability characteristics of biosolids ‘bridge’ between negatively charged floc and particles in the solution-phase. Thus, the polymer brings the solution-phase protein out of the solution into the floc matrix (Murthy et al., b, 2002; Novak et al., 2002).

The proteins in dewatered biosolids are assumed to be the principal cause of odours. During storage of dewatered biosolids a fraction of the proteins became available for degradation and acted as precursors for the compounds found in the odour matrix. To investigate relationships between protein degradation and odour production it is necessary to quantify the proteins in the dewatered cake. Two methods to quantify the concentration of the proteins have been reported by Higgins and Novak (1997) and Ross et al., (2002), respectively. In these studies, the biosolids were centrifuged at different speeds and the supernatant was considered the soluble fraction. Higgins and Novak (1997) considered the labile proteins as those that could be extracted by a dilute salt solution from the pellet that resulted after the first centrifugation. Ross et al. (2002) washed the pellet that resulted after the first centrifugation twice with phosphate buffer. The solution that resulted was centrifuged again and then mixed with Dowex strong cation exchange resin. After decanting the supernatant this was centrifuged twice and this fraction was considered the labile fraction. The protein concentrations were measured using the Hartree method (1972) that is a modification of the method of Lowry et al. (1951).

2.4.3 Odour mitigation

Chemical additives have been evaluated for reduction of odour production during storage of biosolids cakes. Chemical additives impact on both microbial metabolism and the chemistry of volatile sulfur compounds.

Ferric chloride was added prior to polymer conditioning and subsequent dewatering in a medium solids centrifuge. This was found to bind proteins and the iron-bound protein degraded slower than the proteins from the control samples (Higgins et al., 2002). Also, probably the odorous compounds were adsorbed on iron hydroxides, which precipitated. Increasing the FeCl_3 dose from 0 to 125 g per kg of dry solids resulted in decreased peak MM concentrations as well as a delay in the time to peak and it also reduced DMS concentration to very low values. Thus, ferric chloride was found to enhance odour control (Bielefeldt et al, 2002; Higgins et al., 2002).

Organic matter (the substrate) is a carbon source for microbial growth and is needed for formation of new cells, and as an energy source for maintaining life. The microbial catabolic processes include two steps, oxidation of organic matter and reduction of an electron acceptor. The organic matter is the electron donor and the corresponding reduction of an external electron acceptor is performed when either dissolved oxygen, nitrate or sulfate are present. The final products of aerobic and anoxic respiration are carbon dioxide and water, and carbon dioxide, water and nitrogen, respectively. When anaerobic reactions take place, the final products are volatile fatty acids, methane, carbon dioxide and sulfide.

Nitrate can be added to provide an electron acceptor that can promote the degradation of biodegradable substrate under more energy-yielding conditions. The

elevated oxidation-reduction potential leads to the inhibition of sulfate reducing bacteria. The nitrate stimulates the growth of heterotrophic denitrifiers and anaerobic thiobacillus species that compete with sulfate reducing bacteria, and thus reduce their activity. Also, the addition of nitrate provides an oxygen source that promotes the growth of naturally occurring bacteria, which utilize sulfur in their metabolism. Nitrite, the intermediate product of nitrate reduction, may inhibit microbes (Abu-Orf et al., 2002). Potassium nitrate at high dosages was found to reduce MM concentration, but did not have a significant impact on the other volatile sulfur compounds (Higgins et al., 2002). Calcium nitrate with anthraquinone was found to significantly reduce odours due to sulfur compounds (Abu-Orf et al., 2002; Higgins et al., 2002). The quinines inhibit the sulfur reducing bacteria, and thus, when used together (nitrates and quinines) the combined effect leads to a higher degree of sulfate reducing bacterial inhibition. Hence, the odours due to sulfur compounds will significantly decrease.

Oxidizers act on both odorants and non-odorant compounds in the biosolids. These compounds are soluble and have to be added to the cake after dewatering to prevent deflocculation and an increase in the polymer demand. The addition of 44 g/kg dry solids of calcium hypochlorite reduced odours due to volatile sulfur compounds. The analyses were performed after 24 hours of storage (Higgins et al., 2002). A 3% solution of hypochlorite maintained odours below detectable limits for a period of 30 days after treatment (Striebig et al., 1999)

Lime can be used as an inhibitor of biological activity, when added in sufficient quantity to increase the pH (Murthy et al., b, 2001; Higgins et al., 2002). Most organisms

have a favoured pH range for their metabolism and outside of this range the biological activity is reduced.

Because cationic polymers sequester the proteins into the cake, they increase the concentration of proteins that participate in the formation of odorous compounds. Hence, it is necessary to control and minimize the polymer dose (Novak et al., 2002). Higgins et al. (2002) found that a polymer dose of 6.5 g/ kg dry solids produced a lower concentration of methyl mercaptan than a dose of 11 g/ kg dry solids.

Most of the chemicals used, in principal address the decrease of protein availability for the reactions that produce volatile sulfur compounds. Ferric chloride was used to increase the concentration of iron-bound proteins, because these degrade slower, and consequently the odours decrease. An optimal dose of the polymer is necessary to decrease the number of proteins from dewatered cakes available for biodegradation. Nitrate was added to provide a new path for the degradation of proteins. Oxidizers were used to form higher polysulfides that are less odorous chemicals. Inhibitors were added to inhibit the growth of bacteria and microbes.

2.4.4 SPME as a method for measuring odorous compounds

A failure to acknowledge the potential for odours and to work to prevent odour emissions can result in complaints, shutdowns, expensive retrofits and non-acceptance of the finished product. To control odours, they must first be measured. This is not an easy task, because the odour matrix is often very complex (EPA, 2000). Odour measurement techniques fall into two classes. Sensory measurements employ the human nose and measure the effect of the odour as perceived by an observer. Analytical measurements

characterize odours in terms of their chemical composition and attempt to quantify the odorants present. Analytical measurements are complicated by the large number of odorants present, often at concentrations that are close to detection limits (Gostelow et al., 2000). The advantages of analytical methods are their objectivity, repeatability and accuracy. The disadvantages are the following:

- Odorants are often present in a complex matrix, with dozens of compounds. This complicates the analyses considerably and usually necessitates a separation prior to analysis (Grob and Barry, 1995).
- Odorants can be present in very small concentrations. The limit of analytical detection may be above the threshold of smell. Non-odorous compounds can also be present in the sample in much larger concentrations than the odorants (Gostelow et al., 2000).
- It is difficult to relate analytical measurements to the intensity of odour as perceived by a human observer. This is especially the case for mixtures as interactions between different odorants may lead to pleasant or unpleasant effects (Gostelow et al., 2000).

From the large number of analytical methods available for the collection of chemical compounds responsible for biosolids odour, SPME – gas chromatography was selected for examination in this study. Solid phase microextraction (SPME) can be employed to collect and concentrate the odorants from the headspace of biosolids. SPME is a simple, effective adsorption-desorption technique, which eliminates the need for solvents or complicated apparatus for concentrating volatile or non-volatile compounds in liquids or gases (Supelco, Bulletin 923, 1998). When SPME is combined with gas chromatography for analysis, the fiber is transferred to the injection port of the gas

chromatograph, where thermal desorption and transfer of the analytes onto the GC column take place.

SPME integrates sampling and preconcentration in one step. A chemically modified fused silica fiber is exposed in the headspace of the sample or in the liquid sample. Ideally equilibrium is reached between the odour matrix and fiber, but for accuracy and precision, consistency in sampling time is more important than full equilibrium (Supelco, Bulletin 923, 1998). The extraction process is governed by the kinetics of diffusion in the surrounding medium and/or the polymer fiber coating.

Haberhauer et al. (1999) found that SPME has a number of limitations, including the production of artifacts (methyl mercaptan was readily oxidized to dimethyl disulfide, isopropanethiol and isobutanethiol were oxidized into disulfides and dimethyl sulfide was oxidized into dimethyl sulfoxide), the dependence of the extraction efficiency on the relative humidity and low storage stability of the volatile sulfur compounds on the fiber, which leads to the occurrence of decomposition products.

A variety of coated fibers of different polarity and film thickness are commercially available. Pelusio et al., (1995) used a SPME fiber coated with polydimethylsiloxane 100 μm film to identify seven sulfur compounds in black and white truffle aroma. These included dimethyl sulfide (DMS), dimethyl disulfide (DMDS), bis(methylthio)methane ($\text{C}_3\text{H}_8\text{S}_2$), dimethyl trisulfide (DMTS), 1,2,4-trithiolane ($\text{C}_2\text{H}_4\text{S}_3$), tris(methylthio)methane ($\text{C}_4\text{H}_{10}\text{S}_3$) and 2-butanone.

Haberhauer et al. (1999) studied 2 different fibers for separation of volatile sulfur compounds (MM, DMS, isopropanethiol, isobutanethiol). The fibers studied were 100 μm PDMS (polydimethylsiloxane) and 75 μm carboxen – PDMS coated fused silica

fiber. Their results showed that the sensitivity achieved with Carboxen-PDMS 75 μm exceeded the performance of PDMS 100 μm by a factor of 100.

The same 75 μm Carboxen-PDMS fiber was employed to extract the volatile sulfur compounds from saliva and breath (oral malodors) (Payne, 1999). From this matrix MM, DMS, S-methylethanethioate, DMDS, DMTS, dimethyl sulfoxide and methyl (methylthio) methyl disulfide were extracted. Carboxen-PDMS 75 μm was also chosen for extraction of volatile sulfur compounds (CS₂, DMS, DMDS) and TMA from the headspace of biosolids odours (Kim et al. 2002; Murthy et al., a, 2001).

In all of the above studies, SPME was studied in combination with gas chromatography with a mass spectrometer as the detector. As an overall conclusion the 75 μm Carboxen-PDMS fiber had the detection limit for the volatile organic compounds in the low ppt range (0.004 ppb for dimethyl sulfide, 0.05 ppb for methyl mercaptan, 0.01 ppb dimethyl disulfide, 0.004 ppb isobuthanethiol).

Another group of compounds that are of interest in odour matrices includes ammonia and nitrogen containing compounds. Lewis et al. (2002) used two different fibers (PDMS-DVB 65 μm and Carboxen-PDMS-DVB 75 μm) to analyze some common amines: monomethylamine, dimethylamine, trimethylamine, monoethylamine, diethylamine, triethylamine, n-propylamine. The PDMS-DVB was found to have the best results. In a biosolids odour matrix, TMA was the most frequently reported amine, and it was found that Carboxen-PDMS 75 μm was sufficient for analyzing this amine. (Kim et al, 2002; Murthy et al., a, 2001).

Koziel and Spinhirne (2002) studied three different SPME fibers: PDMS/DVB 65 μm , Carboxen/PDMS 75 μm and DVB/carboxen/PDMS 50/30 μm to evaluate their

ability to extract volatile fatty acids (VFAs) from a standard gas. Target VFAs included acetic, propionic, isobutyric, butyric, isovaleric, valeric and hexanoic acids. The most sensitive fiber was found to be DVB/Carboxen/PDMS 50/30 μm . In other studies on the extraction of propionic and butyric acids, an 85 μm polyacrylate fiber was used (Murthy et al., a, 2001; Kim et al., 2002)

The most frequently reported organic compounds from biosolids are organic sulfur compounds and TMA. Based on the above studies a Carboxen-PDMS 75 μm fiber was chosen for this research.

2.5 Conclusions

Odours are a common occurrence at wastewater treatment plants, biosolids processing facilities, and biosolids recycling locations. The literature revealed that the principal contributors to the odours are volatile sulfur compounds, represented by methyl mercaptan, dimethyl sulfide and dimethyl disulfide and trimethylamine. The concentrations associated with the above compounds from dewatered biosolids were reported to be much higher than the odour threshold limits.

Microorganisms can both generate and consume volatile sulfur compounds. Enzymes that catalyze the formation of volatile sulfur compounds from organic material are compiled in Table 2.3. These enzymes have been isolated from soil environments, terrestrial plants, animal manure in soil, wastewater and biosolids and lake sediments.

Methyl mercaptan and dimethyl sulfide were found to be the principal by-products of protein decomposition. Proteins are degraded to methyl mercaptan and dimethyl sulfide by-either aerobic, anaerobic or facultative microorganisms. These processes are enzyme mediated. Carbon disulfide and carbonyl sulfide were another two volatile sulfur compounds found to result from aerobic protein degradation. If enough oxygen is present in the system the aerobic pathways are the principal routes for the formation of organo-sulfur compounds. Thus, dimethyl sulfide, methyl mercaptan, carbon disulfide and carbonyl sulfide are formed. Methyl mercaptan is further aerobically degraded to dimethyl sulfide and by chemical and probably biochemical oxidation to dimethyl disulfide. Dimethyl sulfide is further aerobically degraded to dimethyl sulfoxide and sulfates. If the conditions in the environment become anaerobic it is expected that dimethyl sulfide will be degraded to methyl mercaptan and hydrogen sulfide. Methyl mercaptan, carbon disulfide and carbonyl disulfide are further anaerobically degraded to hydrogen sulfide.

The above described degradation patterns are expected to be found in the stored dewatered biosolids. The predominant degradation pathways in fresh biosolids are expected to be aerobic, and as time passes it is expected that the conditions in the storage facilities will change from aerobic to anaerobic. Hence, the degradation pathways will switch to the anaerobic pathways.

Trimethylamine results from microbial decomposition that involves degradation of proteins. The principal precursor of organosulfur compounds and trimethylamine were proteins. The numerous microorganisms that live in biosolids degrade the proteins and

form the odorous compounds that characterize the biosolids. Other precursors for the formation of odorous compounds, such as ketones, are fats and carbohydrates.

For the collection of the chemical compounds that compose the matrix of the odour a relatively new method was selected. SPME is a new technique that concentrates chemical compounds from the headspace of the biosolids used in combination with gas chromatography.

As indicated above the principal precursors of the odours are proteins. Degradation of these proteins by microorganisms results in the formation of volatile sulfur compounds and trimethylamine. Consequently the methods of mitigation have addressed proteins and microorganisms. Ferric chloride was used for binding some of the proteins in iron-bound proteins, making them less available for degradation. Nitrates were used to enhance the production of new bacteria to compete with the sulfate reducing bacteria. Other chemicals, such as lime, were used to increase the pH of the biosolids and, thus, to decrease the microbial activity. Different process operations as stabilization, anaerobic storage, centrifugation, the use of cationic polymer or lime make the proteins from biosolids more or less available for degradation.

Chapter 3 - Approach, Experimental and Methods

3.1 Project description

The purpose of this research was to determine how storage, anaerobic digestion, and mechanical dewatering affect odor emissions from biosolids cake. Established and new sampling and analytical methods were used to characterize the gas phase of the biosolids that were produced under a variety of process conditions. After the literature review was performed, the protein content of biosolids was analyzed as the principal precursor of the odours.

The project was divided into three principal phases, with each phase corresponding to one set of experiments. The first phase addressed an evaluation of solid phase microextraction as a method for odour measurements. The second phase was dedicated to the characterization of the chemical compounds found in the headspace of the biosolids matrix during one week of storage. The third phase studied the protein content of biosolids as a precursor for biosolids odours.

In the first phase, gas standards were prepared to evaluate SPME as a method for odour measurements and to generate calibration curves. To develop the calibration curves that were used further in this project, the limitations of SPME (revealed by the literature review) were evaluated. One of the SPME variables studied in this phase was the extraction time. The potential generation of artifacts with SPME and the impact of competition on the porous fiber coating were also assessed. The impact of needle coating on the formation of artifacts and competition on the porous fiber were evaluated by testing standards containing a range of odourant concentrations in dry and wet nitrogen.

This was studied with individual standards and in mixtures. The effect of the coated needle on the production of artifacts was evaluated by testing three different SPME assemblies. All assemblies employed the same fiber material, Carboxen-PDMS.

In phase two, mesophilic anaerobic digestion was selected as the stabilization process to be studied, because many of the wastewater treatment plant have implemented this technology. In reviewing the literature, it appeared that mesophilic digestion produced less odorous biosolids inside of the wastewater treatment plant than thermophilic digestion, but more odorous biosolids after a period of storage. In addition, biosolids that originated from digesters operating at temperatures of 35°C, 42°C, 49°C, 55°C and 62°C were evaluated. The effect of cationic polymer addition on the biosolid odours was evaluated in parallel with the effect of digester temperature.

Gas-phase odorous chemicals were measured daily during storage of the dewatered biosolids. Operational parameters were measured simultaneously to determine the influence of these parameters on biosolids odour quality. The impact of oxidation-reduction potential on the biosolids odour was evaluated by establishing aerobic and anaerobic environment in the test vials. To compare the effect of upstream processes (in this case the presence or absence of waste activated sludge) on the biosolids odours, samples of dewatered cake were collected from the Kingston Ravensview wastewater treatment plant and analyzed for odour generation.

Phase three was dedicated to protein analyses. The effect of high solids centrifugation on the proteins was evaluated. Samples used for protein analysis were collected before centrifugation, from the supernatant that resulted after centrifugation and from the dewatered cake. For the dewatered cake, the protein assay was performed every

day for five days of storage. In addition, the protein content of the dewatered cake that was generated in the digesters operating at temperatures of 35°C, 42°C, 49°C, 55°C and 62°C was examined.

3.2 Research approach

Standard analytical tests for total suspended and volatile solids were performed to provide general information about biosolids. The total solids gave indications about the dewatering mechanisms and volatile solids about the quantity of organic material available for degradation. It was anticipated that if a high VS destruction was achieved, the resulting biosolids would be more stable and less odorous.

Headspace analyses for methyl mercaptan, dimethyl sulfide, dimethyl disulfide, and trimethylamines were performed to determine their contribution to odour. They were measured by solid phase microextraction using a gas chromatograph with a mass spectrometer as the detector. The analysis of specific compounds provided insight into the pathways of odour formation and degradation. Sampling of the headspace of the stored biosolids was typically performed daily for a total storage period of five days.

Headspace analyses for methane and carbon dioxide were performed to determine the relationship between redox environment and the formation of odours. It was assumed that anaerobic environments would produce more odorous biosolids than aerobic environments. These analyses would also provide a better understanding of the pathways for odour generation during storage.

Protein analysis was performed as a result of literature reviews that pointed out that proteins are the principal precursors of odour emissions. Anaerobic digestion does not completely degrade proteins. Many of the proteins are bound proteins, but there are

also labile proteins. The labile proteins are the bound proteins that, with applied stress, break down into amino acids and act as precursors for odorous compounds. Some of the bound proteins will become labile proteins with solids processing (polymer addition, shear force applied during the dewatering and anaerobic storage). The measurement of proteins was performed to identify the quantity of substrate available for odor production.

3.3 Experiments

3.3.1 SPME as an analytical method for odorous compounds

Based on a review of the literature, the SPME technique was chosen as the headspace sampling method and a fiber with a Carboxen- Polydimethylsiloxane, 75 μ m film was employed. A gas chromatograph with a mass spectrometer as the detector was used for desorption of target compounds and for their analytical separation. The mass spectrometer detector is usually not considered for low-level volatile sulfur quantification. Selective detectors such as the flame photometric (FPD), pulsed flame photometric (PFPD) and sulfur chemiluminescence (SCD) have been traditionally used in these applications. A common problem with many sulfur selective detectors is hydrocarbon interference, especially from chromatographic coelution. The use of MS and selected ion monitoring (SIM) can largely overcome the coelution problem for many applications (Firor, 2001).

3.3.1.1 Standard preparation and calibration curves

Gas standards were generated using certified Teflon membrane permeation devices (Kin-Tec Laboratories Inc). Table 3.1 presents the compounds for which permeation tubes were available. All the values provided with the calibration certificates were assumed exact and used for a period of six months.

Table 3.1 – Characteristics of permeation tubes for target compounds

Name	MW (g/mol)	Permeation rate (F) (ng/min)	Oven temperature (°C)	Ko
Methyl mercaptan	48.1	1.019	30	0.466
		431	40	
		436		
Dimethyl sulfide	62.1	116	30	0.361
Dimethyl disulfide	94.2	155	40	0.238
Trimethylamine	59.1	324	30	0.379

K_o is a factor that relates the molar mass of the compound to the volume occupied by an ideal gas at standard temperature and pressure and is obtained by dividing 22.4 by the weight of 1 mole of the substance in grams.

The calculation of the standard concentrations was made applying the formula:

$$C = [K_o \times F]/[Q]$$

Where: C - standard concentration (ppm);

: K_o – constant (L/g);

: F – permeation rate (ng/min);

: Q - the flow rate is measured for standard conditions: 0°C and 1 atm (ml/min).

The permeation devices were placed individually and in combinations in a thermostated glass chamber (Kin-Tec Laboratories Inc). A base flow of high purity

(99.99%) nitrogen gas was maintained through the permeation chamber at 50 ml/min, and the concentration of the target compounds was varied using additional dilution gas. The temperature inside the chamber was measured with a temperature probe. The flow was measured at room conditions with an SKC-UltraFlo device and corrected afterwards for standard conditions of 0°C and 1 atm. For humidification of the standards a Perma Pure Inc. Nafion membrane humidifier was used (Figure 3.1)

Figure 3.1 – Humidifier used for wet standard gas preparation

Permeation distillation is the operating principle of the membrane humidifier. The tube-in-shell design allows vapor phase moisture to be added to the gas stream, thereby eliminating the potential for droplets to form in the humidified gas standard. The driving force behind this process is the difference in water vapor pressure across the tubing. Water molecules absorbed onto the surface of the tubing are quickly released into the gas stream.

To increase the humidifier's performance, or water content, either the temperature was increased or the flow rate was decreased. In these experiments a constant flow rate of 50 ml/min was maintained. The temperature was maintained constant at room temperature (approximately 24°C). De-ionized water was used for all experiments to prevent bacterial growth and contamination of the gas stream.

Figures 3.2 and 3.3 present the configurations of the gas handling and sampling apparatus that were employed for generating dry and humid standards, respectively.

Figure 3.2 - Apparatus for the sampling of gaseous dry standards

Figure 3.3 - Apparatus for the sampling of gaseous wet standards

The SPME fibers were exposed to the gas standard in a sampling apparatus located in a temperature-controlled room (23-24°C). The SPME fiber was exposed for five minutes to a continuous flow of the standard that passed through a 40 ml glass vial. The vial had a Teflon-coated septum through which the needle of the SPME device was inserted. For each change of flow and each change in concentration, approximately 20

minutes was allowed to pass before sampling, to ensure a constant concentration in the vial. When the wet standards were used, the dry standards and wet nitrogen were allowed to reach equilibrium for approximately 1 hour. After this period of time the standard gas became saturated with water vapor.

Calibration curves were developed for each compound separately in dry and wet nitrogen flow and for different combinations of target compounds. Calibration curves for the mixture of standards were developed in two stages. Initially, calibration curves were developed for combinations of dry standards of volatile sulfur compounds including MM and DMS, MM and DMDS, DMS and DMDS and a mixture of DMS, DMDS and MM and a mixture of MM and TMA. Finally, calibration curves were developed for mixtures of all four compounds in dry and wet gases.

Gas standards were generated at concentrations starting from the lowest values to the highest values. The ranges of concentrations used in this study are presented in Table 3.2 for each compound individually and in mixtures. The concentration ranges for the gas standards comprised of compounds in a mixture was lower than the concentration range for individual gas standards. For the mixtures two thermostated glass chambers were used (temperature of 30⁰C and 40⁰C) in parallel and through each chamber a minimum flow of 50 ml/min was maintained, thus the minimum flow through the bench was 100 ml/min. In Appendix 1 is presented a summary of concentrations of target compounds in mixtures.

Table 3.2 – Concentrations ranges used for calibration curves

	MM		DMS		DMDS		TMA	
	min	Max	min	Max	min	Max	min	Max
	ppm		ppm		ppm		ppm	
Individual dry standards	0.9	17	0.18	1.24	0.11	0.91	0.42	3.69
Wet and dry mixtures	2.59	10.27	0.12	0.48	0.13	0.38	0.36	1.43

Where:

Min is the minimum value of the gas standard concentration used for the development of the calibration curves.

Max is the maximum value of the gas standard concentration used for the development of the calibration curves.

3.3.1.2 Equilibrium time experiments

SPME is based on partition equilibrium of the analytes between gases sample and the polymer coating of a fused-silica fiber. In normal operation, the fiber is exposed to the sample media until equilibrium is reached, but the time needed to reach the adsorption equilibrium between the sample and the polymer coating can be very long. In these situations, shortening the adsorption time and working in non-equilibrium conditions are desirable. For high accuracy and precision from SPME, consistency in sampling time is more important than full equilibrium. The mass of chemical compounds adsorbed by the SPME fiber coating is related to the concentration in the sample and the sampling interval (Supelco, Bulletin 923, 1998).

The biosolids employed in this study were obtained from the City of Ottawa, Robert O. Pickard Environmental Center (ROPEC), where wastewater treatment sludge is treated in a process that includes mesophilic anaerobic digestion of primary sludge and waste activated sludge and high solids centrifuge dewatering. ROPEC has a high solids centrifuge dewatering system that uses cationic polymers to enhance the dewaterability of the biosolids. Samples were collected from a hopper that collected cake immediately after the centrifuge. The biosolids were put into 500 ml Nalgene bottles for transporting to the laboratory. Two bottles were collected each time and the biosolids were immediately transported to the laboratory. Approximately 10 g of biosolids were transferred into 40 ml

amber glass serum vials. The biosolids occupied approximately one half of the vial volume. The vials were then sealed with a Teflon septum and plastic cap.

The gas that evolved in the headspace was a complicated matrix with a number of compounds that were present at different concentrations. To determine the time of exposure for a mixture of amines it was found that the best time was 1 minute (Lewis et al. 2002). For a mixture of volatile sulfur compounds (CS₂, DMS, DMDS) and TMA it was found that equilibrium was reached after three hours for CS₂, DMS and TMA. For DMDS the equilibrium was not reached even after ten hours (Kim et al., 2002).

In order to determine the equilibrium time in this study a Carboxen-PDMS fiber was exposed to the headspace of the biosolids sample for 5, 10, 15, 20 and 25 minutes. The fiber was subsequently desorbed into the gas chromatograph for analysis.

3.3.1.3 Generation of artifacts

The impact of the SPME needle coating on the formation of artifacts was evaluated by testing a standard containing a range of concentrations of methyl mercaptan in dry and wet nitrogen. The SPME fiber was exposed for five minutes to a continuous flow of the standard that passed through a 40 ml vial. In each case, the fiber was desorbed into the gas chromatograph for analysis.

The impact of needle coating on production of artifacts was evaluated by testing three sets of fiber assemblies obtained directly from SUPELCO. All three fibers were coated with Carboxen-PDMS that was from the same batch. The differences among the assemblies were in the way in which the needle was treated:

- Assembly 1: Carboxen-PDMS, standard – untreated needle
- Assembly 2: Carboxen-PDMS, Silcosteel^R – treated needle
- Assembly 3: Carboxen-PDMS, SulfinertTM – treated needle

The Silcosteel and Sulfinert processes are Restek patented methods for deactivating stainless steel surfaces. The Silcosteel process consists of vapor phase deposition of a micro-sized film of fused silica onto the surface. The fused silica layer passivates the metal surface against corrosion and has the chromatography inertness of fused silica. The Sulfinert treatment is based on the Silcosteel deactivation process. It is reported to be most suitable for low ppb storage and analysis of sulfur and volatile sulfur compounds.

3.3.1.4 Competitive adsorption

Different combinations of standards were used to study competition for sorption sites on the fiber. Initially, combinations of dry standards of volatile sulfur compounds including MM and DMS, MM and DMDS, DMS and DMDS and a mixture of DMS, DMDS and MM were examined. Upon completion of these tests a mixture of methyl mercaptan and trimethylamine was tested. Finally, the dry and wet mixtures of all standards available were tested.

To evaluate the competition onto the adsorption sites the SPME fiber was exposed for five minutes to a continuous flow of the standards that passed through a 40 ml vial and desorbed into a gas chromatograph for further analysis.

3.3.2 Characterization of biosolids odours

3.3.2.1 Sampling points and procedures

For the purpose of understanding the influence of different operational processes on the biosolids odours, samples were collected from three different sources. The first source was dewatered cake from ROPEC. Seven runs were conducted for this experiment, as can be seen in Table 3.3. The data presented in the last three columns of the Table 3.3 are average values based on data available within 7 days of the sampling event. The trial dates represent randomly selected testing events that were several months apart.

Table 3.3 – Period of study for odour characterization.

Run number	Period of time	Max. temperature (°C)	Max. relative humidity (%)	Total precipitation (mm)
Run 1	(08-12)/07/2002	35	83	0
Run 2	(24-27)/07/2002	31	86	1.48
Run 3	(19-26)/08/2002	32	88	0.54
Run 4	(02-13)/12/2002	-1.8	85	1.30
Run 5	(09-13)/12/2002	-4.8	83	0.41
Run 6	(16-20)/05/2003	16	97	2.9
Run 7	(23-27)/06/2003	25	90	0.26

Samples employed in these experiments were collected from the hopper immediately after the centrifuge at ROPEC. The biosolids were transported to the laboratory in 500 ml Nalgene bottles. Once in the laboratory, approximately 10 g of biosolids were transferred into 40 ml amber glass serum vials. The vials were sealed with Teflon septa and capped.

The second source was dewatered cake from the Kingston Ravensview wastewater treatment plant. The Kingston wastewater treatment plant is a chemically enhanced (FeCl_3) wastewater treatment plant. The sludge from the primary clarifier is

stabilized through mesophilic digestion. The stabilized sludge is then centrifuged with a high-speed centrifuge (Humboldt rotary centrifuge). The sample was collected directly downstream from the centrifuge on the 16/01/2003. Immediately after the sample was collected it was transported to the lab.

The third sets of samples were collected from five laboratory scale anaerobic digesters. For this experiment two runs were conducted with run 1 performed in the period 24-30/May/2003 and run 2 in the period 05-11/July/2003. The digesters were operated inside of the environmental laboratory at Carleton University, and the difference among them was the temperature at which they were run. The reactors were operated at 35⁰C (D1), 42⁰C (D2), 49⁰C (D3), 55⁰C (D4) and 62⁰C (D5). All of the reactors were fed with a mixture of primary sludge and waste activated sludge that was obtained from ROPEC. The feeding solution was the same as that used for the mesophilic digesters from ROPEC. The samples for odour measurements were taken from the digester effluents.

Each day when experiment was performed one liter of digester effluent was taken out from each reactor, and this amount dictated the experiments run in this test. The sample was divided into two equal parts. The first 500 ml sample was analyzed for odours after the sample was centrifuged. The second 500 ml sample was mixed with a cationic polymer (FLO POLYMER CA 4600); the same polymer used at ROPEC; and was subsequently analyzed for odours after the sample was centrifuged.

The dosage of polymer used was 10 kg polymer / dry ton of solids that entered centrifugation. The digester effluent from all the reactors had a total solids content around $\pm 3\%$, and assuming the specific gravity of the digested biosolids was 1.2, approximately

180 mg cationic polymer were added to the 500 ml sample. A laboratory centrifuge (Centra GP 8) was used for the dewatering process. The samples were centrifuged for thirty minutes at 5000 rpm. The resulting pellet was considered the dewatered cake, and was subsequently analyzed for odours.

3.3.2.2 Sample preparation

Approximately 10 g of dewatered biosolids was transferred into a 40 ml amber glass serum vial. The vial was then sealed with a Teflon septum and plastic cap. The gas that evolved in the biosolids headspace was sampled by concentrating the target analytes onto a SPME fiber followed by desorption into a gas chromatograph. Duplicate or triplicate bottles were prepared for each run for the ROPEC dewatered cake. For the sample collected from the Kingston wastewater treatment plant four vials were analyzed. Ten vials were prepared for the bench scale digesters, five with and five without polymer. Because of the large number of samples obtained from the bench scale digesters duplicate vials could not be prepared. Also, because of the large number of samples the dewatering of the biosolids and the odour analyses could not be performed on the same day. On the first day, the biosolids were dewatered and the vials were prepared for odour analyses. The ten 40 ml vials were kept over night inside of a refrigerator, to decrease odour production to a minimum value. On the next day the vials were transported to the laboratory, allowed to reach room temperature and the headspace analyses were performed.

3.3.2.3 Headspace analysis

The samples were stored for five days at room temperature. The temperature of the room was controlled at 23-24⁰C. The humidity in the room varied from 20 to 80 %, but it was assumed that this parameter did not influence odour generation, because the vials were sealed. For the five-day period the headspace was sampled daily with SPME and desorbed into the GC. On the first day of storage, the samples were allowed to reach equilibrium between the biosolids and the headspace for approximately one hour, before sampling. The SPME exposure was maintained constant at 5 minutes, to ensure repeatability among the injections. The headspace in the sample represented approximately half of the vial volume. The SPME was exposed above the “cake” at a distance of approximately one half the total headspace length, as is presented in Figure 3.4. The samples were stored for five days and headspace analyses were made for each day of storage. For the Kingston experiment the measurements were made on the first, second and fifth day of storage.

Figure 3.4 – Schematic diagram experimental set-up for odour characterization

3.3.2.4 Evaluation of the redox condition in the stored biosolids

This experiment was repeated three times, over the following periods: run 1: 25/11-09/12; run 2: (02-13)/12 and run 3: (09-16)/12 2002. Samples employed in these experiments were collected from the hopper immediately after the centrifuge and the vials were prepared as for the equilibrium time measurements. The gas that evolved in the headspace was sampled with a 1 ml plastic syringe and a volume of 0.5 ml was manually injected into a gas chromatograph with a thermal conductivity detector. The gas phase was analyzed for methane and carbon dioxide concentration. The samples were stored for twelve days and headspace analyses were made for each day of storage. On each day the injections were made in triplicate.

3.3.2.5 Evaluation of the impact of oxygen availability on the formation/degradation of odorous compounds

In this experiment, the effect of the availability of oxygen on the formation/degradation of odorous compounds was studied. In these tests, the headspace of the dewatered biosolids was flushed with either pure oxygen or with nitrogen to create enhanced aerobic and anaerobic environments respectively.

The dewatered biosolids obtained from ROPEC were transferred into six 40 ml vials, with approximately 10 g of biosolids in each vial. Duplicates were made for control samples, aerobic and anaerobic environments. The control samples consisted of dewatered biosolids with air as a headspace sealed in the vials. Oxygen availability was increased by flowing pure oxygen through the vials, at a constant rate for 10 minutes, and after that the vials were sealed. The flow rate that was chosen was the maximum flow at which the biosolids were not removed from the vials. Oxygen availability was reduced by

flowing pure nitrogen through the vials, at a constant rate for 15 minutes, and after this the vials were sealed. The Teflon seal was then pierced with a needle and more nitrogen was introduced in the vial, for approximately 2 minutes. The flow was chosen on the same consideration as for the enhanced aerobic environments. The samples were stored for 5 days and headspace analyses were made for each day of storage, following the methodology described in Section 3.3.2.3.

3.3.3 Protein content of the biosolids

3.3.3.1 Protein calibration curves

Protein measurement requires standards in order to quantify the results. Bovine serum albumin was used as the standard for protein analyses. The serum albumin was delivered in crystalline state and distilled water was used to dilute the standard to an initial concentration of 0.40 mg/mL. After dilution the bovine albumin serum was kept inside of refrigerator, to avoid degradation of the proteins. In order to ensure accurate results, distilled water was used to dilute all standards and samples. In addition, standards and samples were prepared to the same final volume. Eleven dilutions of bovine serum albumin, with concentrations varying in the range from 0 to 150 µg/mL were prepared. Triplicates were made for each dilution.

The procedure used for the protein assay followed the Hartree-Lowry method (Appendix 2). Sodium potassium tartrate, sodium carbonate, sodium hydroxide, copper sulfate and Folin Ciocalteu were procured from SIGMA Company. The method used for quantity measurements was absorbance of light, and a Milton Roy Spectronic 20D was

used. To ensure repeatable results with the spectrophotometer it was necessary to fill the culture tubes up to a minimum 7.5 ml, and because of that the quantities indicated in the Hartree-Lowry method were multiplied by a factor of 1.5.

3.3.3.2 Sampling points and procedure

Samples were collected from three ROPEC locations: dewatered cake, digester effluent and centrate from the dewatering process. The digester effluent was collected before cationic polymer was added to the dewatering influent.

The protein experiments also examined samples from the bench scale reactors. The sampling points and procedures were the same as those presented in section 3.3.2.1. The samples collected from the laboratory bench scale reactors for protein assay and for odour measurements were taken on sequential days. The samples that were collected on Saturday were analyzed for protein, and additional sample was collected on Sunday and prepared for odour analysis.

3.3.3.3 Sample preparation for protein assay

Three trials were conducted in the ROPEC experiment with run 1 over the period (25-27)/02/2003, run 2 over the period (03-07)/03/2003 and run 3 over the period (21-25)/05/2003. The first run was conducted only for three days, because the quantity of dewatered cake necessary for this experiment was incorrectly calculated.

Ten 250 mL centrifuge bottles were filled with 100 g of dewatered cake and sealed with the lid of the centrifuge bottles. The biosolids occupied approximately half of the bottle volume, and it was assumed that the inside of the bottles had the same

conditions as the inside of the 40 ml vials used for odour measurements. The bottles were stored over a five-day period of time, and each day two bottles were used for the protein assay. For the protein assay the bottles were centrifuged at 10,000 rpm for 15 minutes. The centrate resulting from centrifugation of the bottles was used for measurement of the soluble protein fraction. The soluble fraction was further diluted by a factor of 1:20 with distilled water. The pellet resulting from one of the samples was used for the labile fraction extraction and the pellet resulting from the other sample was used for the total protein extraction. The labile fraction was further diluted by a factor of 1:20 and the total fraction by a factor of 1:100 with distilled water.

For runs 1 and 2, samples were also taken from the digester effluent before cationic polymer was added, and from the centrate resulting from the dewatering process. The protein assay was performed only for fresh samples. Two bottles were filled with digested sludge and two bottles with centrate. A mass of 100 g of either digested sludge or centrate was weighed on a scale and then transferred to a centrifuge bottle. The centrate resulting from centrifugation of these bottles was used for extraction of the soluble protein fraction. The soluble fraction was further diluted by a factor of 1:20 with distilled water. Only the soluble fraction of the centrate was measured because the mass of pellet that resulted after centrifugation was negligible.

Protein measurements were also performed on the cake that originated from the bench scale reactors. The protein assay was performed only for fresh samples. Ten centrifuge bottles were filled with the dewatered cake, two bottles corresponding to each digester. Because the mass of dewatered cake varied from sample to sample, different quantities of dewatered cake were present in the centrifuge bottles. These values are

presented in Table 3.4. The centrate was considered the soluble fraction, and was further diluted by a factor of 1:20 with distilled water. The pellet that resulted after centrifugation was divided in two, one part was used for the measuring labile fraction of proteins and the other part was used for determining the total fraction. The labile fraction was further diluted by a factor of 1:20 and the total fraction by a factor of 1:100 with distilled water.

Table 3.4 – Quantities of dewatered cake used for protein assay

	Without polymer					With polymer				
Amount (gram)	35 ⁰ C	42 ⁰ C	49 ⁰ C	55 ⁰ C	62 ⁰ C	35 ⁰ C	42 ⁰ C	49 ⁰ C	55 ⁰ C	62 ⁰ C
	91	91	91	62	62	130	130	91	91	91

3.3.3.4 Protein extraction time

To determine the best time for extraction of total proteins, extraction times of 1, 2, 3, 4 and 4.5 hours were tested for fresh dewatered biosolids. For this experiment dewatered biosolids from ROPEC were used. Five bottles were filled with dewatered biosolids, with 100 g in each bottle. Because of the results obtained with fresh biosolids only the extraction times of 1, 2 and 3 hours were evaluated over the period of storage. 100 g of dewatered cake was put in each of sixteen centrifugation bottles, four bottles for each day of the remaining storage period (for four days). The extraction time was considered the time spent for mixing of the resulting pellet with a strong alkali solution.

3.4 Analytical methods

3.4.1 Standard tests

The measurement of TS and VS were made in concordance with Standard Methods 2540 G (Total, fixed and volatile solid and semisolid samples) (Greenberg et. al., 1992).

3.4.2 Headspace analysis

3.4.2.1 Volatile sulfur compounds and trimethylamine

At the beginning of each day the inlet of the GC was heated to 250⁰C and the fiber was desorbed into the GC. The fiber was exposed for 5 minutes in the injector to desorb any organic material that could have been collected from the air during overnight storage. For each exposure of the fiber in the headspace considerable attention was paid to the way the exposure was performed. For repeatability of the results, all the experimental conditions were maintained constant, including the distance between needle and biosolids, time of exposure, quantity of biosolids and the temperature in the room. The fibers were very sensitive and the analyst was careful not to put the fiber in contact with the biosolids or with the vial's walls.

An Agilent GC (6890N) that was equipped with an HP 1-50mx0.32 mm ID - 0.52 µm film thickness column and a mass spectrometer as detector (5973N) were employed in this study. The GC and MSD conditions were as follows:

GC parameters (6890N)

Front inlet

Inlet mode: splitless, gas: He

Temperature: 250⁰C

Pressure: 2.04 psi

Total flow: 53.6 ml/min

Column

Mode: constant flow

Detector: MSD (5973N)

He flow: pressure 2.0psi, flow 1.0 ml/min, average velocity: 28cm/sec

Oven

35⁰C hold 15 min

8⁰C/min to 70⁰C hold 15 min

15⁰C/min to 130⁰C hold 2 min

MSD parameters (5973N)

Mass range: 30 to 500

Scans: 0.82 / sec.

Samples: 4

Start time: 0

Threshold: 500

The SIM ions used for each sulfur compound, trimethylamine and other odorous compounds are listed in Table 3.5.

Table 3.5 – Optimized SIM for odor compounds from biosolids

Group	Start time (min)	Target and qualifier ions	Compound
1	4.20	47,45,48	Methyl mercaptan
2	5.0	58,59,42	Trimethylamine
3	5.20	44,58,59	Acetone
4	5.95	62,47,45	Dimethyl sulfide
5	6.31	76,44,78	Carbon disulfide
6	7.00	44,76,61	2-Propanethiol
7	8.41	43,72,57	2-Butanone
8	9.22	43,76,59	1-Propanethiol
9	9.61	61,76,47	Methyl, ethyl sulfide
10	13.52	57,41,90	2-Buthanethiol
11	14.61	41,90,56	1-Propanethiol, 2-methyl
12	19.59	94,79,45	Dimethyl disulfide
13	20.35	79,52,51	Pyridine
14	26.8	108,80,127	Methyl ethyl disulfide
15	33.50	80, 122, 43	Disulfide, methyl propyl
16	38.65	126, 111, 79	Trisulfide, dimethyl

3.4.2.2 Methane and carbon dioxide

To measure the quantity of methane and carbon dioxide, a gas chromatograph equipped with a thermal conductivity detector (SRI 8610C) was used. The column used was an ALLTECH, CTR 1 Column, 6' x 1/4" outer x 1/8" inner diameter. The headspace samples were taken with a plastic, single use syringe. A volume of 0.5 ml of gas from the headspace of the vial was injected into the gas chromatograph. The column temperature was set to 80°C and maintained for seven minutes.

3.4.3 Proteins

3.4.3.1 Soluble proteins

The sample preparation followed the methods described in Section 3.3.3. A sample of dewatered cake with a mass in the range (100 – 200) g was centrifuged at 10,000x g for 15 minutes using a laboratory centrifuge. The supernatant was removed and filtered through a 1.5 µm filter. This fraction was considered to contain the soluble proteins.

3.4.3.2 Labile proteins

Half of the pellet from the laboratory centrifuge was resuspended in 1mM NaOH (pH = 11). The volume of NaOH used (expressed in mL) was equal in absolute value with the weight of the pellet (expressed in grams). The sample was resuspended by manual mixing. The sample was then mixed for 5 minutes in an Orbital Shaker (VWR Scientific Products) at 300 rpm. Five minutes was chosen as the extraction time for labile proteins, because the proteins that are soluble in a dilute salt solution are sensitive to environmental conditions such as excessive stirring. The suspension was then centrifuged at 10,000x g for 15 minutes at 4⁰C. The centrate was removed and allowed to settle in the fridge for 3 hours. This fraction was considered the labile fraction.

3.4.3.3 Total proteins

The total protein fraction represents the labile and bound proteins. The other half of the pellet from the laboratory centrifuged was resuspended in 1N NaOH. The volume of NaOH used (expressed in mL) was equal in absolute value to the weight of the pellet

(expressed in grams). The sample was resuspended by manual mixing. The sample was stirred for two hours in an Orbital Shaker (VWR Scientific Products) at 300 rpm. The suspension was then centrifuged at 10,000x g for 15 minutes at 4⁰C. The centrate was removed and allowed to settle in the fridge for 3 hours. This fraction was considered the total fraction.

The protein concentrations for all three fractions that were extracted were measured using the Hartree (1972) modification of the Lowry et al. (1951) method (Appendix 2). Bovine serum albumin was used as protein standard. It was procured from Sigma-Aldrich Company. Proteins were measured in the liquid-phase of the sample.

3.5 Quality of the data

3.5.1 Precision

Precision measures the reproducibility of repetitive measurements. Analytical precision is a measurement of the variability associated with duplicate or replicate analyses of the same sample in the laboratory. Duplicate samples were analyzed to assess the laboratory precision and the results were reported as the relative percent difference between duplicate measurements. The coefficient of variance was calculated for duplicates.

3.5.2 Accuracy

Accuracy is a statistical measurement of correctness. It reflects the total error associated with a measurement. A measurement is accurate when the measured reported does not differ from the reported value of the standard. For each day of analysis the standards were checked. If the value was produced within 15% of the target it was considered precise.

Chapter 4 - Evaluation of solid-phase microextraction for sampling of biosolids odours

4.1 Equilibrium time measurements

The mass of chemical compounds adsorbed by the SPME fiber coating is related to the concentration in the sample and the sampling interval (Supelco, Bulletin 923, 1998). For high accuracy and precision from SPME, consistency in sampling time is more important than full equilibrium. The experimental set-up for equilibrium time measurements was presented in Section 3.3.1.2.

In order to determine the equilibrium time that would be employed in this study a Carboxen-PDMS fiber was exposed to the headspace of a biosolids sample for 5, 10, 15, 20 and 25 minutes. Figure 4.1 presents a chromatogram for a biosolids sample after 5 minutes exposure time in the headspace. DMDS produced the widest peak and this was explained through the fact that the column peaked at almost the same time as DMDS. The area response of DMDS was the result of both column and chemical compound. In Appendix 3 the library search report for the below chromatogram is presented.

Figure 4.1 - Typical gas chromatograph result

Figure 4.2 presents the area counts for a variety of compounds as a function of sampling time. The difference between the responses at 25 minutes and 5 minutes varied from 8.42% for methyl mercaptan to 231% for trimethylamine.

Figure 4.2 - The response of headspace gas at different exposure time

The pattern for most of the compounds was that the responses after 10 minutes were less than after 5 minutes and after 10 minutes they increased. This behavior was consistently observed in duplicate samples. The above behavior was observed in the summer of 2002. In winter 2002-2003 a new experiment was undertaken to see if this behavior was repeatable. For this experiment the area responses for all compounds of interest increased as the exposure time increased, from 5 minutes to 25 minutes. To double-check this result, an additional run with an exposure time of 5 minutes was performed at the end of the experiment. The area responses for this last run for methyl

mercaptan and dimethyl disulfide were higher than for the 15 and 10-minute exposure times respectively.

Comparing the area responses for odors from summer and winter biosolids it was found that in the summer the number of compounds that appeared on chromatogram were around 65 and in winter around 25, and for compounds of interest (MM, DMS, TMA, DMDS) the concentration in the summer were almost 10 times higher than in the winter.

The observed behavior may have been due to either dynamic competitive adsorption among the wide range of compounds in biosolids headspace or time-varying concentrations due to biological activity in the biosolids during the adsorption. In this study the exact cause of the behavior was not identified. The responses for 25-minute exposure times were consistently higher for all the compounds than the responses for 5 minutes, but in the same range of magnitude. Taking into account the time required for each injection and comparing the responses, the exposure time of 5 minutes was determined to be suitable for all further experiments.

4.2 Calibration curves

The experimental set-up for standard preparation and calibration curves was presented in Section 3.3.1.1. Calibration curves were initially developed for each compound separately. In this study the repeatability amongst injections was tested, for different concentrations of target compounds. For the DMS and TMA standards five injections were made for each point of the calibration curve. The coefficients of variation associated with these replicates are shown in Table 4.1.

Table 4.1 – Repeatability among injections

Dimethyl sulfide		Trimethylamine	
Concentration (ppm)	Coefficient of variance (%)	Concentration (ppm)	Coefficient of variance (%)
0.186	2.51	1.023	1.69
0.235	2.57	1.535	2.19
0.441	2.12	2.047	5.01
0.650	2.94	2.456	6.00

It was found that the relative error among the injections was small and hence for subsequent calibration curves and other injections only duplicates were used. The resulting calibration curves were linear ($R^2 > 0.99$) for all standards, over the range: 0.155-0.73 (DMDS); 0.182-1.24 (DMS); 0.424-1.20 (TMA); 1.617-16.61 (MM) – ppm. The calibration curve for DMDS was linear, with $R^2 = 0.999$, hence it was assumed that the column peak did not influence the behaviour of DMDS response.

The same calibration curves were verified after a period of time. The variation was random, up and down around the expected. From Table 4.2 it can be seen that the method provided consistent results for a variety of compounds when analyzed separately.

Table 4.2 – Verification of the calibration curves

	DMS		MM		DMDS		TMA	
Expected values	0.321	0.855	3.69	9.66	0.287	0.834	0.362	1.43
Values from calibration curves	0.33	0.82	3.83	10.5	0.32	0.8	0.37	1.35
% Difference	2.72	4.01	3.66	8.38	11.5	4.21	2.16	5.93

4.3 Generation of artifacts

From the literature it was found that the most important limitations in SPME use were the potential generation of artifacts and competition on the porous fiber coating. In the next experiments these phenomena were examined. The impact of the SPME steel needle on the production of artifacts, the effect of relative humidity on the

extraction efficiency and occurrence of artifacts, the effect of different needle treatments on the production of artifacts and the competition between odorous chemical compounds and water vapors were examined.

The experimental set-up for generation of artifacts was presented in Section 3.3.1.3. In Figure 4.3 the calibration curves for wet and dry MM are presented. The response for wet MM was approximately half the response for dry MM. The calibration curves revealed a reduction in responses for wet gases that could be either a result of oxidation of methyl mercaptan, of competitive adsorption between methyl mercaptan and water vapour or due to a modification of the fiber by water vapour.

Figure 4.3 - Responses for dry and wet methyl mercaptan gas standards

DMDS was examined as a potential by-product of methyl mercaptan oxidation. When MM was analyzed alone some oxidation of methyl mercaptan to dimethyl disulfide was observed. It is believed that the oxidation happens in the presence of metallic surfaces and/or water vapors (Chin and Lindsay, 1994; Lomans

et al., 2002). The formation of DMDS was observed in spite of the fact that during preparation of the MM gas standard it did not directly contact any metallic surface.

The impact of the SPME needle on the formation of DMDS was evaluated by testing a standard containing a range of concentrations of MM in dry and wet nitrogen. The concentration of MM and DMDS that were measured in the dry gas are presented in Table 4.3.

Table 4.3–Production of dimethyl disulfide as an artifact of dry methyl mercaptan

Methyl mercaptan		Dimethyl disulfide
Expected concentration (ppm)	Concentration from calibration curves (ppm)	Concentration from calibration curves (ppm)
2.27	2.18	0.01
2.66	2.78	0.01
3.91	4.25	0.03
7.98	8.07	0.05
16.64	16.50	0.08

From Table 4.3 it can be seen that a relatively small fraction of the MM was converted to DMDS. Recognizing the conversion of MM to DMDS the final calibration curve for MM was calculated after a mass balance for sulfur was made (eq. 4.1).

$$C_{MM1} = C_{MM2} + 0.5C_{DMDS} \quad (4.1)$$

C_{MM1} = concentration of methyl mercaptan calculated with the data from the calibration certificate for methyl mercaptan

C_{MM2} = concentration of MM resulted after the oxidation of MM into DMDS

C_{DMDS} = concentration of DMDS measured from calibration curve for DMDS

The concentrations of MM and DMDS that were measured in the wet gas are presented in Table 4.4. The calibration curves for wet and dry methyl mercaptan previously described in Section 4.3 were corrected using the above relationship.

Table 4.4 – Production of dimethyl disulfide as an artifact of wet methyl mercaptan

Methyl mercaptan		Dimethyl disulfide
Expected concentration (ppm)	Concentration from calibration curves (ppm)	Concentration from calibration curves (ppm)
0.879	0.889	0.01
1.58	1.59	0.01
4.99	5	0.03
17.3	17.31	0.08

Comparing the values measured for DMDS for dry gas and wet gas it can be seen that the presence of water vapor did not influence the generation of DMDS. The quantities of DMDS were the same as for dry MM. However, the area responses for the wet methyl mercaptan standard gas were half the area responses for dry methyl mercaptan. Because the production of artifacts seemed to be the same for both wet and dry standards, this decrease in area response would appear to be due to competition between methyl mercaptan and water vapor for sites on the porous fiber coating.

Comparing the concentration values for DMDS and MM it was found that the average DMDS concentration was 200 times less than the MM concentrations, over the range of MM concentrations. The highest concentration of MM measured with biosolids during this study period was around 800 ppm. Extrapolating (DMDS resulting from oxidation of MM is 200 times less than MM) suggests that the DMDS concentration in the biosolids sample as a result of oxidation should have been approximately 4 ppm. As will be seen later, the concentrations of DMDS measured from the same sample were around 50 ppm. Hence the DMDS that resulted from the oxidation process represented 8% of the total DMDS. It would appear that the conversion of MM to DMDS would not have a significant impact on the data collected from the biosolids sample.

The standard gas did not come into direct contact with any metal surfaces (fittings, inlet of gas chromatograph) and hence, the only metal surface that came in direct contact with MM was the needle of the fiber. The next part of the research was conducted to study the effect of the presence of the metal from the SPME fiber assembly on the production of artifacts.

4.3.1 Effect of needle coating on the production of artifacts

SPME employs a 1 cm length of fused silica fiber, coated with a polymer, bonded to a stainless steel plunger and installed in a holder that resembles a modified microliter syringe. The holder of the fiber was examined to determine if the presence of this metal might have catalyzed the conversion of MM to DMDS. In this study three sets of fiber assemblies were tested. All three sets of fibers were coated with Carboxen-PDMS and were from the same batch. The differences among the assemblies were in the way in which the needle was treated:

- Assembly 1: carboxen-PDMS, standard – untreated needle
- Assembly 2: carboxen-PDMS, Silcosteel^R – treated needle
- Assembly 3: carboxen-PDMS, SulfinertTM – treated needle

In Figure 4.4 the area counts versus concentration of MM measured with these assemblies in the presence of dry MM standard gas are presented. When the area responses for methyl mercaptan were compared, it was found that fibers F2 and F3 were slightly more sensitive than the standard fiber, but the differences were small.

Figure 4.4 - Responses of three different treated assemblies in the presence of dry methyl mercaptan standard gas

For the lowest concentration (2.5 ppm) the area response for the MM dry gas standard was slightly higher for the Silcosteel treated needle than the other two fibers. For 4 ppm all the responses were the same, and for higher concentrations the responses for the Sulfinert treated fiber were slightly higher the rest. To analyze the data, a “t-test: two-sample assuming equal variance”, a license Microsoft Excel, was performed. The results for t Value were found to be F1/F2 0.21, F1/F3 0.23 and F2/F3 0.01. Interpreting the data it was found that there is not a significant difference in the sample means. A difference could not be detected probably due to small sample size. Of course it is possible that the samples really are from the same population.

Figure 4.5 presents the area counts versus concentration for the fibers in the presence of wet methyl mercaptan. Comparing the responses for methyl mercaptan it was found that Silcosteel treated fiber was slightly more sensitive than the other two fibers. Analyzing the data using a t-test it was also found that the samples either could be from the same population or the sample size was too small.

Figure 4.5 - Performance of three different treated fibers in the presence of wet methyl mercaptan standard gas

The area responses obtained for MM and the concentrations of DMDS are presented in Table 4.5. In Table 4.5, the expected concentrations represent the values that were calculated with the diffusion coefficients that were provided for each permeation tube. The area responses were those generated by the GC-MS. The DMDS concentrations were calculated from the calibration curve that was developed for DMDS alone. From Table 4.5, it can be seen that the DMDS concentrations measured with the Silcosteel and Sulfinert treated assemblies were in some cases higher than for the standard assembly. The same trend (an increase in area response for these two fibers) was also observed for MM. It would appear that the Silcosteel and Sulfinert treated needles enhanced the sensitivity of all the assemblies in concentrating sulfur compounds, although, it is possible that the responses for all fibers to be from the same population. The sensitivity seemed to be slightly higher for the Silcosteel treated needle.

Table 4.5—Measured concentration values for MM and DMDS with three different assemblies

	Conc. ppm.	Area response for MM			Measured DMDM concentration from calibration curve (ppm)		
		F1	F2	F3	F1	F2	F3
Dry standard gas	2.83	4.0×10^7	4.2×10^7	5.2×10^7	0.04	0.04	0.05
	4.03	5.4×10^7	5.6×10^7	6.0×10^7	0.04	0.05	0.05
	8.02	11.5×10^7	13.3×10^7	11.9×10^7	0.06	0.08	0.08
Wet standard gas	1.17	1.2×10^6	2.6×10^6	1.2×10^6	0.01	0.01	0.01
	2.21	1.1×10^6	2.5×10^6	1.4×10^6	0.01	0.01	0.02
	7.36	1.8×10^6	2.7×10^6	1.6×10^6	0.04	0.04	0.05

Methyl mercaptan standards were prepared for a range of concentrations, and using each of the three set of fibers, three area responses (one for each set of fibers) were determined to each concentration. In addition a second standard fiber assembly that was from a different fiber batch, was employed to generate a fourth set of area responses for methyl mercaptan. Comparing the area responses for the MM dry standard gas measured with the standard fiber employed in the above experiment with the area responses measured with a Carboxen-PDMS fiber that was obtained from a different batch it was found that the values measured with the former fiber were higher than the values measured with the latter fiber by approximately (21 to 28)%. The differences between the measured and predicted values for the standard fiber were found to be too high. In gas chromatography analyses, the differences among injections for the same standard concentration have to be as high as $\pm 10\%$ (Grob and Barry, 1995).

To check these values, and implicitly the calibration curve, new injections were performed. The additional tests were performed with the original assembly (F4) that was used for the development of the individual calibration curves. The expected concentrations and the concentrations from calibration curves for F1 and F4 are

shown in Table 4.6. In Table 4.6, the expected concentrations were those that were calculated from diffusion coefficients provided for each permeation tube. Area response measured either with F1 or F4 represents the area given automatically by the GC-MS.

Table 4.6 – MM area responses for two different standard fibers

Expected concentration (ppm)	F4		F1
	Initial calibration	Repeat Calibration	
2.83	2.93×10^7	2.85×10^7	3.97×10^7
4.03	4.23×10^7	4.05×10^7	5.44×10^7
8.02	8.55×10^7	8.02×10^7	11.5×10^7

For fiber F4 the differences between expected values and values from calibration curves were less than 10 %, demonstrating that the calibration curves were correctly developed and the GC-MS was functioning in the right parameters. For fiber F1 the differences were between 20 % and 30 %. Because of the differences measured it was found that when a new fiber is used, new calibration curves have to be made, for good results of the analyses.

For the wet standards there were also found to be high differences between area responses measured with standard fiber from the special lot and the area responses expected from the calibration curve based on known concentrations. These values are presented in Table 4.7.

Table 4.7 – Differences between measured and calculated concentration

Expected MM area response (from calibration curve for known concentration)	Area response for MM measured with F1	%
1916536	1228549	56
3606145	2578149	40
11755250	10205985	15

In conclusion it was found that the area responses for the Carboxen-PDMS 75, Silcosteel treated fiber resulted in higher area counts of both MM and DMDS. This appeared to be the result of an overall increase in sensitivity of the fiber for sulfur compounds. Silcosteel is the most inert material in the reaction with volatile sulfur compounds. Hence the amount of volatile sulfur compounds adsorbed onto the fiber increased. For each fiber new calibration curves have to be generated.

4.3.2 Competitive adsorption

In Section 4.3, it appeared that competition between water vapor and methyl mercaptan for the adsorption sites on the fiber caused a substantial decrease in values for wet methyl mercaptan area responses. Hence, further evaluation of competitive adsorption was performed. After the calibration curves for individual dry and wet MM, DMS, DMDS and TMA were completed, the analysis of gas mixtures was assessed. To study the competition for sorption sites on the fibers, different combinations of standards were used. Initially, combinations of dry standards of volatile sulfur compounds including MM and DMS (Table 4.8), MM and DMDS, DMS and DMDS (Table 4.9) and a mixture of DMS, DMDS and MM (Table 4.10) were examined. Upon completion of these tests, a mixture of methyl mercaptan and trimethylamine was tested. In these tables, the expected concentrations were calculated using the diffusion coefficients provided for each permeation tube. The measured concentrations represent the concentrations that were determined from the individual calibration curves.

Table 4.8 – Mixture of dimethyl sulfide with methyl mercaptan

Compound	Expected concentration (ppm)	Concentration measured (ppm)	Coefficient of variance (%)
DMS	0.357	0.34	7.4
MM	4.58	4.05	3.2
DMDS	0.00	0.03	1.6

Table 4.9 – Mixture of dimethyl disulfide with dimethyl sulfide and methyl mercaptan

Compound	Expected concentration (ppm)	Concentration measured (ppm)	Coefficient of variance (%)
Mixture of dimethyl sulfide and dimethyl disulfide			
DMS	0.365	0.29	6.9
DMDS	0.322	0.33	1.5
Mixture of methyl mercaptan and dimethyl disulfide			
MM	3.31	2.65	0.57
DMDS	0.303	0.33	1.57

Tables 4.8 and 4.9 indicate that binary competition among the volatile sulfur compounds was relatively minor. When MM was mixed with either DMS or DMDS, which are heavier molecules, its measured concentration was slightly lower than expected, on the basis of the single species calibration curve. The MM concentration was 11% less in the presence of DMS and 20 % less than expected in the presence of DMDS. When DMS competed with DMDS, the response of DMS decreased by 21%, while competition was insignificant for DMDS. From Table 4.8 can be seen that 1.5% of the MM was oxidized to DMDS.

In Table 4.10 the concentration values for mixtures that contained all the volatile sulfur compounds used in this study are presented. From Table 4.10, it can be seen that tertiary competition among volatile sulfur compounds was relatively small. In the competition for porous fiber, DMDS seemed to slightly displace the other volatile sulfur compounds (methyl mercaptan and dimethyl sulfide). The values measured for MM and DMS were somewhat lower than the expected values from the individual calibration curves. These differences increased from 0.3% to 20% for MM

and from 13% to 34% for DMS as the concentrations in the mixture increased. Production of DMDS by the oxidation of MM might explain the slightly higher values for the measured DMDS.

Table 4.10 – Mixture of dimethyl sulfide with dimethyl disulfide and methyl mercaptan

Methyl Mercaptan		Dimethyl sulfide		Dimethyl disulfide	
Conc. expected (ppm)	Conc. measured (ppm)	Conc. expected (ppm)	Conc. expected (ppm)	Conc. measured (ppm)	Conc. expected (ppm)
3.17	3.16	0.15	0.13	0.133	0.16
3.3	3.28	0.186	0.14	0.164	0.2
5.69	4.55	0.271	0.18	0.239	0.26
8.52	6.84	0.410	0.27	0.358	0.39

The final dry gas combination that was tested involved methyl mercaptan in a mixture with trimethylamine. Trimethylamine has a basic and nucleophilic nature (Baboul et. al., 1998) and the sulfur – sulfur bond of disulfides is susceptible to cleavage by nucleophiles as follows

From the previous sections it was found that some methyl mercaptan was oxidized to dimethyl disulfide. Consequently it was expected that DMDS produced by the oxidation reaction would react with TMA producing a slight decrease in MM and TMA concentrations. Because of the reaction between the nucleophilic trimethylamine and dimethyl disulfide it was expected that a new compound would be found in the gas phase. Because of the possible reaction among the sulfides it was expected that some dimethyl disulfide would be measured. The data obtained for this test are presented in Table 4.11. In Table 4.11, the expected concentrations were calculated from the diffusion coefficients provided for each permeation tube. The measured concentrations were determined from the individual calibration curves.

Table 4.11 – Mixture of methyl mercaptan and trimethylamine

MM (ppm)			TMA (ppm)			DMDS (ppm)	
Expected	Measured		Expected	Measured		Measured	
	Mean	CV (%)		Mean	CV (%)	Mean	CV (%)
3.32	2.53	5.34	0.46	0.06	9.09	0.02	11.2
4.95	3.64	2.2	0.69	0.16	3.2	0.02	0
8.48	6.42	4.4	1.18	0.42	3.6	0.02	0

Where: CV is the coefficient of variance for the concentration measured from calibration curves (%)

From Table 4.11, it can be seen that the presence of MM had a substantial impact on the measured concentration of TMA. In addition, the measured MM was approximately 30% less than the expected values over the range of concentrations examined. The decrease of TMA was very high with values that were 64% to 87% less than the expected values. Another interesting observation was that DMDS was generated at a constant and low concentration (lower than the value measured for MM without TMA) for the entire range of the MM concentrations. A small reduction of trimethylamine could be explained through the reaction mentioned above. The product of the reaction could not be measured by the analytical method used. However, the observed reductions of both trimethylamine and methyl mercaptan were larger than would be predicted by the above-mentioned reaction. It seems reasonable to assume that TMA reacted with MM. The constant value for dimethyl disulfide, the identification of the possible reaction products and the mechanism of reduction would require further investigations.

Based on the above observations, mixtures of all four compounds in dry and wet gases were prepared. Not all compounds were present at equal concentrations in the mixture. Based on the literature review MM was found to have the higher concentrations in the biosolids headspace followed by DMS and DMDS. The ratio between MM and DMS/DMDS concentrations was chosen to be around 1/20. TMA is

not usually reported as being present in the headspace of mesophilic digested cake and its concentration was randomly chosen. The minimum and maximum values of the concentration of target compounds were dictated by the performances of the permeation tubes presented in Table 3.1. The concentration ranges for each compound that participated in the mixture were presented in Table 3.2.

Figures 4.6-4.9 present the calibration curves for the individual compounds alone and in the dry and wet mixtures. Figure 4.7 includes an additional calibration curve for wet MM alone.

From Figure 4.6, it can be seen that the response for DMS in the wet mixture was approximately half of the response in the dry mixture and this was half of the response of the individual standard gas. When DMS was tested either in a mixture of MM and DMDS or a mixture of MM, DMS and DMDS substantial competition was not observed. The reduced concentration of DMS in the four-compound-mixture would therefore appear to be due to the presence of TMA. The addition of water vapor to the mixture appeared to provide additional competition for DMS on the fiber.

In Figure 4.7 it can be seen that the response of MM in the dry mixture was slightly lower than for the individual standard alone. When water vapors were added to the standard gas, either for the mixture or individual compound, the responses were reduced by a factor of 2. The differences between the area responses for MM in the wet mixture and the wet MM alone were negligible. The reduced values for MM seemed to be primarily due to competition with water vapor. The competition with the other volatile compounds did not influence MM values.

Figure 4.6 - Calibration curves for dimethyl sulfide gas standard

Figure 4.7 - Calibration curves for methyl mercaptan gas standard

The results obtained for MM in the four-compound-dry mixture were somewhat inconsistent with those that were observed in the two-way mixture of MM with TMA. In the binary mixture the values of MM were reduced by approximately 30 % in the presence of TMA over the entire range of concentrations, while in the four-compound-mixture MM was reduced from 1% to 20% over the concentration range. The results would suggest that TMA might have preferentially reacted with one or more of DMS and DMDS in the four-compound-mixture. This conclusion seems to be in agreement with the results from the DMS response presented above.

From Figure 4.8 it can be seen that DMDS was the only compound that was not affected by competition. Furthermore DMDS in the dry mixture had values that were higher than that of individual DMDS. This behavior likely resulted from oxidation of MM to DMDS.

From Figure 4.9, it can be observed that the responses for TMA in wet and dry mixtures were comparable. These results indicate that water vapor is not a significant competitor for adsorption sites with TMA. There were however large differences between the responses for the mixtures and the trimethylamine alone. The reduced TMA response in the presence of the other compounds was consistent with that observation in the binary mixture of TMA and MM. This response may have been due to either competition with the other compounds or as a result of chemical reactions with the other compounds.

Figure 4.8 - Calibration curves for dimethyl disulfide gas standard

Figure 4.9 - Calibration curves for trimethylamine gas standard

From Figures 4.6 to 4.9 it can be seen that SPME provides linear responses over a wide concentration of analytes. The results from this study suggest that for systems containing mixtures of reduced sulfur and nitrogenous compounds it will be difficult to obtain an exact quantitative measurement of these compounds with SPME. The presence of water vapor appeared to have the greatest impact on the analytical results, while competitive adsorption and chemical reactions also had an impact. For the purposes of characterizing the pattern of odor generation from dewatered biosolids in a confined headspace it is reasonable to assume that the headspace will be saturated with water vapor. Hence, use of calibration standards in water-saturated gas should provide reasonably qualitative estimations of contaminant concentrations in biosolids headspaces.

4.4 Conclusions

The most important challenge with SPME is competition between analytes and water vapor on the porous fiber coating and the occurrence of artifacts. The production of artifacts (DMDS) was found to be insignificant, and seemed to not be influenced by water vapor. It was found that the responses of the four analytes were significantly affected by competition with each other and with water vapor. In the latter case, their responses in wet gases decreased by more than a half of the dry gas values. In a real matrix with more compounds than the ones used in this experiment and with heavier compounds at higher concentrations, the impact of competition may increase. Because of the significant disappearance of trimethylamine (either from dry or wet mixture) it was assumed that other compounds were formed as a result of the reaction between volatile sulfur compounds and TMA.

There were differences between fibers and hence it is necessary to generate a calibration curve for each new fiber. For this reason their use is somewhat time consuming. Treatment of the needle in the SPME assembly with inactivating coatings caused slight differences. The small differences could be either due to small sample size and large variability or the samples are from the same population and the responses for all fibers are identical. However, it appeared that the Silcosteel and Sulfinert treated needles enhanced the sensitivity of the assemblies in concentrating sulfur compounds. The sensitivity seemed to be slightly higher for the Silcosteel treated needle.

Because of these reasons this SPME application is only qualitative. SPME can however be used with success for quick screenings for odor compounds.

Chapter 5 - Characterization of biosolids odours

5.1 Experimental

The experimental set-up and sample preparation followed the methodology presented in section 3.3.2. The analytical method for headspace analysis was presented in Section 3.4.2.

Because the mentioned method was developed from gas standards, it was deemed necessary to evaluate its precision for biosolids headspace gas. For the first set of data four replicates were made and the coefficient of variance was calculated. In Table 5.1 the values measured for the most odorous compounds in the second day of storage of the period (24-28)/07/2002 are presented. The coefficients of variance for the measured values were in the range (4.5-13)%, showing the reproducibility of repetitive measurements, and for the subsequent sets of data only duplicates or triplicates were made.

Table 5.1 – Coefficient of variance for four samples taken in period (24-27)/07/2002

	DMS area	MM area	DMDS area	Pyridine area	TMA area
Coefficient of variance	4.50	4.97	12.87	11.64	11.58

5.2 Characterization of odorant production

Seven runs were conducted in this study, as indicated in Section 3.3.2.1. These trial dates represent randomly selected testing events that were several months apart. The dewatering process at ROPEC normally produces biosolids at about 30 % solids. For the period of time under study the dewatered cake characteristics were within the normal

range with the total solids varying from 29% to 31% and the volatile fraction varying from 55.5 to 56.5 %.

In the biosolids headspace were measured more chemical compounds than those for which calibration curves were developed. Thus, Figures 5.1 and 5.2 present the area counts from the GC-MS for the detected compounds as a function of period of storage. Figure 5.1 was typical of that observed in the first three runs and Figure 5.2 for the next four runs. The area responses measured for the first three runs had the highest values. Also, for these runs a large number of sulfur compounds were produced during the storage period.

Figure 5.1 - Odorous compound profiles from dewatered ROPEC cake - Run 1

Figure 5.2- Odorous compound profiles from dewatered ROPEC cake- Run 6

The area response is a characteristic of the detector used in combination with the gas chromatograph. The high values for area responses did not necessarily imply high concentrations. A comparison between area responses among different compounds could not be made because the detector sensitivity is different from compound to compound. For example an area response for methyl mercaptan of 12.5×10^8 corresponded to a concentration of 208 ppm and an area response for dimethyl disulfide of 13.8×10^8

corresponded to a concentration of 10.8 ppm. It can be seen that for almost the same area responses values the methyl mercaptan concentration was 19 times higher than dimethyl disulfide. However, despite this limitation, the area responses allowed the characterization of how a compound varied during the period of storage and between sampling events.

The total and volatile solids characteristics of the dewatered biosolids used for these experiments had similar values for all seven runs, but very high variations were recorded in the area responses of the odorous compounds. The odour generation seemed to have the same pattern for all the runs: on the first day DMS and CS₂ were present, on the second day DMDS (for very high area responses, as for runs 1, 2 and 3, DMDS was measured from the first day of storage), MM and TMDS were detected. The trend in DMS was to increase over the period of storage. CS₂ was measured on the first day and after that it could not be found in the biosolids headspace. DMDS was observed to peak on the third or fifth day of storage. MM peaked on the fourth day of storage and DMTS on the second or third day of storage. During the runs performed in the warm weather months (runs 1, 2, 3) trimethylamine and pyridine could be measured starting on the second day of storage. These observations suggest that the wastewater temperature influenced the appearance of trimethylamine. As the temperature increased the trimethylamine production increased. For runs 1 to 5 other sulfur compounds were recorded, which appeared after day 4 of storage. The number of these compounds decreased as the area responses for the main compounds decreased.

The most odorous biosolids were recorded in the summer months and the least malodor was recorded in the spring and winter period. The digester temperature was

constant during the summer and winter (approximately 35⁰C) and the temperature of the biosolids stored in the vials was constant (approximately 23⁰C). Hence, it seems reasonable to assume that warm temperature affected the processes upstream of the digesters.

The average monthly values for the ratio of primary sludge to waste activated sludge that was treated in the summer (July, August) and winter (December) 2002 were obtained from ROPEC operational personal. It was found that the ratio was 1.6, 1.5 and 1.7 (kg TS primary sludge/day)/(kg TS waste activated sludge/day) for July, August and December, as can be seen in Table 5.2.

Table 5.2 – The total solids content for the digester influent (ROPEC data)

	Primary sludge (kg TS/day)	TWAS (kg TS/day)	K (kg primary TS/kg TWAS TS)
July	41577	26684	1.6
August	36342	24062	1.5
December	45586	25807	1.7

It can be seen that the ratio had almost the same values for summer and winter. The lower ratio value of 1.5 corresponded to the higher volatile sulfur concentrations from stored dewatered cake recorded at end of July-beginning of August. The higher ratio value of 1.7 corresponded to a much lower volatile sulfur concentrations from stored dewatered cake. Although the ratio difference was not significant, it appeared that for lower ratio values (and correspondingly larger waste activated sludge flow rates) corresponded to the enhanced production of volatile sulfur compounds from dewatered cake.

In part of the period when this study was performed one of the digesters was out of order for repairs. The hydraulic retention time of the digesters, the overall volatile

solids reduction inside the digesters and the number of digesters in service were obtained from ROPEC operational personal. The data are presented in Table 5.3.

Table 5.3 – The digesters characteristics (ROPEC data) for the seven runs

	HRT			Overall volatile solids loading (kg VS)	Overall volatile solids reduction (%)	Number of digesters in service
	In primary digesters (days)	In dewatering feed digester (days)	Overall (days)			
Run 1	20	6	26	48,657	63	4
Run 2	19	5	24	48,134	57	4
Run 3	17	6	23	47,858	57	4
Run 4	11	5	16	50,774	63	3
Run 5	12	5	17	57,325	60	3
Run 6	14	6	20	59,299	62	3
Run 7	13	5	18	53,477	65	3

Volatile solids loading and reduction values are usually determined every other day. The above values represent the average based on data available within 7 days of the sampling event. In the last column, the number of digesters including the primary digesters and the dewatering feed digester is presented. From Table 5.3 it can be observed that there were differences between the first three runs that produced the higher amounts of volatile sulfur compounds and the last four runs. For the first three runs the hydraulic retention times were higher by about 4 to 9 days than for the last four runs. Also, the first three runs had one more primary digester. The volatile solids loading for the last four runs was higher, but the overall volatile solids reduction was higher. From this data and meteorological data presented in Table 3.3 it appeared that longer hydraulics time, larger number of digesters and higher ambient temperature produced a more odorous dewatered cake as the final product.

5.3 Volatile sulfur compounds and trimethylamine quantification

For the quantification of the concentrations of dimethyl sulfide, dimethyl disulfide, methyl mercaptan and trimethylamine the calibration curves developed in the Chapter 4 were used. The values measured for the five days of storage were much higher than the values used for the calibration curves. To be confident that the calibration curves were used without further errors, the values measured from the samples should be in the range of concentration of the calibration curves. Thus, the reported concentration values do not necessarily correspond to emission rate from the dewatering process from the wastewater treatment plant, and they should be considered somewhat qualitative in nature.

Organic matter is the precursor of odours in biosolids. Total solids and volatile solids are usually used to characterize the organic matter. Over the different periods of study the concentrations of odorous compounds measured in the biosolids headspace varied significantly.

The pattern of generation for methyl mercaptan, dimethyl sulfide, dimethyl disulfide and trimethylamine can be seen in Figures 5.3, 5.4, 5.5 and 5.6. For dimethyl sulfide (Figure 5.3) the maximum peak concentration was 55.11 ppm/gram dry solids in run 2 as compared to 7.26 ppm/gram dry solids, which was the peak concentration in run 6.

Figure 5.3 – Dimethyl sulfide concentration vs. period of storage

The concentration profiles measured in the warm months had almost the same values and approximately the same pattern of development. The same trend was observed also for the last four months. After analyzing the data from all runs, it appeared that DMS peaked earlier for high concentrations and later for small DMS concentrations. The concentrations measured in the warm months were (6.5 – 8) times higher than the concentrations measured in the cold months.

In run 4, the experiment was conducted for 12 days. From Figure 5.3 it can be seen that the DMS concentration was almost constant, from day five to day eleven, after which it decreased. The value measured on the last day of storage was 0.072 ppm/gram dry solids, which was much less than that measured on the first day, when 0.837 ppm/gram dry solids were recorded.

The initial rate of formation of dimethyl sulfide was high for warm months and low for cold month. In warm months the DMS response had high slope values, thus the

rate of dimethyl sulfide formation was much higher than the rate of degradation for the first four days. Comparing the DMS slopes for the cold months it appeared that the rate of formation was still higher than the rate of degradation for the first five days, but there was more balance between formation and degradation. The presence of DMS can be explained through two mechanisms. The formation of DMS was stimulated by the conditions in the vial or the destruction of the formed DMS was inhibited by the conditions in the vial. Probably both mechanisms applied. Initially oxygen is present in the biosolids and the vial. The presence of oxygen stimulated the production of DMS and inhibited its destruction. As the conditions in the vial changed from aerobic to anaerobic the methanogens would start to degrade DMS and to form methane. From the values measured in runs 3 and 4 it appeared that for a period of few days equilibrium was reached between the formation and degradation of DMS, followed by a very active period for degrading organisms that made DMS almost disappear.

Dimethyl disulfide (Figure 5.4) also demonstrated different responses in warm and cold months. The maximum concentration was measured on the fourth day of run 1 and had a peak value of 15 ppm/gram dry solids, while the minimum peak concentration was observed on the third day of run 7 with a value of 0.345 ppm/gram dry solids.

Figure 5.4 – Dimethyl disulfide concentration vs. period of storage

For runs 1, 2 and 3 the maximum DMDS concentration registered was in the range of 15 to 11.5 ppm/gram dry solids, and DMDS could be measured in the headspace of the fresh biosolids. DMDS peaked on the fourth day for runs 1 and 2, and on the fifth day for run 3. The concentrations of DMDS for these runs had almost the same values and patterns. For the rest of the runs DMDS peaked on the third day.

For both sets of data (from warm and cold months) it seemed that the concentration of dimethyl disulfide was the net result between the rate of formation and the rate of degradation over the period of time. For the first period, when the dimethyl disulfide concentration was increasing, the rate of formation was much higher than the rate of degradation. The period of time required for the formation of the maximum peak concentrations seemed to be equal to the period of time required for the destruction of the

formed DMDS. For the second period the rate of degradation was much higher than the rate of formation, and DMDS concentrations decreased.

Figure 5.5 – Methyl mercaptan concentration vs. period of storage

Methyl mercaptan could not be measured in the headspace of the fresh biosolids for all the periods analyzed. The pattern in concentrations of methyl mercaptan (Figure 5.5) could be grouped in three categories. In runs 1 and 2 the concentration of methyl mercaptan increased over the duration of the test and had a maximum value of 270 ppm/gram dry solids in run 1 and 279 ppm/gram dry solids in run 2. The third test was run for eight days. Methyl mercaptan increased over the first five days to a peak value of 149 ppm/gram dry solids, and then decreased to 145 ppm/gram dry solids on the last day of the test. In runs 4, 5, 6 and 7 the concentrations peaked on the fourth day of storage, with peak values ranging from 45 ppm/gram dry solids (run 5) to 16 ppm/gram dry solids (run 7). In run 4 the methyl mercaptan concentration on the twelfth day of storage was

only 0.507 ppm/gram dry solids. This was 8 times less than the concentration recorded on the second day of storage and 41 times less than maximum recorded concentration.

The microorganisms that are responsible for breaking down protein to form methyl mercaptan seemed to be more active over the first five days of storage for the first three runs, and for the first four days for the rest of the runs, than the organisms which degraded methyl mercaptan. For the first three runs, the slopes of the formation curves were higher than for the other four runs, thus for the first three runs the formation of methyl mercaptan was more intense than for the latter runs. The formation of MM could be explained through the same mechanisms as for DMS. For the first interval of the test the formation of MM was stimulated by the conditions in the vial or the destruction of already formed MM was inhibited (for example by the presence of the oxygen in the vial). The formation mechanisms were predominant for the first 4 days. As the conditions in the vials changed from aerobic to anaerobic the destruction mechanisms became predominant.

Trimethylamine (Figure 5.6) seemed to be primarily produced during the summer months. The trimethylamine concentrations during the period of storage varied randomly, and conclusions could not be drawn from the results. The maximum concentration value of 9.9 ppm/gram dry solids was measured on the fifth day of the first run. The mean values for each run varied from 1.89 ppm/gram dry solids (run 3) to 2.82 ppm/gram dry solids (run 2).

Figure 5.6 – Trimethylamine concentration vs. period of storage

In summary, the concentrations measured in the biosolids headspace appear to be the net result of formation and degradation processes. The odorous compounds were formed, reached equilibrium between the rate of formation and the rate of degradation, and after that degraded to unidentified compounds. It seemed that early in the storage, the formation rate was faster than the degradation. The limiting parameter in the formation-degradation mechanism was likely the availability of substrate for conversion to reduced sulfur compounds. For all seven runs it was observed that the formation of odorous compounds followed two patterns, one for the first three runs and the other for the next four. This could be explained through two hypotheses. In the first hypothesis, for the first three runs, the degradation mechanisms were initially inhibited, and at some point this inhibition was overcome. For the slow, low peaks (runs 4, 5, 6 and 7), the degradation mechanisms were less affected by the inhibition. In the second hypothesis the formation mechanism was inhibited during the last four runs. For the fast, high peaks (runs 1, 2 and

3), the inhibition of formation was less. Both of these mechanisms seemed to be directly affected by the presence of oxygen in the system. When oxygen was available in the system the formation of volatile sulfur compounds was recorded. As the conditions changed from aerobic to anaerobic it seemed that the methanogens used the volatile sulfur compound as a substrate for methane production, and the disappearance of volatile sulfur compounds was recorded.

To compare the strengths of the sludge odours a unitless odour index as proposed by Striebig et al. (1999) was used. The unitless odor index was determined by dividing the concentration of a compound in the headspace ($C_{\text{headspace}}$ in ppm) by its odour threshold ($C_{\text{threshold}}$ in ppb) given in Table 2.1, Chapter 2, as indicated in equation 1. The overall odour was considered as the sum of the individual odours, and was calculated as follows, for each day of the period of storage for all seven runs.

$$\text{ODI} = C_{\text{headspace}}^i / C_{\text{threshold}}^i \quad (5.1)$$

Where i represents each of the four standards used in this study.

$$\text{ODI}_{\text{overall}} = \text{ODI}_{\text{DMS}} + \text{ODI}_{\text{DMDS}} + \text{ODI}_{\text{MM}} + \text{ODI}_{\text{TMA}} \quad (5.2)$$

Figure 5.7 – Chemical composition of odours for run (08-12)/07/2002

Figure 5.8 – Chemical composition of odours for run (24-27)/07/2002

Figure 5.9 – Chemical composition of odours for run (19-26)/08/2002

Figure 5.10 – Chemical composition of odours for run (02-13)/12/2002

Figure 5.11 – Chemical composition of odours for run (09-13)/12/2002

Figure 5.12 – Chemical composition of odours for run (16-20)/05/2003

Figure 5.13 – Chemical composition of odours for run (23-27)/06/2003

In Figures 5.7 to 5.13 the odour generation over time for each of the seven runs are presented. From these figures it can be seen that for fresh biosolids, dimethyl sulfide was the most odorous and concentrated compound. After one day of storage methyl mercaptan dominated the odour index although dimethyl sulfide was present in important concentrations.

It was found that the odour index ratio for methyl mercaptan/dimethyl sulfide was in the same range for all seven runs, as follows: run 1 (6 to 12), run 2 (3.5 to 10), run 3 (1.2 to 6.6), run 4 (1.3 to 14), run 5 (1.7 to 8.4), run 6 (1.9 to 11) and run 7 (1.6 to 7.4). It seems that there was a strong relationship between these two compounds. Both of these compounds have the same proteins (methionine) as precursors. Probably proteins were degraded into MM and DMS in a constant ratio. The small differences measured among the seven runs for the MM/DMS ratio could be explained through the environmental conditions in the storage vials. These conditions promote the biochemical reaction between MM and DMS, as can be seen in Figure 2.4. The difference in concentrations

amongst the runs was likely a consequence of the availability of organic sulfur predecessors.

For the first three runs trimethylamine was the third important contributor to the odour index. For the other runs trimethylamine could not be measured. However, the values of the trimethylamine odour index were comparable to the values calculated for the dimethyl disulfide odour index. In Figure 5.14 the overall odour index for all seven runs is presented.

Figure 5.14 – Strengths of the biosolids odours for seven runs

The most odorous biosolids was collected in run 2, followed by run 1, run 3, run 5, run 4, run 6 and run 7 respectively. An odour index greater than 1 means that the odour threshold had been exceeded. The odour strength from dewatered biosolids ranged from 612,000 to 38,000 - times greater than the odour threshold, having as a base for calculation one-gram of dry solids. This result confirms that the odours from dewatered ROPEC cake are very malodours. The overall odour index variation followed the variation for the methyl mercaptan odour index. Methyl mercaptan and dimethyl sulfide were the major contributors to the overall odour but dimethyl disulfide and trimethylamine influenced its final value.

5.4 Characterization of storage conditions

Microbial transformations of organic matter in biosolids are basically biochemical processes that involve changes of chemical components initiated by living organisms. The main organic compounds from which volatile sulfur organic compounds and trimethylamine originate are proteins. The biomass makes use of the organic matter in the biosolids as the carbon source for microbial growth needed for the formation of new cells and it is the source of energy needed for maintaining life. The substrate utilization for growth of biomass takes place in parallel to its conversion for energy purposes. The energy accumulated in the organic matter is made available to the microorganisms by degradation processes that involve oxidation of the organic matter, and hence the organic matter is the electron donor. The corresponding reduction of an external electron acceptor is performed when either dissolved oxygen, DO (aerobic process), nitrate (anoxic

process) or sulfate (anaerobic process) is present (Hvitved-Jacobsen, 2002). Aerobic redox reactions have carbon dioxide and water as final products. Anoxic redox reactions have nitrogen, carbon dioxide and water as final products.

Anaerobic fermentations do not require an external electron acceptor. The organic substrate undergoes a balanced series of oxidative and reductive reactions where the organic matter that is reduced in one step is oxidized in another. The fermentation products, in addition to fermentable substrate, can be used by sulfate reducing bacteria that make use of sulfate as the terminal electron acceptor. Methanogenic bacteria also utilize the low molecular weight fermentation products to obtain energy, producing methane as an end product. Anaerobic redox reactions have methane, carbon dioxide, hydrogen sulfide and water as final products.

For the following experiments methane was considered as an indicator of the oxidation-reduction conditions in of the biosolids. The experimental set-up was presented in Section 3.3.2.4, and the analytical method used was presented in Section 3.4.2.2. The injections were made in triplicate, for which the coefficient of variance was in the range 0.5 to 17%. In Figures 5.15 and 5.16 the profiles for methane and carbon dioxide generation versus storage time are presented.

Figure 5.15 - Headspace methane fraction during storage

Figure 5.16 - Headspace carbon dioxide fraction during storage

The pattern of CO₂ and CH₄ generation was the same for all the tests with CH₄ absent in the fresh biosolids. After one day of storage anaerobic conditions were created in the vials as indicated by CO₂ and CH₄ concentrations that increased over the first eight days of the tests. The methane concentration from the ninth to fifteenth days remained almost constant at the values measured on the eighth day. The carbon dioxide concentration from the ninth day to the fifteen days remained relatively constant. The concentrations measured for methane and carbon dioxide did not vary as much from run to run as the values measured for sulfur compounds.

On the first day of the tests the redox conditions in the biosolids were likely mostly aerobic. As storage proceeded oxygen was consumed and this was reflected by a change in the redox conditions as indicated by the methane production. Under changing aerobic/anaerobic conditions in the biosolids sealed in the 40 ml vial, low molecular weight organics that are produced under aerobic conditions may be degraded anaerobically. The variation of methane, methyl mercaptan, dimethyl sulfide and dimethyl disulfide concentrations for tests that were conducted over the periods (02-13)/09/2002 and (09-13)/09/2002 respectively, are presented in Figures 5.17 and 5.18.

Figure 5.17 – CH₄ and odorous compound responses versus time for period (02-13)/12/2002

Figure 5.18 – CH₄ and odorous compound responses versus time for period (09-13)/12/2002

From the above figures it seemed that carbon disulfide was produced only during aerobic conditions. It appeared only on the first day, when aerobic conditions were prevalent. CS_2 has homocysteine, cysteine and cystine as precursors, and these are degraded only in aerobic or waterlogged soils (Taylor, 1992). The aerobic-water logged soils represented the conditions for fresh biosolids. From equations 10 and 12 presented in Chapter 2 the products that result from carbon disulfide degradation are methane and hydrogen sulfide. Hydrogen sulfide could not be detected in the biosolids headspace. However, it may have been precipitated as iron sulfides as it was produced.

Dimethyl sulfide could also be measured in fresh biosolids. Aerobic organisms such as *Thiobacillus sp.*, *Pseudomonas sp.* may have generated dimethyl sulfide. The area responses for dimethyl sulfide increased significantly over the storage period. Under anaerobic conditions dimethyl sulfide may have been generated from methionine. The results suggest that as anaerobic conditions became established, the production of volatile sulfur compounds was stimulated. From the fifth day to the tenth day the concentration of dimethyl sulfide was relatively constant, suggesting that the dimethyl sulfide produced after the fifth day was consumed by methanogens. From the eleventh day probably the population of the microorganisms that used dimethyl sulfide as substrate was in high concentrations and also the availability of proteins as precursor of DMS was reduced. Hence, the rate of degradation was much higher than the rate of formation and the dimethyl sulfide concentration measured on the twelfth day of storage had a very low value. From Figure 2.4, Chapter 2 it can be seen that the final products of the dimethyl sulfide degradation are carbon dioxide, methane and hydrogen sulfide.

After the first day of anaerobic conditions (second day of storage) methyl mercaptan, dimethyl disulfide and dimethyl trisulfide appeared. Methyl mercaptan peaked on the fourth day of storage. Methyl mercaptan may be formed by either aerobic catabolism of dimethyl sulfide or anaerobic catabolism of methionine. The aerobic oxidation could be catalyzed by methane monooxygenase species. Methyl mercaptan could not be measured in the headspace of the fresh biosolids. During the transition period from aerobic to anaerobic conditions it appears that the oxygen in the environment inhibited the methanogens that could further degrade the methyl mercaptan. As the conditions in the vial became anaerobic, the anaerobic microorganisms degraded proteins to form methyl mercaptan. The limiting parameter in MM formation seemed to be the protein availability. The decrease in MM concentration is likely due to depletion of substrate and enhanced methanogens, as the conditions became more strongly reducing. The process of degradation of methyl mercaptan to carbon dioxide, methane and hydrogen sulfide was presented in Figure 2.4, Chapter 2.

Under changing conditions from aerobic to anaerobic conditions, the low molecular organics such as methyl mercaptan and dimethyl sulfide may have been converted aerobically (chemical and probably biochemical oxidation) to dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). DMTS peaked on the second or third day. It was subsequently degraded with DMDS as the probable product.

Hydrogen sulfide was expected to be one of the final products of the degradation, but it could not be measured in the biosolids headspace. Hydrogen sulfide could be further degraded to thiosulfate, elemental sulfur and sulfate, but this is unlikely under anaerobic conditions. Hydrogen sulfide could be precipitated by free metals. It is also

possible that the SPME fiber and GC conditions used in this study were not appropriate for hydrogen sulfide collection.

5.5 Conditions responsible for the formation and destruction of odorous compounds

From the previously described studies it appeared that the aerobic environment promoted production of carbon disulfide, dimethyl disulfide and dimethyl trisulfide. Methyl mercaptan was believed to be a product of the anaerobic environment. Dimethyl sulfide was initially produced in aerobic conditions, but its concentration increased significantly when the condition changed from aerobic to anaerobic.

The recognition thresholds of the compounds produced by aerobic environments have higher values than for compounds produced by anaerobic environments (CS_2 – 7.7 ppb; DMDS – 2.5 ppb; DMTS – 10 ppb; DMS – 1 ppb and MM – 0.5 ppb – Table 2.1). Hence, aerobic products may be less odorous than compounds produced by anaerobic environments. For a better understanding of the pathways in the generation of odorous compounds, the oxidation-reduction potential of the biosolids were studied. Aerobic environments can affect biodegradation processes in more than one way. In the aerobic respiration process the complex organic molecules – the electron donors – are broken down by passing electrons to molecular oxygen that is reduced. Also, the oxygen can be directly involved as a reactant in some of the intermediate steps. Hvitved-Jacobsen (2002) concluded that volatile substances discharged into and produced in an aerobic wastewater system are often efficiently removed.

From the previous experiments it was observed that if the period of storage was long enough the organic matter was consumed and the concentration of volatile sulfur compounds became less than that observed in the first day of storage. For some compounds such as dimethyl disulfide and dimethyl trisulfide the concentrations were found to be below the detection limits for the twelfth day of storage. To better understand the conditions that are responsible for formation and destruction of odorous compounds it was decided to create environments that had enhanced and depleted oxygen content. These were achieved by flushing the headspace of the vials with oxygen and nitrogen gas respectively.

Three sets of samples, including control samples, aerobic samples, and anaerobic samples were created. The samples were stored for five days and headspace analyses were conducted on each day of storage, following the methodology described in Section 3.3.2.5. The analytical procedures for headspace analysis were presented in Section 3.4.2.1.

5.5.1 Experimental results

The tests were repeated twice with the first test run being performed over the period 16-20/05/2003 and second test run over the period 23-27/06/2003. For each test duplicate bottles were tested. Figures 5.19 – 5.25 present the headspace concentrations, where available, and the area counts where concentrations were not available for the odorous compounds detected in the tests. The values presented in the graphs represent the average of the duplicates made in each test. The coefficients of variance calculated for each compound measured in each of the tests performed in both runs are presented in Table 5.4. The pathways for odour generation seemed to be the same for the duplicates.

Table 5.4 – The range of coefficient of variance for two different runs

		Coefficient of variance (%)						
		Acetone	DMS	CS ₂	MM	DMDS	2-But.	DMTS
Control	R1	0.9-24	9-14	18	6-44	10-65	9-13	36-50
	R2	2-25	0.3-25	25	3-57	12-54	ND	0.2-40
Oxygen	R1	0.8-9	10-20	0.8-21	14-33	3-36	0.6-10	0.8-25
	R2	2-25	3-26	3-16	3-31	0-38	0.8-19	4-37
Nitrogen	R1	ND	2-31	ND	10-45	0-11	ND	ND
	R2	ND	1-11	ND	2-7	0-25	ND	ND

Figure 5.19- Methyl mercaptan concentrations vs. period of storage

Figure 5.20 - Dimethyl disulfide concentrations vs. period of storage

Figure 5.21- Dimethyl sulfide concentrations vs. period of storage

Figure 5.22 - Dimethyl trisulfide area responses vs. period of storage

Figure 5.23 - Carbon disulfide area responses vs. period of storage

Figure 5.24 – Acetone area responses vs. period of storage

Figure 5.25 - 2-Butanone area responses vs. period of storage

There were two runs and duplicates were made in each run. There was variability between duplicates and between runs, but the patterns of responses of chemical compounds in each of the three tests performed (control, aerobic and anaerobic samples) were almost the same for both runs. An interpretation of the previously presented figures was difficult to perform and further simplifications were considered. The coefficients of variance calculated for the values measured in run 1 and run 2 for each of the three tests performed are presented in Table 5.5. The coefficients of variance were considered small enough to use average values calculated from the two runs for the explanation of the impact of biosolids environment on the odour production.

Table 5.5–The range of the coefficients of variance for values measured for odorous compounds in each of the three tests performed in run 1 and run2

	Coefficient of variance (%)						
	MM	Acetone	DMS	CS ₂	2-Butanone	DMDS	DMTS
Control	7-25	5-21*	25-26	28	37 [^]	10-52	16-54
Nitrogen	3-40	ND	6-23	ND	ND	50-75	ND
Oxygen	5-20	6-20	16-35	14-45 [@]	1-12	3-24	19-40

* - acetone could not be detected in control samples on the first day of run 2

[^] - 2-butanone in the second run was detected in control samples only on the fifth day

[@] - carbon disulfide could not be detected in oxygenated samples on the fifth day of the second run

The average values for methyl mercaptan concentrations are presented in Table 5.6. Comparing the oxygenated and anaerobic environment with the control sample for the five days of storage, presented in Figure 5.19 and Table 5.6, it seemed that methyl mercaptan production followed different patterns.

Table 5.6 – Average methyl mercaptan concentrations as a function of biosolids environment and the period of storage

Period of storage	Control (ppm)	Anaerobic (ppm)	Oxygen enhanced (ppm)
Day 1	0	0.56	0
Day 2	4.1	54.6	0.3
Day 3	47.3	32.7	1.3
Day 4	60.5	24.5	3.9
Day 5	44.9	18.9	11.1

For the control and aerobic samples methyl mercaptan could not be measured on the first day of storage. MM peaked for the control on the fourth day, for the anaerobic environment on the second day and for the aerobic conditions its concentration increased over the period of storage. The value measured for the second day of the anaerobic bottles was the highest value observed in any of the bottles. However on the fifth day the value measured for the anaerobic environment was smaller than the control and in the same range as the aerobic samples.

The formation mechanism in the control bottles took time and seemed to be influenced, as previously determined, by the substrate availability and the oxygen content. As the oxygen was consumed and the anaerobic environment was established in the bottles the methanogens became active and used methyl mercaptan as a substrate. For the oxygenated samples the limiting parameter seemed to be the concentration of oxygen. The available substrate was degraded to other sulfur compounds such as dimethyl sulfide and carbon disulfide by aerobic organisms and hence, the concentration of methyl mercaptan was low over the test period. However, concentrations were starting to increase at the end of the test when the anaerobic conditions were likely becoming established. In the anaerobic bottles the production of methyl mercaptan was rapid and

then decay also occurred quickly. The anaerobic conditions promoted a quick degradation of proteins and after one day of storage the methanogens started to use methyl mercaptan as substrate.

From Figure 5.20, it appeared that dimethyl disulfide concentration increased over the period of storage for the oxygenated bottles. The concentration value measured on the fifth day of this test was the maximum value recorded for the experiment. The anaerobic environment seemed to produce the smallest quantities of dimethyl disulfide, and peaked on the second day. DMDS peaked on the third day for control samples. In Table 5.7, the average values for dimethyl disulfide are presented.

Table 5.7 – Average dimethyl disulfide concentrations as a function of biosolids environment and the period of storage

Period of storage	Control (ppm)	Anaerobic (ppm)	Oxygen enhanced (ppm)
Day 1	0.005	0.0075	0.0025
Day 2	0.31	0.26	0.22
Day 3	1.45	0.06	0.62
Day 4	0.44	0.03	0.92
Day 5	0.3	0.02	2.18

For the control samples, as was previously observed, dimethyl disulfide was aerobically converted (chemical and probably biochemical oxidation) from methyl mercaptan and dimethyl sulfide. For the oxygen enhanced samples the concentration of dimethyl disulfide increased over the period of storage. The oxygen likely reacted with methyl mercaptan and dimethyl sulfide and probably formed dimethyl disulfide. As the concentration of methyl mercaptan and dimethyl sulfide increased in the oxygen environment, the concentration of dimethyl disulfide increased. In the anaerobic environment, probably some oxygen was trapped in the biosolids matrix. The trapped

oxygen was consumed in one day of storage to generate oxidation products, such as dimethyl disulfide. Dimethyl disulfide started to be degraded after two days of storage, under anaerobic conditions.

From Figure 5.21, it could be seen that dimethyl sulfide had the highest concentrations in the anaerobic environments, followed by the control sample and the smallest concentrations were for the aerobic environment. For all the environments, the DMS increased over the period of storage. The dimethyl sulfide concentrations for the control and anaerobic samples were similar and were almost eight times higher than for the aerobic environment, as can be seen in Table 5.8.

Table 5.8 – Average dimethyl sulfide concentration as a function of biosolids environment and the period of storage

Period of storage	Control (ppm)	Anaerobic (ppm)	Oxygen enhanced (ppm)
Day 1	1.25	1.33	0.16
Day 2	4.65	7.96	0.89
Day 3	10.46	17.29	1.87
Day 4	18.77	23.82	3.28
Day 5	21.45	25.69	3.59

The formation of dimethyl sulfide under anaerobic conditions seemed to be more productive than under aerobic conditions. This can be explained in two ways. Under aerobic conditions, an important fraction of dimethyl sulfide is further transformed into dimethyl disulfide, dimethyl trisulfide and probably other oxidation products that could not be identified. Also, under aerobic conditions, it seemed that proteins are the substrate for the formation of other volatile sulfur compounds, such as carbon disulfide. As was seen previously under anaerobic conditions, methanogens started to degrade dimethyl sulfide after a long period of storage (after 11 days of storage). One explanation

could be that methanogens preferentially degraded methyl mercaptan, and after that dimethyl sulfide.

From Figures 5.22 to 5.25, it can be observed that the anaerobic environment did not produce dimethyl trisulfide, carbon disulfide, acetone and 2-Butanone respectively. For the control samples, dimethyl trisulfide rapidly increased to a maximum value recorded on the second day. The rate of degradation was as fast as the rate of formation, and after three days of the study dimethyl trisulfide could not be measured in the biosolids headspace. For the oxygen enhanced samples, DMTS increased over the period of storage. The peak value for the aerobic samples was 1.4 times higher than the peak value of the control samples.

Carbon disulfide (Figure 5.23) was produced by the control sample only for the first day. The formation of carbon disulfide in oxygen-enhanced samples took only one day, and it decreased over the rest of the period of storage. The value measured on the first day in the aerobic environment was four times higher than the control sample, and only the value registered on the fifth day of storage in the aerobic environment was smaller than the value registered on the first day for the control sample.

Acetone patterns (Figure 5.24) were almost the same for the control and aerobic samples, with higher values for the aerobic environment. After one day of storage, the area responses for acetone were almost constant for the period of storage, and the values recorded for the aerobic environment were three times higher than for the control samples.

For 2-Butanone (Figure 5.25) the control samples had a different response for the two runs. In run 1, 2-Butanone was measured only on the fifth day of storage, and for run

2 it appeared after the second day of storage. For the aerobic environment 2-Butanone was recorded after the first day, and after the second day had almost the same values all the period of storage, having the same variation for both runs.

Anaerobic conditions seemed to produce the smallest number of odorants, with the complete absence of dimethyl trisulfide, carbon disulfide, acetone and 2-butanone. The concentrations of methyl mercaptan produced in the anaerobic environment were lower than for the control sample and on the fifth day of storage had almost the same values as for aerobic environment. The dimethyl disulfide concentrations produced in anaerobic conditions had values that were smaller than either the control or the aerobic samples. Dimethyl sulfide was the only volatile sulfur compound that was enhanced by anaerobic conditions, with concentrations higher than the control and aerobic samples.

Aerobic conditions produced lower concentrations of dimethyl sulfide, dimethyl disulfide and methyl mercaptan than the control samples. For all the other compounds aerobic conditions produced higher values than the control samples. From Figures 5.19, 5.20, 5.21 it can be seen that as the run progressed and anaerobic conditions were likely established, the concentrations of dimethyl sulfide, dimethyl disulfide and methyl mercaptan increased over the period of storage. In the first day of storage in oxygen enhanced conditions, the concentrations of all the above compounds were lower than the concentration produced by control and anaerobic samples. From the above observations, it appeared that the aerobic environment acted to inhibit the production of volatile sulfur compounds. After five days of storage, when the condition in the bottles probably changed from oxygen enhanced into anaerobic, the concentrations of methyl mercaptan were as high as the concentration produced in anaerobic conditions after five days of

storage. The concentrations of dimethyl disulfide were higher than the concentrations measured after five days of storage for the control sample and anaerobic conditions. Dimethyl trisulfide, carbon disulfide, acetone and 2-Butanone seemed to be the product of aerobic conditions.

To compare the strength of sludge odours, a unitless odour index, as proposed by Striebig et al. (1999) was used. The odour index variation over the period of storage calculated for each of the environments tested is presented in Figure 5.26. The concentrations used to calculate the overall odour index were those presented in Table 5.6, 5.7 and 5.8.

Figure 5.26 – Strengths of the biosolids odours for control, anaerobic and oxygen enhanced conditions

From Figure 5.26, it can be seen that both anaerobic and oxygen enhanced environments produced less odorous biosolids compared with the control samples, with much lower values for the latter environment. The fresh biosolids and biosolids stored for

one day under anaerobic conditions produced higher volatile sulfur concentrations than the concentration produced by the control and oxygen enhanced environments, decreasing afterwards. The oxygen-enhanced samples produced lower concentrations, but this concentration seemed to increase with the period of storage.

The early rate of formation of methyl mercaptan under anaerobic conditions was very fast. Under anaerobic conditions that were similar to those experienced by the biomass in the upstream digester, the existing biomass was able to rapidly convert the readily available proteins to produce methyl mercaptan quickly. In addition, the microorganisms that degraded methyl mercaptan were also able to rapidly adjust to the anaerobic environment in the vials to degrade methyl mercaptan. Under oxygen-enhanced conditions, either methyl mercaptan was not produced or it was rapidly oxidized upon production of dimethyl disulfide and dimethyl trisulfide. The fact that MM concentrations were increasing with the period of storage suggested that the environment was becoming more reducing and less oxidation of the already produced methyl mercaptan occurred. In the control samples the anaerobic microorganisms were probably early inhibited by the available oxygen, this resulting in an increase rate of formation and decrease in rate of destruction. However, as oxygen became depleted methyl mercaptan formation increased but it appears that microorganisms that lead to the destruction of methyl mercaptan may have still been somewhat inhibited. The concentrations of methyl mercaptan after five days of storage in the control samples were higher than the concentrations after five days of storage in anaerobic samples.

Both, anaerobic and aerobic organisms produced dimethyl sulfide. The dimethyl sulfide concentration increased over the period of storage for all conditions studied. The

formation mechanisms in the anaerobic and control conditions seemed to be similar. The rate of formation in the oxygen-enhanced samples was substantially reduced as compared with the formation rates in the anaerobic and control samples. The anaerobic organisms seemed to be more prolific than the aerobic ones, and probably the anaerobic pathways for dimethyl sulfide production were the most important. In all the conditions for which dimethyl sulfide was measured, it appeared that the rate of destruction had lower values than the rate of formation. In oxygenated environments, the oxygen probably inhibited the biomass that utilized dimethyl sulfide as a precursor. In the anaerobic environment, the destruction mechanisms were inhibited early by any traces of oxygen trapped into the biosolids matrix. The increase of dimethyl sulfide concentration over the period of storage could be explained by preferential consumption of methyl mercaptan by biomass and only when this compound was not available did the microorganisms start to consume dimethyl sulfide. This could explain the different patterns between methyl mercaptan and dimethyl sulfide, taking into account that both of them have the same precursors.

Carbon disulfide was generated during the aerobic conditions. This experiment seemed to concur with the conclusions of Taylor (19992) that stated that the predecessors of CS_2 are proteins and these are degraded only in aerobic or waterlogged soils.

Dimethyl disulfide and dimethyl trisulfide were probably produced by chemical oxidation of methyl mercaptan. As oxygen availability increased, the DMDS concentrations and the area responses for DMTS increased over the period of storage. Only small concentrations of DMDS were measured in the anaerobic environment indicating only limited formation of dimethyl disulfide under these conditions.

In conclusion, the oxygen-enhanced environment significantly reduced the concentrations of the volatile sulfur compounds with very low odour thresholds and enhanced the production of others that have higher odour thresholds. However, maintaining the environment aerobic is challenging. The anaerobic environment generated reduced concentrations of volatile sulfur compounds over the five-day period of storage. It is believed that the limiting factor in the production of volatile odour compounds was the availability of the proteins to participate as the raw material in the degradation process. As the conditions in the vials changed from aerobic to anaerobic, it is believed that the methanogens, and probably other bacterial species utilized methyl mercaptan first and then dimethyl sulfide as substrate. The final products were probably methane, carbon dioxide, water and hydrogen sulfide. The hydrogen sulfide could not be detected in the biosolids headspace.

5.6 Inter-plant comparison

To compare the effect of the wastewater and sludge treatment processes on the dewatered biosolids odours a sample was collected from the Kingston Ravensview wastewater treatment plant. The sampling procedures were presented in section 3.3.2.1. The experimental set-up was presented in section 3.3.2.3 and the analytical methods in section 3.4.2. The total solids and volatile solids fraction of the dewatered biosolids produced by the Kingston wastewater treatment plant were 32 % and 44%, respectively.

From discussion with the operating personal it was found that odours from the Kingston biosolids were not problematic, and this was attributed to the low volatile solids content of the biosolids. Hence, it was hypothesized that concentrations of odorous

compounds measured in this sample would be lower than the concentrations from the ROPEC sludge.

The area counts for the chemical compounds found in the headspace of the samples are presented as a function of the period of storage in Figure 5.27. The odorous compounds found in the headspace of the fresh biosolids from Kingston were dimethyl sulfide, carbon disulfide and acetone. After one day of storage, methyl mercaptan, dimethyl disulfide, 2-butanone and dimethyl trisulfide appeared. Dimethyl trisulfide was only measured after one day of storage, and carbon disulfide was only measured from fresh biosolids. Dimethyl sulfide increased over the period of storage while methyl mercaptan and dimethyl disulfide peaked in the interval from day two to day four. Trimethylamine was not present in the odour matrix, for the period of storage studied.

Figure 5.27 - Odorous compounds profile from dewatered biosolids Kingston

Figures 5.28, 5.29 and 5.30 present a comparison between the methyl mercaptan, dimethyl sulfide and dimethyl disulfide concentrations from Kingston dewatered cake and from ROPEC dewatered cake. For the purposes of comparison data from run 5 with ROPEC biosolids was employed. Run 5 was conducted at the time nearest to the Kingston run.

Figure 5.28 - Methyl mercaptan generation at two different plants

Figure 5.29 - Dimethyl sulfide generation at two different plants

Figure 5.30 - Dimethyl disulfide generation at two different plants

From the above figures, it can be seen that the concentrations of the odorous compounds from the Kingston biosolids were less than half of the concentrations recorded at ROPEC. It should be noted that Run 5 for biosolids from ROPEC registered almost the smallest values of all the ROPEC runs. The differences between the two wastewaters treatment plants include the absence of an activated sludge process at the Kingston wastewater treatment plant, the use of ferric chloride to enhance primary settling at Kingston and the different type of centrifuge. The stabilization process was the same for both wastewater treatment plants, but the quality of the sludge that entered the digestion unit was quite different. It appeared that the concentration patterns for compounds of concern were similar to the concentration patterns measured in the biosolids headspace from ROPEC, with reduced sulfur concentrations for Kingston samples. This similarity suggested that the same mechanisms produced the volatile sulfur compounds in the Kingston's biosolids headspace. Because of the difference in the concentration values between the two wastewater treatment plants it appeared that the limiting parameter in these mechanisms was the protein availability.

The digester influent at ROPEC is a mixture of activated and primary sludge. The ROPEC digester influent is high in biomass, as a result of the waste activated sludge, and hence rich in proteins. During the digestion most of the proteins are consumed, but still after digestion some of the proteins remain undegraded. The influent to the Kingston digester is composed of only primary sludge. The primary sludge production is enhanced by the addition of 10 mg/L ferric chloride and of 0.4 mg/L of cationic polymer to the wastewater. The Kingston digester influent has less organic matter and more inorganic compounds. Because of the absence of waste activated sludge process and because of the

chemically enhanced primary sludge probably the Kingston digester effluent is less rich in available proteins than the ROPEC effluent digester.

Both wastewater treatment plants have high solids centrifuges. At the ROPEC total solids content of the dewatered biosolids was 30 % while the Kingston biosolids had a value of 32 %. However, the volatile solids contents were much different with a value of 56% observed at ROPEC and 44 % at Kingston. The low volatile solids content of the Kingston biosolids likely contributed to the reduced generation of odorous compounds as compared to the ROPEC biosolids.

5.7 Conclusions

The concentration of volatile sulfur compounds in the dewatered biosolids seemed to be a function of the ratio of primary sludge to waste activated sludge and the hydraulic retention time. The lower ratio primary sludge-waste activated sludge and the longer hydraulic time produced the most odorous biosolids.

The principal odorous compounds found in the dewatered biosolids headspace were methyl mercaptan, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, carbon disulfide, acetone, 2-butanone and trimethylamine. Carbon disulfide seemed to be generated during aerobic conditions. Dimethyl sulfide was generated during aerobic processes but when the conditions in the vials become anaerobic the concentration of dimethyl sulfide increased significantly. After one day of storage methyl mercaptan, dimethyl disulfide and dimethyl trisulfide were measured. Methyl mercaptan was the product of anaerobic degradation. Anaerobic degradation of proteins seemed to be the most important pattern for protein degradation during the storage of biosolids.

Formation and degradation of dimethyl sulfide were found to be related to the formation and degradation of methyl mercaptan. Both compounds have as precursors the same proteins. They started to be degraded when the conditions changed from aerobic to anaerobic. Methyl mercaptan was degraded first and after this compound was depleted dimethyl sulfide was used as a substrate.

Dimethyl disulfide was produced by chemical and biochemical oxidation of methyl mercaptan. Dimethyl trisulfide was produced by chemical oxidation of methyl mercaptan in the presence of oxygen. Trimethylamine could be measured only in the summer months. There were substantial differences between summer and winter-spring months in the concentration of odorants.

After a period of storage when the conditions in the vials were essentially anaerobic, the concentration of target analytes started to decrease. In most of the cases, by the last day of storage, the values were lower than for the first day of storage. Establishment of anaerobic biosolids conditions seemed to be appropriate to be used as method of odour mitigation, producing a smaller number of odorants at lower concentration than the aerobic biosolids environment. An oxygen-enhanced environment reduced the concentrations of volatile sulfur compounds that have low odour thresholds and resulted in the generation of compounds that have higher odour thresholds. However, maintaining a highly aerobic environment will be challenging.

Odours from biosolids seemed to be enhanced when the influent sludge contained waste activated sludge. The waste activated sludge is rich in proteins that are not totally degraded in the stabilization process, with some of them remaining available for further degradation during the period of storage. Chemically enhanced primary clarification with

ferric chloride and cationic polymer and different dewatering system associated with the absence of activated sludge decreased by half the odours from dewatered biosolids.

Chapter 6 - Protein – odour relationship

From the literature review, it appeared that the principal precursors of odours from biosolids are proteins. In this study, the biosolids were characterized in terms of soluble, labile and total proteins. It was assumed that only the soluble and labile proteins would be degraded by microorganisms to produce odorous compounds. The experimental methods that were used for the extraction of each protein fraction were presented in Section 3.3.3. The analytical methods were presented in Section 3.4.3. Triplicate analyses were made for each measurement of the protein concentrations.

6.1 Protein calibration curves

The measurement of protein content of an unknown requires an assay in which a known quantity of protein concentration is used as a reference. Hence, protein determination requires standards, in order to quantify the results. The experimental set-up and the method used for development of the protein calibration curve were presented in Section 3.3.3.1. The calibration curve for proteins, when bovine serum albumin was used as protein standard is presented in Figure 6.1.

Figure 6.1 - Protein calibration curve

The calibration curves was generally linear, with an $R^2 = 0.95$. The standard curve gave the mass of proteins in the test tube and not a concentration. The concentration of the unknown samples was determined from the measured mass of protein, the initial volume of the sample and the dilution factor.

6.2 Protein extraction time

For extraction of the labile proteins a time of 5 minutes was chose as the extraction time. The reasons for choosing this time were presented in Section 3.3.3.4. The experimental set-up for the extraction time for total proteins was also presented in the same section. The analytical method used for the extraction of proteins was presented in section 3.4.3. To determine the best time for extraction of total proteins, extraction times of 1, 2, 3, 4 and 4.5 hours were tried. For each extraction time triplicates were performed and the coefficients of variance are presented in Table 6.1. The concentration value was

calculated as an average value among triplicates, as a result of the small values calculated for the coefficients of variance ($<10\%$). The results are presented in Figure 6.2.

Table 6.1 – Coefficients of variance for concentrations of total protein

Extraction time (hour)	1	2	3	4	4.5
Coefficient of variance (%)	1.61	2.86	6.25	2.18	3.39

Figure 6.2 – Total protein concentration as a function of extraction time

The total protein concentration increased as the extraction time increased, but the difference between one hour of extraction and 4.5 hours were minimal. The concentration corresponding to the 4.5 h extraction time was only 1.023 times higher than concentration corresponding to the 1 h extraction time. As a result, extraction times of 1, 2 and 3 hours were employed for total protein measured in the subsequent testing.

The experiment presented above was performed with fresh biosolids. The effect of period of storage on the extractability of total protein was tested. The variation of total proteins as a function of extraction time and period of storage is presented in Figure 6.3.

Figure 6.3- Total protein concentration as a function of extraction time and period of storage (100 g biosolids basis)

On the basis of the data presented in Figure 6.3, an extraction time of 2 hours was chosen as the extraction time for total proteins in further experiments. The 2-hour extraction time seemed to have the most stable concentration values over the period of storage. With the one-hour extraction time, the concentration of total proteins was lower for the first three days of storage than the concentration for the 2-hour extraction time, and was higher for day 4 and 5. With the three-hour extraction time, the concentration was higher than the concentration for the 2-hour extraction time for days 1, 2 and 5, and smaller for days 3 and 4.

6.3 Protein behaviour during cake storage

For this experiment, the dewatered biosolids samples were collected from ROPEC. In addition to the measurements for protein from the unknown samples, a protein standard with concentration of 100 µg/ml was run, to verify the calibration curves.

6.3.1 Cake characterization

The methodology for sample preparation was presented in Section 3.3.3.2. The experimental method was presented in section 3.3.3.3. The samples for labile and soluble fractions were diluted by a factor of 1:20 with distilled water and the samples for total fraction were diluted by a factor of 1:100 with distilled water. The analytical method performed for protein quantification was presented in section 3.4.3. The protein

concentrations over the period of storage for total, soluble and labile fractions are presented in Figures 6.4, 6.5 and 6.6 respectively.

Figure 6.4- Total protein concentrations vs. period of storage

Figure 6.5 - Soluble protein concentrations vs. period of storage

Figure 6.6 - Labile protein concentrations vs. period of storage

From Figure 6.4, it can be seen that the concentration of total proteins decreased over the period of storage, but there were differences among concentrations from run to run. On the third day of run 1 the concentration of total protein was half the concentration recorded on the first day. For run 2 the rate of protein degradation was slower. On the fifth day of storage only 21% of the total protein was degraded by the microorganisms. In the third run, protein disappearance was steady with 50% removal of total proteins after the five days of storage. Thus, the rate of degradation of total protein and by extension the generation of odorous compounds, as by-products of the degradation processes, appears to be quite variable.

The response of soluble proteins over the period of storage differed between runs, as can be seen in Figure 6.5. Also, comparing the concentration values recorded for soluble proteins with the concentration values recorded for total proteins, it was found

that the latter were much higher (from 10 to 30 times higher) than the former values. In run 1, the concentration of soluble proteins decreased by 50% over the period of storage. In run 2, the concentration increased over the period of storage, with non-detectable concentrations of soluble protein on the first day. In run 3, the concentration of soluble proteins generally decreased over the period of storage. In conclusion, for runs 1 and 3 the soluble proteins were in the liquid phase from the beginning of the tests, and were consumed over the period of storage. For run 2, the polymer probably bridged all of the protein molecules into the biosolids matrix, and in the first day none of the proteins existed in the soluble fraction. The increase in the soluble protein concentration over the period of storage could not be explained.

The labile protein concentrations (Figure 6.6) generally decreased over the period of time in the first and third runs. For run 2, the labile protein concentrations decreased over the first three days of storage but increased on the fourth day. This increase was attributed to the random variability. Hence, the labile protein concentrations for all runs did not seemed to change much over the period of storage.

All the measurements were made in triplicates. In table 6.2 are presented the coefficients of variance for soluble, labile and total protein concentrations. From the low values of these coefficients ($<10\%$), it seemed that the average concentration values used for Figures 6.4, 6.5 and 6.6 correctly characterized the pattern of protein destruction.

Table 6.2– Coefficients of variance for concentrations of total, labile and soluble proteins from cake samples over the period of storage

Protein fraction	Period of study	Coefficient of variance (%)				
		Day 1	Day 2	Day 3	Day 4	Day 5
Total	Run 1	0	3.76	1.76	-	-
	Run 2	3.41	15.67	5.35	1.11	1.25
	Run 3	2.86	6.95	5.03	0.24	2.78
Soluble	Run 1	17.96	9.62	1.87	-	-
	Run 2	ND	0.07	0.32	0.08	0.04
	Run 3	1.85	0.58	3.87	2.14	1.38
Labile	Run 1	6.52	2.33	6.13	-	-
	Run 2	0.58	0.50	0.008	0.58	0.01
	Run 3	4.22	2.83	1.95	5.22	1.29

To characterize the odour generation, it was initially assumed that the proteins available for degradation were only the soluble and labile protein. The total proteins measured above contain both labile and bound proteins. On the assumption that only labile protein could be degraded it was expected that the bound protein fraction would be constant over the period of storage. The labile and bound fractions that compose the total protein are presented in Figures 6.7, 6.8 and 6.9 for each run individually. The bound fraction was calculated as the difference between the total protein concentrations and labile protein concentrations.

Figure 6.7 – Labile and bound protein concentrations vs. period of storage in Run 1

Figure 6.8 – Labile and bound protein concentrations vs. period of storage in Run 2

Figure 6.9 – Labile and bound protein concentrations vs. period of storage in Run 3

From the above Figures can be seen that the assumption made earlier was incorrect. It appeared that the labile fraction was almost constant. Instead, the bound fraction decreased over the period of storage, producing an overall decrease in the total protein concentration. The results suggest that during the period of storage probably the bound proteins were degraded to labile proteins and these new labile proteins were further degraded to odorous compounds, producing an overall steady labile fraction.

The protein disappearance in the samples should translate into the generation of sulfur-bearing byproducts. For example the degradation of methionine is believed to result in the generation of the methyl mercaptan, dimethyl sulfide and their oxidation products, such as dimethyl disulfide and dimethyl trisulfide. The degradation of cysteine is mainly believed to result in generation of hydrogen sulfide and carbon disulfide.

To evaluate the potential generation of sulfur bearing compounds from the observed disappearance of proteins, a typical protein content was assumed. Methionine typically represents 2% of the amino acids in beef flesh and 2.5 % of the amino acids in casein (Batstone et al., 2002). For the purposes of estimating sulfur production, it was assumed that the methionine mass in the biosolids represented 2% of the total protein mass. Cysteine is considered the precursor of hydrogen sulfide and carbon disulfide, and these two compounds were either not detected in the biosolids headspace (hydrogen sulfide) or not quantified (carbon disulfide). Thus, this amino acid was not considered in the evaluation of potential generation of sulfur bearing compounds from proteins..

The methionine mass was expressed as $\mu\text{g S/gram dry solids}$. The empirical formula used is presented in equation 6.1.

$$M_{\text{methionine}} = \frac{2 \text{ g methionine}}{100 \text{ g protein}} \times \frac{MW_{\text{sulfur}}}{MW_{\text{methionine}}} \times M_{\text{protein}} \quad (6.1)$$

Where:

$M_{\text{methionine}}$ is the concentration of methionine expressed as $\mu\text{g S/gram dry solids}$;

MW_{sulfur} is the molecular weight of sulfur (32 mol/gram);

$MW_{\text{methionine}}$ is the molecular weight of methionine (135 mol/gram);

M_{protein} is the mass of proteins ($\mu\text{g protein /gram dry solids}$) measured in the dewatered cake.

To evaluate the fraction of the methionine used to produce methyl mercaptan, dimethyl sulfide and dimethyl disulfide, it was necessary to express the mass of the compounds mentioned above as $\mu\text{g S/gram dry solids}$. The sum of all odorous compounds measured and quantified in the biosolids headspace is presented in equation 6.2.

$$M_{\text{odorous compounds}} = \frac{MW_{\text{sulfur}}}{MW_{\text{methyl mercaptan}}} M_{\text{methyl mercaptan}} + \frac{MW_{\text{sulfur}}}{MW_{\text{dimethyl sulfide}}} M_{\text{dimethyl sulfide}} + \frac{MW_{\text{sulfur}}}{MW_{\text{dimethyl disulfide}}} M_{\text{dimethyl disulfide}} \quad (6.2)$$

Where:

$M_{\text{odorous compounds}}$ is the mass of all odorous compounds quantified in the biosolids headspace, expressed as $\mu\text{g S/gram dry solids}$;

$M_{\text{methyl mercaptan}}$ is the mass of methyl mercaptan measured in the biosolids headspace ($\mu\text{g methyl mercaptan/gram dry solids}$);

$M_{\text{dimethyl sulfide}}$ is the concentration of dimethyl sulfide measured in the biosolids headspace ($\mu\text{g dimethyl sulfide /gram dry solids}$);

$M_{\text{dimethyl disulfide}}$ is the mass of dimethyl disulfide measured in the biosolids headspace ($\mu\text{g dimethyl disulfide /gram dry solids}$);

MW_{sulfur} is the molecular weight of sulfur (32 mol/gram);

$MW_{\text{methyl mercaptan}}$ is the molecular weight of methyl mercaptan (48 mol/gram)

$MW_{\text{dimethyl sulfide}}$ is the molecular weight of dimethyl sulfide (62 mol/gram);

$MW_{\text{dimethyl disulfide}}$ is the molecular weight of dimethyl disulfide (94 mol/gram).

The concentrations measured for odorous compounds in the biosolids headspace were expressed as ppm. To perform the above calculations, it was necessary to convert units. At 1 atm and 25⁰ C, one m³ of any perfect gas contains 40.87 moles. One ppm is 40.87 micromoles. The weight concentration of any gaseous pollutant is presented in equation 6.3.

$$C\left(\frac{\mu\text{g}}{\text{gram dry solids, m}^3}\right) = C\left(\frac{\text{ppm}}{\text{g dry solid}}\right) \times 40.87 \times MW\left(\frac{\text{g}}{\text{mol}}\right) \quad (6.3)$$

To calculate the mass of odorous compounds in the biosolids headspace, the weight concentration was multiplied by the volume of headspace, as shown in equation 6.4.

$$M\left(\frac{\mu\text{g}}{\text{gram dry solids}}\right) = C\left(\frac{\mu\text{g}}{\text{gram dry solids, m}^3}\right) \times V_{\text{headspace}} (\text{ml}) \times \left(\frac{\text{m}^3}{10^6 \text{ ml}}\right) \quad (6.4)$$

Where: $V_{\text{headspace}}$ is the volume of the headspace above biosolids = 20 ml

For the purposes of data analysis, the methionine mass values measured in run 3 and expressed as $\mu\text{g S/gram dry solids}$ as shown in equation 6.1, were analyzed in parallel with the sum of mass of odorous compounds, expressed as $\mu\text{g S/gram dry solids}$. The data used to characterize the mass of odorous compounds were those measured in run 6, in the Chapter 5. The resulting values are presented in Figure 6.10.

Figure 6.10 – Variation of the mass of precursor and by products on a sulfur basis

The methionine mass decreased over the period of time to produce 37.1 µg S/gram dry solids. This decrease was related to the increase of odorous compounds mass. A value of 1.7 µg S/gram dry solids was calculated for the increase in mass for odorous compounds over the period of storage. From these data can be seen that approximately 5.3 % of the sulfur found in the structure of methionine was used for the production of volatile sulfur compounds. This relatively small percent of transformation of sulfur from methionine to volatile sulfur compounds can be explained in five ways. First explanation is based on the fact that the data presented for protein and odour concentrations were not from the same period of time, and from the previous studies it was seen that there were differences between each set of tests. Second, not all the volatile sulfur compounds were quantified; for example dimethyl trisulfide and carbon disulfide were detected but not quantified. As a third explanation it was assumed that some other volatile sulfur

compounds were present in the headspace (as hydrogen sulfide) but they could not be detected with the method used. A fourth explanation was found in the probably that some percent of the sulfur from methionine structure was transformed in the inorganic form, and precipitated into the biosolids matrix. The last explanation could be found in the percent of amino acids assumed to be methionine. Maybe in the case of biosolids the percentage of 2% was too high.

6.3.2 Protein mass balance around centrifuge

In order to evaluate the behaviour of protein through the centrifuge samples were taken from three collection sites: digester effluent before cationic polymer was added, dewatered cake and centrate resulting from the dewatering process. The ROPEC digested sludge had a total solids content of 2.5%. The centrate had a solids content of 750 mg TS/L centrate. The protein concentrations for digested sludge and cake were determined for each gram of dry solids. The protein concentrations for the centrate were calculated for each ml of centrate. Approximately 100 ml of centrate were used for protein quantification. The protein concentrations are presented in Table 6.3.

Table 6.3 - Proteins concentration for the dewatering process

	Dewatering influent without polymer		Dewatered cake		Supernatant	
	R1	R2	R1	R2	R1	R2
	µg proteins/gram dry solids				µg proteins/ml centrate	
Total	9146	9251	10209	8786		
Soluble	2281	1017	560	0	14.4	13.6
Labile	2584	1214	806	785		

For the purposes of developing a mass balance on total protein, the mass of total protein that entered and left the centrifugation process was calculated. The influent flow was estimated as 15000 m³ digested sludge/day after discussion with ROPEC operating personal. The solids recovery in the centrifuge was found be to be 97%. The calculations are presented in the next paragraph. The centrate was studied only for the soluble fraction, because the mass of pellet that resulted after centrifugation was negligible.

The influent flow rate was 15000 m³ digested sludge/day. The total solids content in the digested sludge was 2.5%. From these two values the total solids flow of digested sludge was calculated in equation 6.5.

$$\text{Mass}_{\text{digested sludge}} = Q_{\text{influent}} \times \rho_{\text{digested sludge}} \times \text{TS}_{\text{digested sludge}} \quad (6.5)$$

Assuming $\rho = 1000 \text{ kg/m}^3$, the mass flow of the total solids in the influent was computed to be 375 ton of TS/day.

The centrifuge recovery was initially assumed to be 95 %. With this value the mass of total solids in the dewatered cake (equation 6.6) and the flow rate for the dewatered cake (equation 6.7) were calculated. The total solids content in the dewatered cake was 30 % and the gravity of total solids was assumed 1200 kg/m³.

$$\text{Mass}_{\text{dewatered cake}} = 0.95 \times \text{Mass}_{\text{digested sludge}} = 356.3 \text{ kg TS/day} \quad (6.6)$$

$$\begin{aligned} Q_{\text{dewatered cake}} &= \text{Mass}_{\text{dewatered cake}} / (\text{TS}_{\text{dewatered cake}} \times \rho_{\text{dewatered cake}}) \\ &= 989.6 \text{ m}^3 \text{ dewatered cake/day} \end{aligned} \quad (6.7)$$

Performing a mass balance the flow rate of centrate, the total solid flow rate and the mass of total solids were calculated. The computation is showed in equations 6.8 and 6.9.

$$Q_{\text{centrate}} = Q_{\text{influent}} - Q_{\text{dewatered cake}} = 14010.4 \text{ m}^3 \text{ centrate/day} \quad (6.8)$$

$$\text{Mass}_{\text{centrate}} = \text{Mass}_{\text{digested sludge}} - \text{Mass}_{\text{dewatered sludge}} = 18.75 \text{ ton TS/day} \quad (6.9)$$

But the ROPEC centrate had a solid content of 750 mg TS/L. With this value the mass of total solids in the centrate was calculated in equation 6.10.

$$\text{Mass}_{\text{total solids in centrate}} = 750 \text{ .g TS/L} \times Q_{\text{centrate}} = 10.51 \text{ ton/day} \quad (6.10)$$

Because of the differences recorded in the equations 6.9 and 6.10 it was found that the solids recovery of 95% was too low. Recalculating to have the same value from both equations 6.9 and 6.10 a solids recovery of 97 % was found. The corresponding values for this recovery are presented in Figure 6.11.

Figure 6.11 – Mass balance for the centrifugation process

The dosage of cationic polymer was 10 kg/dry ton of solids that entered to centrifugation. The cationic polymer was not considered in the calculation of the protein mass balance. In Table 6.4 are presented the mass of proteins involved in the mass balance.

Table 6.4 – Protein mass balance

	Mass In (ton protein/day)		Mass Out (ton protein/day)			
	Digested sludge		Dewatered cake		Centrate	
	R1	R2	R1	R2	R1	R2
Total	3.43	3.47	3.72	3.20	-	-
Soluble	0.86	0.38	0.20	0	0.20	0.19
Labile	0.97	0.46	0.29	0.29	-	-
TOTAL= Total + Soluble	4.29	3.85	3.92	3.20	0.20	0.19
Mass balance	R1	R2	R1		R2	
	4.29	3.85	4.12		3.39	

Where:

- Mass In for digested sludge = Protein concentration (column 2 or 3 from Table 6.3) x Mass total solids in digested sludge (937.5 ton/day);
- Mass Out for dewatered cake = Protein concentration (column 4 or 5 from Table 6.3) x Mass total solids in dewatered cake (927.5 ton/day);
- Mass Out for centrate = Protein concentration (column 6 or 7 from Table 6.3) x Q_{centrate} (13762.5 m³/day)

From the data presented in Table 6.4 it appeared that the closure of the mass balance on proteins was quite good. There were likely some errors induced by the uncertainty of flows in and out. All the flows used in the above calculations were found using average values provided by the operators from ROPEC. Also it is expected that the concentration of protein in the centrate would be slightly higher. In the above protein mass balance the mass of total proteins in centrate was considered negligible compared with the mass of soluble proteins in centrate and because of that the mass of total proteins was not quantified.

Comparing the protein mass in and out it was found that during the centrifugation most of the proteins were transferred into the biosolids matrix. This transfer of proteins from the solution phase into the liquid phase was presumably enhanced by polymer

addition. Also it seems that centrifugation converted most of the soluble and labile protein that was present in the influent into bound protein in the dewatered cake.

6.4 Conclusions

The concentration of total proteins decreased over the period of storage, but there were differences among concentrations from run to run. The labile and soluble proteins did not have a repeatable pattern over the period of time for the three tests performed. The most important precursor of volatile sulfur compounds was found to be the bound fraction of protein. Only around 5% of the sulfur content of methionine molecules was used to produce methyl mercaptan, dimethyl sulfide and dimethyl disulfide. The rest of the sulfur could be used for the production of other volatile sulfur compounds that were either not detected with the analytical method used or were not quantified. Also, a fraction of the sulfur could have been transformed into inorganic sulfur and precipitated into the biosolids matrix.

During the centrifugation most of the proteins were transferred into the biosolids matrix. From the mass balance for labile and total proteins it was found that labile and soluble protein fractions from the influent were transformed during the centrifugation process, into bound proteins in dewatered cake. Polymer acted as a trap of proteins, taking them out of solution and transferring them into the biosolids matrix.

Chapter 7 - The effect of digester temperature on biosolids odours

The impact of digester temperature on the protein content of the digested biosolids and the odour production from these biosolids were investigated. The effluents from bench scale digesters operating on ROPEC sludge in the Environmental Engineering Laboratory at Carleton University were employed for this purpose. The sampling points and procedures were explained in section 3.3.2.1 for odour tests and in Section 3.3.3.2. for protein tests. Sample preparation for odour tests was presented in Section 3.3.2.2 and for protein tests in Section 3.3.3.3. The headspace experimental analyses were shown in Section 3.3.2.3. The analytical methods used were explained in Section 3.4.

7.1 Experimental results for the solids content

The digester effluents were centrifuged in a laboratory centrifuge to generate a cake that was subsequently used to evaluate odour production. Cakes were generated with and without polymer addition. The total solids content and the volatile fraction of the produced cakes are summarized in Table 7.1.

Table 7.1 – Total solids and volatile fraction of centrifuged digested effluent

		Total solids (%)		Volatile fraction (%)	
		Run 1	Run 2	Run 1	Run 2
Without polymer	35 ⁰ C	16.0	13.5	56.6	53.7
	42 ⁰ C	16.4	14.5	57.6	55.8
	49 ⁰ C	16.9	14.5	57.4	55.4
	55 ⁰ C	18.5	18.2	57.3	55.2
	62 ⁰ C	23.1	20.2	57.3	55.7
With polymer	35 ⁰ C	12.6	10.8	63.9	64.1
	42 ⁰ C	12.0	11.9	64.0	63.2
	49 ⁰ C	12.0	11.4	66.8	65.6
	55 ⁰ C	11.9	12.2	64.9	61.0
	62 ⁰ C	18.2	15.5	61.8	61.1

The digested sludge from all the five reactors typically had a total solids content in the range of 2.5 – 3.0 %. The total solids of the dewatered cake seemed to increase with the digester temperature for samples with or without polymer. Thus, it appeared that the higher the digester temperature, the better dewaterability of the sludge. The volatile fraction seemed to have lower values for sludges that originated from digesters operating at 35⁰C and 62⁰C, followed by 55⁰C. Higher values were recorded for sludge that originated from 49⁰C and 42⁰C reactors either with or without polymer addition. Thus, dewatered biosolids that originated in reactors for which the operating temperature was in the optimum range for microbial activity had a lower fraction of volatile solids.

Some differences were found among the total and volatile solids from run 1 and run 2. On the weekend before collecting the samples for run 2 the power was down in the environmental lab (all five reactors were found at the operating temperature of 35⁰C), and probably this influenced the total and volatile content of the dewatered samples from run 2. The total solids content for samples without polymer were higher than the sample with polymer, but the quantity of dewatered cake from the sample with polymer was approximately double that without polymer. The volatile solids content was higher for the

samples with polymer because of the addition of organic matter through the cationic polymer, increasing the capture of organic solids into the biosolids matrix. Because of the differences between run 1 and run 2 (high volatile content for run 1) it was expected that the odours and proteins from run 1 would have higher concentrations than those from run 2.

The total solids content was in the range (10-23) %, and was much lower than the solids content obtained at ROPEC. In addition, the laboratory centrifuge was not able to reproduce the shearing forces that affect the biosolids inside of the high solids centrifuge that is used at ROPEC. It was believed that the dewatered cake obtained with the laboratory centrifuge had the properties of cake from a full-scale low solids centrifuge.

7.2 Experimental results for the protein measurements

After the dewatered samples were prepared, the protein measurements were performed. The concentration of soluble, labile and total proteins from dewatered cake, as a function of the digester temperature and the dosage of the cationic polymer (0 or 10 kg/dry ton solids) are presented in Figures 7.1, 7.2 and 7.3, respectively. In these figures, 1, 2, 3, 4 and 5 represent the dewatered cake originating in reactors 35⁰C, 42⁰C, 49⁰C, 55⁰C and 62⁰C without polymer and 1p, 2p, 3p, 4p, 5p represent the dewatered cake that originated from the same reactors, but with polymer addition. As expected, there were differences among runs, with higher values for the first run, but both runs had the same trend in protein concentrations.

Figure 7.1 - Soluble protein concentrations vs. digester temperature and polymer dosage

Figure 7.2 - Labile protein concentrations vs. digester temperature and polymer dosage

Figure 7.3 - Total protein concentrations vs. digester temperature and polymer dosage

The protein concentrations for the first run had higher values for almost all fractions and for almost all samples, as were expected. The mass of organic material was directly related with the fraction of volatile solids. The higher content of total solids and volatile fraction, the higher content of organic matter was expected.

The soluble protein concentrations from both samples with or without polymer recorded the higher values for sludges that originated in digesters at 55°C, 62°C and 49°C respectively. The lower values were recorded for sludges that originated in digesters at 42°C and 35°C.

The soluble protein concentrations were lower in samples with polymer. The polymer probably increased the capture of soluble proteins, and transferred them into the biosolids matrix. Thus, polymer transformed some of the soluble proteins into labile or

bound proteins. It appeared that polymer removed more than proteins. If the polymer removes more proteins than other substance, then the protein concentration in the solids is expected to increase. If the polymer removes less proteins than other substances, then the protein concentration in the solids is expected to decrease.

In the above paragraph it was assumed that polymer addition transferred the soluble protein from the solution phase into the biosolids matrix transforming some of them into labile and bound proteins. Checking the values for labile protein concentrations for samples with or without polymer it was found that the labile concentration values for samples with polymer had lower values than for samples without polymer. Hence as an overall conclusion, it appeared that either the labile and soluble proteins were transformed into bound proteins when polymer was added or polymer increased the solids mass into the biosolids, thus producing a decrease in overall protein concentration.

The effect of digester temperature on labile protein concentrations was difficult to determine, because of the substantial differences between the two runs. However, it appeared that higher concentration for labile proteins was recorded for the reactor at 55⁰C, for both samples, with or without polymer addition.

The total protein concentrations had lower values in samples with polymer addition for sludge that originated from reactors operating at 35⁰C and 62⁰C for both runs and from the reactor operating at 42⁰C for run 1. The total protein concentration had higher values in samples with polymer for sludge that originated from the reactors at 49⁰C and 55⁰ C and from the reactor at 42⁰C for run 2. The polymer addition to the sludge that was digested at 35⁰C and 62⁰C seemed to produce a decrease in total protein concentration. This can be explained by an overall increase in solids mass in biosolids

matrix. The polymer added to sludge digested at 49°C and 55°C seemed to produce a transformation of proteins from soluble and labile into bound proteins, producing an overall increase in the total protein concentrations. For sludge from the digester at 42°C the effect of polymer on the total proteins could not be found, because there were substantial differences between runs.

Because the substantial differences between replicate runs it was not possible to determine if there was a difference between reactors with respect to the production of total proteins. However, for samples without polymer it appeared that the reactor at 35°C produced higher values. For samples with polymer addition the reactors at 35°C, 49°C and 55°C had higher values and the reactors at 42°C, 62°C had lower values.

The protein concentrations for almost all fractions were higher for samples without polymer. It is believed that polymer acted as a bridge, taking soluble material out of solution and incorporating it into the solids. It appeared that the polymer removed more materials from solution than only the proteins. Because of this the solids volume increased, producing a decrease in protein concentration. For samples from the reactors at 49°C and 55°C the polymer seemed to transform some of the protein from soluble and labile into bound proteins, producing an overall increase in total protein concentration.

7.3 Impact of digester temperature on odour formation

Two runs were conducted to characterize odour generation over the five-day period, immediately following the protein assay. In the first run the biosolids originating from only four digesters (35°C, 49°C, 55°C and 62°C) were analyzed. The total solids content of cakes from the bench scale reactors had lower values than for the ROPEC samples and hence for this experiment the biosolids volume occupied less than half of the vial volume. Thus, the quantity of oxygen in the biosolids headspace was higher than in the ROPEC experiment. Also because the lower amount of solids in the bench-scale biosolids the quantity of oxygen uptake for chemical and biochemical reactions would be reduced compared with ROPEC biosolids. Thus, the oxygen was probably present in the vial for a longer period than in the ROPEC biosolids.

For this experiment the distance between the biosolids and the fiber exposed to collect the odorants was higher than for the experiments in which ROPEC dewatered biosolids was employed. The increased headspace volume would produce a dilution of the odorous compounds in the headspace. Also, because of the oxygen presence, probably the odour pathways would be different as compared with ROPEC odour pathways. All the concentrations were calculated on a dry solid basis.

The odorants detected in this study were dimethyl sulfide, dimethyl disulfide, methyl mercaptan and carbon disulfide. Dimethyl trisulfide and trimethylamine were not detected. The concentrations of methyl mercaptan from the samples with and without polymer as a function of storage time are presented in Figures 7.4 and 7.5. The concentrations of dimethyl sulfide from samples with and without polymer are presented in Figures 7.6 and 7.7 and the concentrations of dimethyl disulfide from samples with or without polymer are presented in Figures 7.8 and 7.9 as a function of storage time. The area responses for carbon disulfide as a function of storage time are presented in Figures 7.10 and 7.11. Because of the high differences recorded in the concentration values between run 1 and run 2 only one set of data was analyzed. Initially, both sets of data were analyzed in parallel, but this seemed to add confusion in the interpretation of the data. In figures 7.4 to 7.11 only the values recorded in the second run were presented. This set of data was chosen because in this run samples from all reactors were analyzed. In Appendix 4 are attached figures with concentrations of the volatile sulfur compounds for both runs.

Figure 7.4 – Methyl mercaptan concentration for samples without polymer vs. period of storage and digester temperature recorded in second run

Figure 7.5 – Methyl mercaptan concentration for samples with polymer vs. period of storage and digester temperature recorded in second run

Figure 7.6 – Dimethyl sulfide concentration for samples without polymer vs. period of storage and digester temperature recorded in second run

Figure 7.7 – Dimethyl sulfide concentration for samples with polymer vs. period of storage and digester temperature recorded in second 2

Figure 7.8 – Dimethyl disulfide concentration for samples without polymer vs. period of storage and digester temperature recorded in second run

Figure 7.9 – Dimethyl disulfide concentration for samples with polymer vs. period of storage and digester temperature recorded in second run

Figure 7.10 – Carbon disulfide area responses for samples without polymer vs. period of storage and digester temperature recorded in second run

Figure 7.11 – Carbon disulfide area responses for samples with polymer vs. period of storage and digester temperature recorded in second run

The overall observation derived from the above figures was that the concentrations of volatile sulfur compounds from samples with or without polymer addition originated from all bench-scale reactors had lower values compared with ROPEC concentrations. This decrease in concentrations could be explained through either the absence of shear during the centrifugation or the reduced mass of solids in vials. From the literature review was found that shear breaks down the bonds between proteins, making the proteins more available for biodegradation. The shear force could not be reproduced for the samples originating from the bench-scale reactors. In addition the total solids in the samples that originated from the bench-scale reactors had much lower values as compared with the total solids from ROPEC samples. However the reduction in the odour peaks was much greater than the difference in the mass of solids. Hence, this would suggest that the former mechanism was more responsible for the decrease in the concentration of volatile sulfur compounds.

From Figures 7.4 and 7.5 can be seen that the concentration of methyl mercaptan had higher values for almost all samples with polymer. There was no apparent pattern in methyl mercaptan concentrations with temperature. However, methyl mercaptan could be detected in the biosolids headspace only from the second day of storage. Probably the higher amount of oxygen in the biosolids headspace and the lower uptake of oxygen by the microorganisms from the biosolids matrix resulted in more persistent aerobic conditions in the vials.

The values recorded for dimethyl sulfide concentrations were higher for samples with polymer (Figure 7.7) than for samples without polymer (Figure 7.6). Dimethyl sulfide was consistently produced at levels much higher in the samples from the reactor at 62⁰C than from the other digesters.

The concentrations of dimethyl disulfide measured from samples with polymer (Figure 7.9) had higher values than the concentrations recorded from samples without polymer (Figure 7.10). Dimethyl disulfide concentrations from both samples, with or without polymer addition, had very small concentrations, and were primarily produced from the reactor at 62⁰C.

Carbon disulfide (Figures 7.10 and 7.11) had almost the same values for area responses for samples with or without polymer. The samples originating from reactors at 35⁰C and 42⁰C did not produce carbon disulfide. The pattern of carbon disulfide appeared to be consistent with the pattern recorded from ROPEC samples, with the observation that the values recorded from samples that originated from the bench-scale reactors produced higher area responses than the samples from the ROPEC samples. The values presented in Figures 7.10 and 7.11 were compared with the values from run 6 of the ROPEC samples.

From the analysis of the proteins content in the biosolids matrix it was found that the concentrations of proteins in the fresh biosolids without polymer were higher than the concentrations of proteins in the fresh biosolids with polymer. Because of this observation it was expected that higher concentrations of odorous compounds would be produced from samples without polymer than from samples with polymer. However, the results of the odour study showed that samples with polymer produced larger quantities of odorous compounds than samples without polymer. The results suggest that the polymer trapped the proteins inside of the biosolids matrix.

The highest concentrations of odorous compounds were recorded for reactors at 62°C and 49°C, followed by the reactor at 55°C. The lower concentrations were recorded for the reactor at 42°C and for the reactor at 35°C the volatile sulfur compounds could not be detected in the biosolids headspace.

To compare the strengths of the sludge odors a unitless odor index, as was already presented in Chapter 5, Section 5.3 was used. In Figures 7.12 and 7.13 are presented the odour indices of the biosolids from the five digesters with and without polymer as a function of period of storage for both runs performed.

Figure 7.12 – Odour index from biosolids without polymer vs. digester temperature and period of storage

Figure 7.13 – Odour index from biosolids with polymer vs. digester temperature and period of storage

From Figures 7.12 and 7.13 it was found that the most odorous biosolids originated from the digester at 62°C, followed by digesters at 49°C, 55°C, 42°C and 35°C both, with and without polymer. It can be seen that the most odorous biosolids come from less traditional digesters. It is known that temperature plays an important role in the metabolic activities of the microbiological population. The optimum temperatures for microbial growth are believed to be 35°C (mesophilic) and 55°C (thermophilic). Because the most odorous biosolids were found to be those from digesters 49°C and 62°C digesters, probably the microorganisms responsible for the decomposition of organic matter in the stabilization process did not adapt at these temperatures.

Also, the strength of the odours seemed to follow the same pattern as the solids content of the biosolids they originated from. The biosolids with the higher total solids content produced the higher concentrations for volatile sulfur compounds. It was found that the higher digester temperature produce better dewaterability of the sludge and higher concentration of volatile sulfur compounds in the dewatered biosolids.

Although in chapter 5 it was found that the bound fraction of the proteins probably played an important role in the formation of odorous compounds, in the following analysis only the labile and soluble fractions were considered. In the previous chapter the proportion of bound proteins that participate in the biochemical reactions was not directly analyzed. In this portion of the study the results suggest a greater dependence of odours on labile and soluble protein. The highest concentrations of labile and soluble proteins in fresh biosolids were found to be in the biosolids from the digester at 55°C, followed by 62°C, 49°C, 35°C and 42°C digesters (Tables 7.1 and 7.2). These trends were similar to those observed in odour production. Thus, the higher the concentrations of

labile and soluble protein fractions, the higher concentrations of volatile sulfur compounds were expected.

7.4 Comparison between the dewatered biosolids produced with high and low solids centrifuges

7.4.1 Protein comparison

The dewatered sludge from digester 1, with an operating temperature of 35⁰C, had total solids in the range of (10.8-12.5)% and a volatile solids fraction in the range of 64% for the sample with polymer addition. The response of this biosolids was compared with the dewatered biosolids from ROPEC, which had 30% total solids and a volatile solids fraction of 56 %. The differences between the two biosolids were the centrifuge used and the fact that first digester was a bench-scale digester and second was a full-scale digester. A low solids centrifuge produced the laboratory biosolids and a high solids centrifuge produced the ROPEC biosolids. The data from run 1, Section 7.2 were compared with the data from run 3, Section 6.3.1, because both runs were performed in the same month. The protein concentrations measured in the two biosolids samples are presented in Figure 7.14.

Figure 7.14 - Soluble, labile and total proteins concentration for two different dewatered biosolids

The concentrations of the total and labile fractions from the laboratory biosolids had higher values than those extracted from the ROPEC biosolids (high solids centrifuge). The soluble fraction seemed to have slightly higher values for the ROPEC biosolids.

In chapter 2, it was found from the literature overview that an increase in shear results in an increase in labile protein. Comparing the data from Figure 7.15 it seemed that the high shear increased only the soluble proteins. This result could be a consequence of the fact that the biosolids obtained with high and low solids centrifuge had different solids content.

7.4.2 Odour comparison

The odours from the sample obtained after the centrifugation of the digested sludge from reactor 1, when cationic polymer was added to enhance the dewatering process, were compared with the odours from the ROPEC dewatered biosolids, over a five-day period of time. The data from run 1, Section 7.3 were compared with the data from run 6, Section 5.2. In run 1 a laboratory scale centrifuge was used, and was considered to be comparable to a low solids centrifuged, and for run 6 a high solids centrifuge was used. The volatile sulfur compounds recorded for the two mention runs are presented in Table 7.2.

Table 7.2 – Comparison between biosolids odours from low and high solids centrifuge
(all the values are reported per gram dry solids)

	MM		DMS		DMDS		CS ₂		DMTS	
	Concentration ppm/gram dry solids		Concentration ppm/gram dry solids		Concentration ppm/gram dry solids		Area response million/gram dry solids		Area response million/gram dry solids	
	ROPEC	Lab	ROPEC	Lab	ROPEC	Lab	ROPEC	Lab	ROPEC C	Lab
Day 1	0.0	0	0.3	0.104	0.00	0	2.37	0	0	0
Day 2	1.44	0	1.48	0	0.11	0	0	0	26.34	0
Day 3	16.87	0	3.03	0	0.62	0	0	0	13.23	0
Day 4	24.43	0	6.11	0	0.21	0	0	0	0	0
Day 5	18.72	0	7.03	0	0.13	0	0	0	0	0

From the above table it can be seen that the odours from the biosolids generated by the high solids centrifuge were much higher than the odours from the biosolids generated by the low solids centrifuge. These results were obtained despite the fact that the concentration of proteins recorded higher values for the biosolids that were dewatered with low solids centrifuge.

However, to have more certain results the samples for odour analysis should be prepared on the basis of dry weight and not wet weight, as they were prepared in these tests. In this way the solids, and respectively the organic matter in the vial would have the same basis. Even if the values measured for protein concentration from low-solids centrifuge had an overall concentration value higher than from the high-solids centrifuge the actual protein mass in the vials from the low-solids centrifuge had lower values than from high-solids centrifuge. This can be explained through the fact that the protein mass in the vial with biosolids produced by low-solids centrifuge was lower than the protein mass in the vial with biosolids produced by high-solids centrifuge due to the lower total

solids content of the former biosolids (TS from low-solids centrifuge 11% and from high-solids centrifuge 30%). Because of that the lower amount of protein from the biosolids produced by low-solids centrifuged were faster aerobically degraded to dimethyl sulfide and carbon disulfide.

The addition of polymer captured the proteins inside of the biosolids matrix. When subjected to high shear in the centrifuge the captured protein became available for odour generation during storage.

7.5 Conclusions

The protein concentrations for almost all fractions were higher for samples without polymer. It is believed that polymer acted as a bridge, taking the soluble material out of solution and incorporating it into the solids. It appeared that the polymer removed from solution more than only the proteins. Because of that the solids volume increased, producing a decrease in protein concentration. For total protein fraction for samples from reactors 49⁰C and 55⁰C the polymer seemed to transform some of the protein from soluble and labile into the bound proteins, producing an overall increase in total protein concentration.

It was found that samples with polymer addition produce larger quantities of odorous compounds than samples without polymer. The results suggest that the polymer trapped the proteins inside of the biosolids matrix.

The odorants detected in these biosolids were dimethyl sulfide, dimethyl disulfide, methyl mercaptan and carbon disulfide. Dimethyl trisulfide and trimethylamine were not detected. The most odorous biosolids originated in the digester at 62⁰C,

followed by digesters 49°C, 55°C, 42°C and 35°C both, with and without polymer. It can be seen that the most odorous biosolids came from less traditional digesters. It was found that the higher digester temperature the better dewaterability of the sludge resulted in higher concentration of volatile sulfur compounds being produced by the dewatered biosolids.

The odours from biosolids that were generated by a high-solids centrifuge were much higher than the odours from biosolids generated by a low-solids centrifuge. Even if the values measured for protein concentration from low-solids centrifuge had an overall concentration value higher than from high-solids centrifuge the actual protein concentration in the vials from low-solids centrifuge had lower values than from high-solids centrifuge. This can be explained through the fact that the protein fraction in the vial with biosolids produced by low-solids centrifuge was lower than the protein fraction in the vial with biosolids produced by high-solids centrifuge, due to the lower total solids content of the former biosolids (TS from low-solids centrifuge 11% and from high-solids centrifuge 30%). Because of that the lower amount of protein from the biosolids produced by low-solids centrifuged were degraded aerobically to dimethyl sulfide and carbon disulfide. The addition of polymer captured the proteins inside of the biosolids matrix. When subjected to high shear in the centrifuge the captured protein became available for odour generation during storage.

Chapter 8 – Conclusions

The analytical method used to collect and analyze the odorants from the biosolids headspace was SPME (Carboxen-Polydimethylsiloxane, 75 μm film) connected with gas chromatography using a mass spectrometer as the detector. It was found that the SPME application is mostly qualitative, but can however be used with success for quick screenings for odour compounds.

The most important challenge with SPME is competition between analytes and water vapor on the porous fiber coating. The production of artifacts (DMDS) was found to be insignificant (only 8% of the DMDS measured in the biosolids headspace was the result of MM oxidation), and was not be influenced by water vapor. It was found that the responses of the four analytes were significantly affected by competition with each other and with water vapor. In the latter case their responses in wet gases decreased by more than a half of the dry gas values. In a real matrix with more compounds than the ones used in this experiment and with heavier compounds at higher concentrations the impact of competition may increase. Because of the significant disappearance of trimethylamine (either from dry or wet mixture) it was assumed that other compounds were formed as a result of the reaction between volatile sulfur compounds and TMA.

There were differences between fibers in the range from 20 to 30 % and hence it is necessary to make a calibration curve for each new fiber. For this reason their use is somewhat time consuming. Treatment of the needle in the SPME assembly with inactivating coatings did not cause a significant difference in responses. However, the Silcosteel and Sulfinert treated needles enhanced the sensitivity of the assemblies

in concentrating sulfur compounds.

The principal odorous compounds found in the dewatered biosolids headspace were methyl mercaptan, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, carbon disulfide, acetone, 2-butanone and trimethylamine. The concentration of volatile sulfur compounds in the dewatered biosolids seemed to be a function of the wastewater and sludge treatment processes. The influent ratio of primary sludge to waste activated sludge to anaerobic digester and the digester hydraulic retention time appeared to influence the dewatered biosolids odours. The lower ratio primary sludge-waste activated sludge and the longer hydraulic retention time produced the most odorous biosolids. Also the odours from biosolids were enhanced when the influent sludge contained waste activated sludge. For five bench-scale anaerobic reactors it was found that the digester temperature influenced the odours from dewatered biosolids. The most odorous biosolids originated in the digester at 62°C, followed by digesters 49°C, 55°C, 42°C and 35°C both, with and without polymer addition. The most odorous biosolids came from less traditional digesters. Chemically enhanced primary clarification with ferric chloride and cationic polymer seemed to decrease the odours from dewatered biosolids. The odours from biosolids that were generated by a high-solids centrifuge contained MM, DMS, DMDS, CS₂ and DMTS over the five days period of storage and the odours from biosolids generated by a low-solids centrifuge contained only DMS from fresh biosolids at a concentrations three times less than ROPEC concentration. It was found that biosolids with polymer addition produce larger quantities of odorous compounds than samples without polymer.

Carbon disulfide was generated during aerobic conditions. Dimethyl sulfide was

generated during aerobic processes but when the conditions in the vials become anaerobic the concentration of dimethyl sulfide increased significantly. After one day of storage methyl mercaptan, dimethyl disulfide and dimethyl trisulfide were measured. Methyl mercaptan was the product of anaerobic degradation.

Formation and degradation of dimethyl sulfide were found to be related to the formation and degradation of methyl mercaptan. Both compounds have as precursors the same proteins. They started to be degraded when the conditions changed from aerobic to anaerobic. Methyl mercaptan was degraded first and after this compound was depleted dimethyl sulfide was used as a substrate.

Dimethyl disulfide was produced by chemical and probably biochemical oxidation of methyl mercaptan. Dimethyl trisulfide was produced under aerobic conditions, probably by chemical oxidation of methyl mercaptan in the presence of oxygen. Trimethylamine could be measured only in the summer months. There were substantial differences between summer and winter months in the concentration of odourants.

After a period of storage, when the conditions in the vials were essentially anaerobic the concentration of target analytes started to decrease, and in most of the cases, by the last day of storage, the values were lower than for the first day of storage. Establishment of anaerobic biosolids conditions seemed to be appropriate to be used as method of odour mitigation, producing a smaller number of odorants at lower concentration than the aerobic biosolids environment. An oxygen-enhanced environment significantly reduced the concentrations of volatile sulfur compounds that have low odour thresholds and resulted in the generation of compounds that have higher odour

thresholds. However, maintaining a highly aerobic environment will be challenging.

Proteins are the principal precursor of odorous compounds in dewatered biosolids. The concentration of total proteins decreased over the period of storage, but there were differences among concentrations from run to run. The labile and soluble proteins did not have a repeatable pattern over the period of time. The most important precursor of volatile sulfur compounds was found to be the bound fraction of protein.

Only around 5% of the sulfur content of methionine molecules was used to produce methyl mercaptan, dimethyl sulfide and dimethyl disulfide. The rest of the sulfur could be used for the production of other volatile sulfur compounds that were either not detected with the analytical method used or were not quantified. Also, a fraction of the sulfur could have been transformed into inorganic sulfur and precipitated into the biosolids matrix.

During the centrifugation most of the proteins were transferred into the biosolids matrix. The protein concentrations for almost all fractions were higher for samples without polymer. It is believed that polymer acted as a bridge, taking the soluble material out of solution and incorporating it into the solids. When subjected to high shear in the centrifuge the captured proteins become available for odour generation during storage. It appeared that the polymer removes from solution more than only the proteins. Because of that the solids volume increased, producing a decrease in protein concentration.

As a final conclusion one can say that the processes involved in odours generation are much more complex than this study could cover. In order to provide an algorithm these processes, much more parameters are to be taken into account and also over a larger period of time.

Chapter 9 – Recommendations

For the future work in this area it is recommended that the following to be considered.

1. A larger number of standards for the development of the calibration curves should be used. The supplementary standards should contain carbon disulfide, dimethyl trisulfide, hydrogen sulfide, acetone and 2-Butanone. Also, the standards should provide a larger range of concentrations, especially an increase of the upper limit.
2. The products of the chemical reactions between trimethylamine and volatile sulfur compounds should be determined.
3. The experiments for the conditions responsible for the formation and destruction of odorous compounds should be repeated and the storage conditions (methane and carbon dioxide generation) should be evaluated.
4. The impact of bound proteins on biosolids odours should be determined. It is recommended that the samples used for protein assay to be kept on ice until the assay is performed.
5. The experiments for the impact of digester temperature on odour formation should be repeated, because of the high differences recorded in the concentration values between performed runs.
6. The biosolids odours from the bench-scale reactors using laboratory low and high solids centrifuge should be measured.

One of the goals of this project was to determine the mechanisms and pathways of volatile sulfur compounds and trimethylamine production from dewatered biosolids in order to provide a foundation for the development of odour control. The main obstacle was the fact that many parameters need to be taken into account and not all the parameters were easy to identify. Parameters that might be considered include the wastewater and sludge processes within the plant, protein concentrations, biosolids environmental conditions, odorous concentrations and analytical method used.

Recently the mathematicians have introduced a new mathematical device called neural networks that can be used in the cases where an algorithm cannot be experimentally found but a large number of examples are available. Neural Networks are analytic techniques modeled after the (hypothesized) processes of learning in the cognitive system and the neurological functions of the brain, being capable of predicting new observations (on specific variables) from other observations (on the same or other variables) after executing a process of so-called learning from existing data. The use of them is that once a large collection of input-output examples is used for training such a mathematical device, it could find a rule in odours generation.

Chapter 10 - References

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Appendix 1 - Summary of concentrations of target compounds in mixtures

Individual standards

	MM		DMS		DMDS		TMA	
Concentration value (ppm)	Min	Max	Min	Max	Min	Max	Min	Max
	0.9	17	0.18	1.24	0.11	0.91	0.42	3.69

Mixture of two standards

MM + DMDS		
Concentration values (ppm)	3.31	0.30
	9.18	0.83
MM + DMS		
Concentration values (ppm)	1.67	0.148
	3.08	0.271
	9.65	0.851
DMS + DMDS		
Concentration values (ppm)	0.365	0.322
MM + TMA		
Concentration values (ppm)	3.32	0.46
	4.95	0.693
	8.48	1.18

Mixture of three standards

MM + DMS + DMDS						
	MM		DMS		DMDS	
	dry	wet	dry	wet	dry	wet
Concentration values (ppm)	3.17	2.77	0.15	0.13	0.13	0.12
	3.9	3.24	0.19	0.15	0.16	0.14
	5.69	4.07	0.27	0.19	0.24	0.17
	8.52	5.25	0.41	0.25	0.36	0.22
	-	8.39	-	0.40	-	0.35

Dry mixture of all available standards

	MM	DMS	DMDS	TMA
Concentration values (ppm)	2.59	0.12	0.11	0.34
	3.00	0.14	0.13	0.40
	3.62	0.17	0.15	0.46
	4.84	0.23	0.20	0.63
	10.27	0.48	0.43	1.43

Wet mixture of all available standards

	MM	DMS	DMDS	TMA
Concentration values (ppm)	2.75	0.13	0.13	0.36
	3.2	0.15	0.16	0.42
	4.03	0.19	0.19	0.51
	5.21	0.25	0.24	0.68

Appendix 2 – Determination of protein

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Determination of Protein: A Modification of the Lowry Method That Gives a Linear Photometric Response

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The value of the method developed by Lowry, Rosebrough, Farr, and Randall (1) for assay of protein concentration is apparent from its widespread adoption. In their critical assessment of the method these authors refer to its two disadvantages: (a) that the color yields of different proteins vary considerably, and (b) that the relationship between color yield and protein concentration is not linear. The former is inescapable, arising as it does from the complexity of the reactions of proteins in alkaline solution with cupric tartrate and the Folin-Ciocalteu reagent (2).

During a chemical study of the Cowper's gland mucin of the boar (3), I found that the color yield in protein assays was well below that to be expected from a knowledge of the carbohydrate content. However, the color yield could be increased considerably by incubating the mucin with a more concentrated alkaline copper tartrate reagent at temperatures above ambient. It would appear that the multiple anionic site-oligosaccharide side-chains of the mucin molecule (4) effectively shield the polypeptide. Experiments with other proteins revealed somewhat smaller increases in color yields under similar circumstances. As the color yields increased so calibration curves approached straight lines. By the following adaptation of the Lowry procedure it is possible to establish a linear relationship between concentration of a soluble protein and color yield. This adaptation is based in part upon modifications introduced by Miller (5).

MATERIALS

Folin-Ciocalteu reagent was obtained from British Drug Houses Ltd., Poole, Dorset, England. Bovine serum albumin (cryst.) and gamma-globulin were from Armour; trypsin, RNase A, and insulin were crystalline products from Sigma. Ovalbumin was isolated from egg white

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and recrystallized three times (6). Other reagents were analytical grade. *Solution A*: 2 gm potassium sodium tartrate and 100 gm Na_2CO_3 are dissolved in 500 ml 1 *N* NaOH and diluted with water to 1 liter.

Solution B: 2 gm potassium sodium tartrate and 1 gm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are dissolved in 90 ml water and 10 ml 1 *N* NaOH is added.

Solution C: 1 vol Folin-Ciocalteu reagent is diluted with 15 vol water. This solution (prepared daily) should be between 0.15 *N* and 0.18 *N* when titrated to pH 10 with 1 *N* NaOH. If the acidity exceeds 0.18 *N* it should be adjusted with NaOH.

Partitioning the tartrate between A and B prevents crystallization in B but provides the tartrate concentration necessary for the assay. Stored in polyethylene bottles at room temperature, A and B remain clear, and usable, for at least six months.

METHODS

Assays were carried out in medium-weight test tubes of 13 mm diameter. The tubes should be matched for wall thickness.

Protein samples are diluted to 1 ml with water and treated with 0.9 ml A. A blank and a standard are set up in the same way. The tubes are placed in a water bath at 50° for 10 min, cooled to room temperature (21-25°), and treated with 0.1 ml B. The solutions are left at room temperature for at least 10 min, then 3 ml C is forced in rapidly to ensure mixing within 1 sec. The tubes are again heated at 50° for 10 min and cooled to room temperature. Absorbances are read in 1 cm cuvettes at 650 nm.

Protein assays were also carried out by the original procedure (1) except that A was read at 650 nm and all volumes were doubled. This brought the assay volume to 5.2 ml, which is 0.2 ml above that of the new procedure. To enable comparisons to be made, the appropriate corrections have been applied in Fig. 1 and Table 1.

RESULTS AND DISCUSSION

When the new method was applied within the range 15-110 μg protein, there was direct proportionality between weight of protein and A_{650} for the proteins listed in Table 1. Experimental points for standard protein solutions showed deviations in absorbance of not more than 3% from the best straight-line fit. The sensitivity of the method can be increased by using 2 cm cuvettes or by scaling down reagent columns. Absorbances at 750 nm are 30% higher than those at 650 nm but the straight-line fit of standard points is not so good as the former wavelength. Color yields are higher than those obtained by the original method and this difference

TABLE 1
Comparison of Two Methods of Protein Assay: Responses of Different Proteins

Protein	Procedure of Lowry <i>et al.</i> (1) with double volumes					Present procedure	
	A_{680} for 80 μ g protein*	Approx. linear regions of calibration curve (μ g protein)		Rel. slopes of curves (region b)	Ratio of A_{680} values for 30 μ g and 70 μ g protein	A_{680} for 80 μ g protein	Rel. slopes of curves
		(a)	(b)				
Bovine serum albumin	0.325	*	30-80	[1.00]	0.467	0.400	[1.00]
Ovalbumin	0.395	*	30-80	1.31	0.492	0.433	1.06
RNase A	0.450	0-30	30-70	1.50	0.478	0.572	1.40
Trypsin	0.444	0-30	30-80	1.42	0.465	0.515	1.26
Gamma-globulin	0.382	0-30	30-80	1.21	0.470	0.437	1.07
Insulin	0.571	0-15	15-80	1.92	0.495	0.720	1.76

* Values were adjusted by the factor 5.2/5.0 (see text).

* Color yield from 15 μ g was less than half of yield from 30 μ g.

E. F. HARTREE

FIG. 1. Calibration curves for protein analysis: (I) present method; (II) method of Lowry *et al.* (1). A values for II were corrected by the factor 5.2/5.0 to make the two curves directly comparable (see text).

becomes marked as protein concentration rises (Fig. 1). In both methods results are less reproducible at protein levels below 15 μ g. Curves may become etched if repeatedly used for this assay. It is advisable to soak them briefly in 6N HCl before the final rinsing of the day.

Like the original procedure the present assay is influenced by sucrose and by Triton X-100 (Table 2). The lack of effect of sucrose on the uncorrected A values is fortuitous: with the chosen concentration of serum albumin the depression in A caused by sucrose was compensated for by the color yield of the sucrose itself, which was evaluated from protein-free blank runs. However, errors caused by both sucrose and Triton X-100 can be greatly reduced if a constant concentration of either

TABLE 2
Effects of Sucrose and Triton X-100 on Color Yield of Serum Albumin Assayed by the Present Method*

Solution	Relative color yield	
	Uncorr.	Corr.
50 μ g albumin	[100]	
+0.2 M sucrose	103	92
+0.5 M sucrose	101	77
+1.0 M sucrose	83	58
+0.02% Triton X-100	111	106
+0.05% Triton X-100	131	116
+0.1% Triton X-100	133	108

* Protein was added as 1 ml solution containing indicated concentrations of the two reagents. Values were corrected for color yields obtained in absence of protein.

reagent is present in all assay tubes, including blanks and protein standards.

Calibration curves obtained by the original method (1) are, as a rule, approximately linear for the range 30–80 μg (or 15–40 μg if reagent volumes are not doubled). With three proteins, RNase A, trypsin, and gamma-globulin, approximate linearity is also observed for the range 0–30 μg (Fig. 1, Table I). The two sections of the calibration line are, however, not collinear. If one of the three proteins is used as a standard, it is possible to construct, from a knowledge of A_{550} for 70 μg protein, a fair approximation to a calibration curve: A_{550} for 30 μg is assumed to equal $0.47 [A_{550} \text{ for } 70 \mu\text{g}]$ (Table I) and the curve is drawn in the form of two straight sections from 0–30 and 30–70 μg . The deviations of such a curve from one that is based upon a series of different standards will be greater than the deviations of standards from a straight line in the present method. However the errors so introduced may be no greater than errors that arise in the original method from another uncertainty namely, that the form of the relationship between A and weight of protein varies among proteins. In other words, if A^{100} and A^2 are the absorbances observed with 100 μg and $x \mu\text{g}$ protein, respectively, plots of $(100/A^{100}) + (x/A^2)$ against x for different proteins do not coincide. This can lead to errors in relative concentrations among solutions of a single protein or of a given protein mixture.

When assay conditions produce a linear response protein concentrations calculated from A_{550} are consistent among themselves but represent, e.g., "albumin equivalents" per milliliter rather than absolute protein concentrations. Since it is often the case that knowledge of relative protein concentrations in a series of "unknowns" has more relevance than precise information on absolute protein content the advantage of the present method is clear. Furthermore, water-insoluble fractions obtained during cell fractionation dissolve readily in reagent A at 50° and no special procedure for insoluble material (1) is necessary. On the other hand, the original method, being carried out entirely at room temperature, is more convenient for automated analyses.

SUMMARY

1. The Lowry, Rosebrough, Farr, and Randall method for protein assay has been modified so as to give a higher color yield with bovine serum albumin and with five other pure proteins.
2. Under the new conditions there is direct proportionality between absorbance at 650 nm and weight of protein within the range 15–110 μg .

ACKNOWLEDGMENT

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Appendix 3 - The library search report for a chromatogram recorded for odorants from biosolids headspace

Information from Data File:

File: C:\MSDCHEM\ODOR\DATA\V91207S2.D

Operator: monica

Date Acquired: 12 Jul 2002 9:33

Method File: VARIANTA

Sample Name: sample2

Misc Info:

Vial Number: 1

Search Libraries: C:\DATABASE\wiley275.l Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak

Integration Events: Chemstation Integrator - RAY.E

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.36	10.25	C:\DATABASE\wiley275.l			
			Acetaldehyde (CAS) \$\$ Ethanal \$...	184	000075-07-0	3
2	3.81	5.62	C:\DATABASE\wiley275.l			
			NITROMETHANE-D3 \$\$ Methane-d3,	777	013031-32-8	7
3	4.26	13.81	C:\DATABASE\wiley275.l			
			Methanethiol (CAS) \$\$ Mercaptom...	298	000074-93-1	90
4	4.84	0.65	C:\DATABASE\wiley275.l			
			Carbon dioxide (CAS) \$\$ Dry ice...	178	000124-38-9	2
5	5.00	0.33	C:\DATABASE\wiley275.l			
			Methanamine, N,N-dimethyl- (CAS...	621	000075-50-3	86
6	5.17	1.37	C:\DATABASE\wiley275.l			
			2-Propanone (CAS) \$\$ Acetone \$...	505	000067-64-1	84

7	5.33	1.38	C:\DATABASE\wiley275.1			
			Nitrogen oxide (N2O) (CAS) \$\$ N...	221	010024-97-2	3
8	5.89	26.67	C:\DATABASE\wiley275.1			
			Methane, thiobis- (CAS) \$\$ 2-Th...	830	000075-18-3	95
9	6.47	3.32	C:\DATABASE\wiley275.1			
			Methyl-d3 1-Dideuterio-2-propen...	1498	000000-00-0	7
10	6.98	0.55	C:\DATABASE\wiley275.1			
			2-Propanethiol (CAS) \$\$ Isoprop...	2045	000075-33-2	91
11	7.10	1.13	C:\DATABASE\wiley275.1			
			TRIDEUTEROACETONITRILE \$\$	144	002206-26-0	2
12	7.80	0.72	C:\DATABASE\wiley275.1			
			Carbon dioxide (CAS) \$\$ Dry ice...	179	000124-38-9	4
13	8.40	2.12	C:\DATABASE\wiley275.1			
			2-Butanone (CAS) \$\$ Methyl ethy...	1357	000078-93-3	80
14	9.22	0.36	C:\DATABASE\wiley275.1			
			1-Propanethiol (CAS) \$\$ Propane...	2042	000107-03-9	87
15	9.61	0.28	C:\DATABASE\wiley275.1			
			Ethane, (methylthio)- (CAS) \$\$...	2055	000624-89-5	96
16	12.87	0.05	C:\DATABASE\wiley275.1			
			2-Butanone, 3-methyl- (CAS) \$\$...	3352	000563-80-4	64
17	13.50	0.14	C:\DATABASE\wiley275.1			
			2-Butanethiol (CAS) \$\$ sec-Buty...	4412	000513-53-1	97
18	14.62	0.09	C:\DATABASE\wiley275.1			
			1-Propanethiol, 2-methyl- (CAS)...	4415	000513-44-0	94
19	15.48	0.26	C:\DATABASE\wiley275.1			
			2-Pentanone (CAS) \$\$ Methyl pro...	3325	000107-87-9	90
20	16.41	0.21	C:\DATABASE\wiley275.1			
			1-Propene, 3-(methylthio)- (CAS...	3951	010152-76-8	50
21	17.02	0.38	C:\DATABASE\wiley275.1			
			PHOSPHANOXYD, TRIMETHYL-	4515	000000-00-0	4
22	17.89	0.13	C:\DATABASE\wiley275.1			
			Propane, 1-(methylthio)- (CAS) ...	4423	003877-15-4	45

23	18.20	0.03	C:\DATABASE\wiley275.l			
			DIMETHYLAMINE-D3	240	020786-94-1	38
			Methanethiol (CAS) \$\$ Mercaptom...	295	000074-93-1	35
			Propanal, 3-(methylthio)- (CAS)...	8093	003268-49-3	32
24	19.58	9.13	C:\DATABASE\wiley275.l			
			Disulfide, dimethyl (CAS) \$\$ 2,...	4732	000624-92-0	97
25	20.23	0.87	C:\DATABASE\wiley275.l			
			Pyridine (CAS) \$\$ Azine \$\$ CP 3...	2165	000110-86-1	94
26	21.55	2.88	C:\DATABASE\wiley275.l			
			Benzene, methyl- (CAS) \$\$ Tolu...	4560	000108-88-3	94
27	22.39	0.19	C:\DATABASE\wiley275.l			
			Hexane, 2,5-dimethyl- (CAS) \$\$...	12478	000592-13-2	11
28	22.97	0.10	C:\DATABASE\wiley275.l			
			Oxirane, 2-methyl-2-(1-methylet...	6919	072221-03-5	72
29	24.74	0.03	C:\DATABASE\wiley275.l			
			4-Octene, (E)- (CAS) \$\$ trans-4...	10970	014850-23-8	93
30	24.93	2.64	C:\DATABASE\wiley275.l			
			Cyclotrisiloxane, hexamethyl- (...	106848	000541-05-9	91
31	25.36	0.17	C:\DATABASE\wiley275.l			
			1-Pentanethiol (CAS) \$\$ Pentane...	8275	000110-66-7	50
32	25.52	1.08	C:\DATABASE\wiley275.l			
			3-Octene, (E)- (CAS) \$\$ (E)-3-O...	10962	014919-01-8	91
33	26.28	0.11	C:\DATABASE\wiley275.l			
			2-Octene (CAS) \$\$ oct-2-ene \$\$...	10954	000111-67-1	94
34	26.78	0.07	C:\DATABASE\wiley275.l			
			2-Pyridinamine, 4-methyl- (CAS)...	9058	000695-34-1	80
			Methyl ethyl disulfide \$\$ Disul...	8939	020333-39-5	74
			Methyl ethyl disulfide \$\$ Disul...	8940	020333-39-5	64
35	27.72	0.48	C:\DATABASE\wiley275.l			
			Cyclotrisiloxane, hexamethyl- (...	106848	000541-05-9	90
36	28.07	0.28	C:\DATABASE\wiley275.l			
			Cyclohexane, 1,4-dimethyl- (CAS...	11176	000589-90-2	58
37	28.36	0.24	C:\DATABASE\wiley275.l			

			Butane, 2-methyl-2-(methylthio)...	14208	013286-92-5	52
38	28.69	0.20	C:\DATABASE\wiley275.l			
			Cyclooctane, (1-methylpropyl)- ...	53899	016538-89-9	59
39	29.19	0.60	C:\DATABASE\wiley275.l			
			2-Hexanone, 5-methyl- (CAS) \$\$...	12151	000110-12-3	94
40	30.15	0.10	C:\DATABASE\wiley275.l			
			METHYLLAURATE	8718	000000-00-0	95
41	30.40	0.06	C:\DATABASE\wiley275.l			
			2-Pyrazoline, 1-isopropyl-5-met...	17854	026964-54-5	80
42	31.18	0.15	C:\DATABASE\wiley275.l			
			p-Xylene	8705	000106-42-3	94
43	32.25	0.07	C:\DATABASE\wiley275.l			
			Nonane, 2-methyl- (CAS) \$\$ 2-Me...	30026	000871-83-0	59
44	33.18	0.09	C:\DATABASE\wiley275.l			
			2-Hexanone, 5-methyl- (CAS) \$\$...	12144	000110-12-3	43
45	33.51	0.15	C:\DATABASE\wiley275.l			
			Methyl i-propyl disulfide \$\$ Di...	15440	040136-65-0	91
46	34.21	0.08	C:\DATABASE\wiley275.l			
			Benzene, 1,4-dimethyl- (CAS) \$\$...	8676	000106-42-3	64
47	34.68	0.19	C:\DATABASE\wiley275.l			
			1H-Indole, 2,3-dihydro-4-methyl...	22533	062108-16-1	32
48	34.95	0.15	C:\DATABASE\wiley275.l			
			Cyclohexane, 1-ethyl-4-methyl-,...	18295	006236-88-0	83
49	35.20	0.12	C:\DATABASE\wiley275.l			
			Cyclohexane, 1-ethyl-2-methyl- ...	18359	003728-54-9	78
50	35.79	0.03	C:\DATABASE\wiley275.l			
			1H-Indol-5-ol (CAS) \$\$ 5-Hydrox...	22482	001953-54-4	43
51	35.87	0.03	C:\DATABASE\wiley275.l			
			3-Nonene (c,t)	18349	020063-77-8	18
52	36.22	0.52	C:\DATABASE\wiley275.l			
			Nonane (CAS) \$\$ n-Nonane \$\$ She...	19788	000111-84-2	97
53	36.53	0.20	C:\DATABASE\wiley275.l			
			Cyclohexane, 1,3-dimethyl-, tra...	11156	002207-03-6	59

54	36.70	0.03	C:\DATABASE\wiley275.1			
			Cyclohexane, 1-ethyl-4-methyl-,...	18294	006236-88-0	86
55	37.09	0.12	C:\DATABASE\wiley275.1			
			Tetradecane (CAS) \$\$ n-Tetradec...	83545	000629-59-4	42
56	37.27	0.23	C:\DATABASE\wiley275.1			
			Cyclohexane, (1-methylethyl)- (...)	18279	000696-29-7	50
57	37.38	0.16	C:\DATABASE\wiley275.1			
			Octane (CAS) \$\$ n-Octane \$\$ Oct...	12446	000111-65-9	58
58	37.56	0.11	C:\DATABASE\wiley275.1			
			ethylidimethylthiophene	27914	000000-00-0	83
59	37.73	0.15	C:\DATABASE\wiley275.1			
			Cyclopentane, (1-methylbutyl)- ...	28345	004737-43-3	47
60	37.81	0.26	C:\DATABASE\wiley275.1			
			Cyclohexane, propyl- (CAS) \$\$ P...	18269	001678-92-8	91
61	38.01	0.60	C:\DATABASE\wiley275.1			
			ALPHA.-PINENE \$\$ DIHYDRO-para-...	25368	000080-56-8	96
62	38.14	0.39	C:\DATABASE\wiley275.1			
			Octane, 2,6-dimethyl- (CAS) \$\$...	30052	002051-30-1	90
63	38.30	0.16	C:\DATABASE\wiley275.1			
			2-(2-THIENYL)PROPANAL \$\$ 2-Thio...	27444	063362-05-0	78
64	38.45	0.50	C:\DATABASE\wiley275.1			
			1,3,2-DIOXABOROLANE, 4,4-DIMETH	29103	000000-00-0	53
65	38.65	0.78	C:\DATABASE\wiley275.1			
			Trisulfide, dimethyl (CAS) \$\$ 2...	17326	003658-80-8	96
66	38.76	0.59	C:\DATABASE\wiley275.1			
			C3-BENZENE	15073	000000-00-0	91
67	39.02	0.74	C:\DATABASE\wiley275.1			
			1,1,2,3-tetramethylcyclohexane ...	28402	006783-92-2	93
68	39.29	0.90	C:\DATABASE\wiley275.1			
			Phenol (CAS) \$\$ Izal \$\$ ENT 181...	4823	000108-95-2	87
69	39.35	0.83	C:\DATABASE\wiley275.1			
			Hexane, 2,2,5,5-tetramethyl- (C...	30096	001071-81-4	64
70	39.52	0.45	C:\DATABASE\wiley275.1			

				Benzene, 1-ethyl-3-methyl- (CAS...	14960	000620-14-4	70
71	39.76	0.93		C:\DATABASE\wiley275.l			
				l-.beta.-Pinene \$\$ Bicyclo[3.1....	25214	018172-67-3	93
72	39.91	0.40		C:\DATABASE\wiley275.l			
				1-Decene (CAS) \$\$ Dec-1-ene \$\$...	28264	000872-05-9	43
73	40.01	0.18		C:\DATABASE\wiley275.l			
				(E)-9-((2'S,3'R)-3-HYDROXY-6'-(...	212728	071117-50-5	37
74	40.20	0.62		C:\DATABASE\wiley275.l			
				1,2,4-Trimethylbenzene	15086	000095-36-3	89

Tue Dec 03 15:59:33 2002

Appendix 4 - The concentrations of volatile sulfur compounds for two different runs recorded from samples with or without polymer as a function of storage time

Methyl mercaptan concentration for samples without polymer
vs. period of storage and digester temperature

Methyl mercaptan concentration for samples with polymer
vs. period of storage and digester temperature

Dimethyl sulfide concentration for samples without polymer
vs. period of storage and digester temperature

Dimethyl sulfide concentration for samples with polymer
vs. period of storage and digester temperature

Dimethyl disulfide concentration for samples without polymer
vs. period of storage and digester temperature

Dimethyl disulfide concentration for samples with polymer
vs. period of storage and digester temperature

Carbon disulfide area responses for samples without polymer
vs. period of storage and digester temperature

Carbon disulfide area responses for samples with polymer
vs. period of storage and digester temperature