Understanding the effect of changing urban water management on sewer emissions

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A thesis submitted for the degree of Doctor of Philosophy at The University of Queensland in 2014
School of Chemical Engineering
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Abstract

Sewer emissions, such as hydrogen sulfide, methane and volatile organic sulfur compounds (VOSCs) can cause severe problems to sewer systems and the overall environment, including sewer pipe corrosion, malodor nuisance and greenhouse effect. In the past few decades, various studies have been carried out to understand, to model and to mitigate the effects of sulfide and methane emitted from sewers. However, with the perceived implementation of new urban water management practices, like water demand management or decentralized water management, the sewer conditions will change accordingly. These changes may affect existing in-sewer biochemical processes as well as the sewer emissions. Therefore, the overall aim of this thesis is to understand the effect of changing urban water management on three major sewer emissions, i.e. hydrogen sulfide, methane and VOSCs.

The effect of reduced water consumption (RWC), achieved by water demand management, on sulfide and methane production in rising main sewers was investigated through laboratory tests and mathematical modeling. Under RWC conditions, both sulfide and methane concentrations increased in rising main sewers. The increase of sulfide concentration was mainly due to the longer hydraulic retention time (HRT), as the sulfide-producing activity of sewer biofilms was not significantly affected. Whereas, the higher methane concentration under RWC condition was caused by both enhanced methanogenic activity and the longer HRT. The mathematical modeling revealed that the volumetric chemical dosing rate for sulfide mitigation would increase; however, due to the lower flow rate, the daily chemical dosing cost would decrease.

The microbial community structure and activities of sewer biofilms under RWC conditions was investigated using a combination of microelectrode measurements, molecular techniques and mathematical modeling. It was seen that sulfide was mainly produced in the outer layer of the biofilm, between 0 - 300 μm, which was in good agreement with the distribution of sulfate reducing bacteria (SRB). SRB had a higher relative abundance of 20% on the surface layer, which decreased gradually to below 3% at the depth of 400 μm. In contrast, methanogenic archaea (MA) mainly inhabited in the inner layer of the biofilm, with their relative abundances increasing from 10% at a depth of 200 μm to 75% at a depth of 700 μm, into the biofilm. The biofilm modeling indicated that the coexistence and spatial structure of SRB and MA in the biofilm were due to differences in the microbial types, their proposed metabolic transformations and substrate utilization.
The effect of iron-rich coagulation sludge, discharged from decentralized systems, on sulfide and methane production in rising main sewers was investigated through laboratory studies. It was observed that the application of the iron-rich coagulation sludge significantly reduced the total dissolved sulfide concentration in sewers. The decrease of dissolved sulfide concentration was mainly due to the precipitation between iron and sulfide, but other reactions might be also involved. The results indicated that iron-rich coagulation sludge could be used to control sulfide levels in sewer systems. The addition of sludge slightly increased the total chemical oxidation demand (tCOD) concentration (by approximately 12%), but slightly decreased the soluble chemical oxidation demand (sCOD) and methane formation by 7% and 20%, respectively.

In order to understanding the transformation of VOSCs under different sewer conditions, an efficient method was developed to measure dissolved VOSCs in wastewater. This method used gas chromatography with a sulfur chemiluminescence detector (GC-SCD) and a static headspace technique. The method is simple and rapid, as it requires no pre-concentration treatment of samples. It has low detection limits (<1.0 ppb) and good linearity (>0.999). The recovery ratio tests and real wastewater sample analysis demonstrated that this method was suitable for routine VOSCs measurement in wastewater. In addition, sample preservation tests showed that VOSCs in wastewater samples could be preserved for at least 24 hours by acidification (pH ~1.1). Thus, this method can be used for both laboratory studies and field measurements.

With use of the GC-SCD method, the degradation of methanethiol (MT), a predominant VOSC in rising main sewers, was investigated under different biofilm development conditions. MT degradation was found to be strongly dependent on the methanogenic activity of sewer biofilms. The MT degradation rate accelerated with the increase of methanogenic activity of sewer biofilms, resulting in MT accumulation in sewers with relatively low methanogenic activities, and MT removal with higher methanogenic activities. A modified Monod-type kinetic expression was developed to describe MT degradation kinetics in anaerobic sewers, in which the maximum degradation rate was correlated to the maximum methane production rate through a power function. It was also found that the MT concentration had a linear relationship with the acetate concentration, which might be used for preliminary assessment of MT presence in anaerobic sewers.

The research outcomes of this thesis indicated that changes in urban water management practices would affect the in-sewer processes and sewer emissions. These unintended impacts should be considered in sewer management in future.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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### Publications included in this thesis


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Contributions by others to the thesis

This thesis includes contributions made by others, particularly in the chemical analysis of wastewater and reactor samples. These contributions are acknowledged as follows:

- Dr. Beatrice Keller-Lehmann, Susan Cooke, Jianguang Li and Nathan Clayton operated ion chromatography (IC), gas chromatography (GC), inductively coupled plasma-optical emission spectrometry (ICP-OES) and Flow Injection Analyzer (FIA) to analyse dissolved sulfur species, volatile fatty acids, dissolved methane, metal and dissolved nitrogen species.

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Statement of parts of the thesis submitted to qualify for the award of another degree

None.
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wastewater, biofilms, sewer, sulfide, methane, volatile organic sulfur compounds, urban water management, environmental analytical chemistry, mathematical modelling

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Figure 2-2. The dissociation of H$_2$S at different pH, generated with dissociation constants pK$_{a1}$=7 and pK$_{a2}$=14.

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<tr>
<td>AdoMet</td>
<td>S-adenosylmethionine</td>
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<tr>
<td>AED</td>
<td>Atomic emission detector</td>
</tr>
<tr>
<td>APS</td>
<td>Adenosine-phosphosulphate</td>
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<tr>
<td>BOD</td>
<td>Biological oxygen demand</td>
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<tr>
<td>CH$_4$</td>
<td>Methane</td>
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<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>DMDS</td>
<td>Dimethyl disulfide</td>
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<tr>
<td>DMS</td>
<td>Dimethyl sulfide</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>DMSO$_2$</td>
<td>Dimethyl sulfone</td>
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<tr>
<td>DMSP</td>
<td>Dimethylsulfoniopropionate</td>
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<tr>
<td>FIA</td>
<td>Flow Injection Analyzer</td>
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<td>FID</td>
<td>Flame ionization detector</td>
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<td>FPD</td>
<td>Flame photometric detector</td>
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<tr>
<td>GC</td>
<td>Gas chromatography</td>
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<td>H$_2$S</td>
<td>Hydrogen sulfide</td>
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<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
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<tr>
<td>IC</td>
<td>Ion chromatography</td>
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<tr>
<td>ICP-OES</td>
<td>Inductively coupled plasma-optical emission spectrometry</td>
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<tr>
<td>MA</td>
<td>Methanogenic archaea</td>
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<tr>
<td>MDL</td>
<td>Method detection limit</td>
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<td>MPR</td>
<td>Methane production rate</td>
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<td>MS</td>
<td>Mass spectrometer</td>
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<td>MT</td>
<td>Methanethiol</td>
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<tr>
<td>NOM</td>
<td>Natural organic material</td>
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<tr>
<td>PFPD</td>
<td>Pulsed flame photometric detector</td>
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<tr>
<td>PT</td>
<td>Purge and trap</td>
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<tr>
<td>RWC</td>
<td>Reduced water consumption</td>
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<tr>
<td>SCD</td>
<td>Sulfur chemiluminescence detector</td>
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<tr>
<td>sCOD</td>
<td>Soluble chemical oxygen demand</td>
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<tr>
<td>SMM</td>
<td>S-methyl-methionine</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid-phase microextraction</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SPR</td>
<td>Sulfide production rate</td>
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<tr>
<td>SRB</td>
<td>Sulfate-reducing bacteria</td>
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<tr>
<td>tCOD</td>
<td>Total chemical oxygen demand</td>
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<tr>
<td>TKN</td>
<td>Total kjeldahl nitrogen</td>
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<tr>
<td>TKP</td>
<td>Total kjeldahl phosphate</td>
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<tr>
<td>TSS</td>
<td>Total suspended solid</td>
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<tr>
<td>VFA</td>
<td>Volatile organic fatty acids</td>
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<tr>
<td>VOSC</td>
<td>Volatile organic sulfur compound</td>
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<tr>
<td>WSAA</td>
<td>Water Services Association of Australia</td>
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<td>WWTP</td>
<td>Wastewater treatment plants</td>
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Chapter 1 Introduction

1.1 Background

Sewers, designed for collecting and conveying wastewater can also be regarded as bioreactors since the retention of wastewater allows various microbial processes to occur. However, the in-sewer processes can produce volatile or gaseous compounds and the emission of these compounds can cause problems within the sewer maintenance as well as to the environment. Hydrogen sulfide is a well-known sewer emission, causing odor nuisance to the surrounding areas and inducing sewer pipe corrosion. Studies and practices to control hydrogen sulfide production and emission from sewer systems have been conducted in the past several decades (US EPA 1974, Hvitved-Jacobsen et al. 1988, Zhang et al. 2008). Methane is recently found to be produced in sewers in significant amounts (Guisasola et al. 2008). Since methane is a potent greenhouse gas, its emission from sewer systems could contribute to the global warming effect. Therefore, more studies have been conducted to understand and control methane emissions from sewer systems (Foley et al. 2009, Guisasola et al. 2009, GWRC 2011, Jiang et al. 2013, Gutierrez et al. 2014).

However, the well studied in-sewer processes and the existing sewer management strategies may be affected by potential changes in urban water management practices. These changes are aimed to cope with the global water crisis, one of the greatest human development challenges in early 21st century. Specifically, nowadays, a large proportion of the world’s population is confronted with water scarcity due to climate change and increasing human activities (Vörösmarty et al. 2000, HDR 2006, Jury and Vaux Jr 2007). Water pollution caused by anthropologic activities also aggravates the global water crisis. Due to lack of suitable water treatment or sanitation systems, currently more than one third of the world’s population face a lack of safe drinking water (Schwarzenbach et al. 2010). As a result, many countries are implementing new water management practices to overcome these issues. However, the new practices will result in changes to the urban water cycle which may consequently affect in-sewer processes.

Water demand management is a commonly used urban water management strategy to meet the water supply and demand balance by reducing the water consumption rate. However, reduced water consumption (RWC) changes both the composition and flow of wastewater discharged to sewer systems. By comparing wastewater characteristics before and after RWC, several studies imply that more concentrated wastewater is discharged to sewers with RWC (Dezellar and Maier 1980, Parkinson et al. 2005, Sharma et al. 2005, Cook et al. 2010, Min and Yeats 2011). In addition, decreased wastewater discharge would cause a reduced flow rate, thereby resulting in longer
hydraulic retention times (HRT) of wastewater in sewers (Zornes et al. 2011). All these changes could have unintended impacts on the in-sewer biotransformation processes potentially adding to the sulfide and methane emission problems (Zornes et al. 2011, Marleni et al. 2012).

Decentralized water management is another emerging strategy to cope with global water crisis. This strategy aims to provide water, wastewater and stormwater services at the allotment, cluster and development scale (Cook et al. 2009). These systems could increase flexibility and reduce energy consumption for water management and lower the costs of infrastructure replacement as well. However, the operation of decentralized systems can generate some waste products, such as coagulation sludge, which is produced during coagulation processes used in decentralized drinking water or water recycling systems to remove natural organic material (NOM), colour or turbidity. Unlike centralized systems often containing sludge treatment processes, due to the relatively small scale of the decentralized systems, the coagulation sludge is usually dumped into the sewer systems directly (US EPA 2013). Since the sludge could be high in metals and organic matter, the occurrence of this in the sewer might also affect the in-sewer microbial processes and consequently the sulfide and methane emissions.

In addition to sulfide and methane, volatile organic sulfur compounds (VOSCs) emitted from sewer systems are attracting increasing attention recently. VOSCs are believed to an important odorant in wastewater due to a combination of malodorous characteristics, high volatility and low odor thresholds. It is suggested that VOSCs should be considered in the design and assessment of odor abatement systems (Sivret et al. 2013a). However, the studies of VOSC are always hindered by complicated detection methods and the reactive nature of these compounds. The implementation of new urban water management practices could also potentially affect the transformation of VOSCs in sewer systems. Detailed studies of VOSC transformations in sewer systems under different conditions will provide useful information to the water industry for planning odor management strategies in a range of sewer conditions.

### 1.2 Objectives of the thesis

The aim of this Ph.D thesis is to understand the effects of changing urban water management on sewer emissions. In particular, the effect of reduced water consumption and coagulation sludge discharge on sulfide and methane production was studied. Transformations of VOSCs in sewer systems were also investigated under different sewer biofilm conditions.
1.3 Organization of the thesis

This thesis is organized into nine chapters.
Chapter 1 gives a general introduction to the background, objectives and organization of this thesis.
Chapter 2 presents a comprehensive literature review highly relevant to the thesis topic.
Chapter 3 describes three research objectives of this thesis.
Chapter 4-8 presents the detailed background, methods and results of each research objective in the form of research articles.
Chapter 9 summarizes the significant outcomes of this work and discusses the synthesis of the research outcomes as well as the recommendations for future research.
Chapter 2 Literature Review

The literature review below summarizes the findings of previous studies that are highly related to the thesis topic. Section 2.1 gives succinct overviews of sewer systems and sewer emissions. In Section 2.2, aspects of sulfide and methane production in sewers are reviewed in detail. Section 2.3 summarizes the current findings on volatile organic sulfide compounds, in terms of detection methods and biological transformation pathways. In Section 2.4, the changing urban water management strategies are introduced.

2.1 Overview of sewer systems and sewer emissions

2.1.1 Development and function of sewer systems

Sewer systems firstly appeared in many ancient civilizations that include the Chinese, Roman, Egyptian and Greek, where they were mainly used to convey storm runoff to prevent flood (Gray 1940, Hvitved-Jacobsen 2002). Not until the middle 19th century, did it become a hygienic and sanitary installation for the collection of municipal wastewater, which helped reduce the spread of epidemics. Nowadays, sewers are an indispensable part of urban water system. A sewer system is defined as a network of pipelines and ancillary works that conveys sewage to wastewater treatment plants (WWTPs) or other places for disposal (WSAA 2002). With increasing urbanization and the requirement for improved living conditions, the scale of sewer systems continues to expand in both developing and developed countries.

Depending on the function of the sewer, the network can be classified as a sanitary sewer, a storm sewer or as a combined sewer (Hvitved-Jacobsen 2002):

1. Sanitary sewers are constructed to collect and transport sewage from residential areas, commercial districts and industries. In most situations, sanitary sewers divert sewage to a WWTP, where sewage can be treated before its disposal to watercourses.
2. Storm sewers are developed for transport of storm water collected from surfaces with poor water permeability, such as from streets, roofs and cement courts. These sewers function only in wet-weather and usually transport the runoff water directly into the natural water bodies without treatment. In some cases, detention ponds, serving as treatment systems, are built as a part of such sewer networks.
3. Combined sewers collect and transport both municipal wastewater and urban runoffs. During dry-weather periods they operate like sanitary sewers. However, in wet weather,
they serve the purpose for collecting runoff as well. As a result, the flow conditions shift regularly and overflow structures are always included as part of detention basin design.

2.1.2 Design of sewer networks

The six main parts for sewer network design include: catchment design, flow estimation, pipe size selection, sewer layout, maintenance and ancillary structure, and property connection (WSAA 2002).

Based on different locations and sizes of sewer pipes, municipal sewers include reticulation sewer, branch sewer and trunk sewer (WSAA 2002). Reticulation sewers collect wastewater directly from customer properties (residential, commercial and industrial) and the pipe diameters are usually no more than 300 mm. Branch sewers connect reticulation sewers in a reticulation area or a group of reticulation areas. The diameters of branch sewers are normally between 375 mm and 600 mm. Trunk sewers are principal sewers with diameters no less than 675 mm that connect the branch sewers and transport the sewage to a treatment facility.

Where possible, the flow in a sewer is designed to be conveyed by gravity, which is achieved by setting a suitable gradient in the pipeline (WSAA 2002). This kind of sewer is defined as a gravity sewer and this design will save on energy costs for sewage transportation. According to the sewerage code of Australia (WSAA 2002), the slope of the gravity sewer should ensure a velocity of wastewater that is no less than 0.7 m/s for self-cleansing, and no more than 3.0 m/s to avoid septicity caused by high velocity. Gravity sewers can be full or partially full depending on the operation.

However, steep terrain and other variations in local landscape may preclude or limit the viability of a gravity sewer. When this happens, one solution is to pump sewage by pumping stations through a pressure sewer (rising main) to a gravity sewer or another pumping station for further transport to a WWTP. The pressure sewers are always full and the velocity range is regulated between 0.7 m/s and 3 m/s (WSAA 2007).

2.1.3 Sewer emissions

Sewer systems can be regarded as bioreactors as the retention of wastewater in sewers allows various microbial processes to occur (Hvitved-Jacobsen et al. 2002). Different microbial processes take place in one or more of the five phases in a sewer pipe, these phases include the water phase, sewer biofilms, sewer sediments, the sewer gas phase and sewer pipe walls (Figure 2-1). The
microbial processes can produce volatile or gaseous compounds, which can be emitted to atmosphere causing environmental problems.

Figure 2-1. A schematic presentation of distinct conditions and typical processes occurring in (A) pressure and (B) gravity sewers, modified from Jiang (2010). Briefly, pressure sewers are under anaerobic conditions, where fermentation, sulfate reduction and methane formation would occur. Gravity sewers are subject to both aerobic and anaerobic conditions depending upon the re-aeration. Aerobic biotransformation of organic compounds and oxidation of hydrogen sulfide would happen under aerobic conditions while fermentation and sulfate reduction would take place in anaerobic water phase, biofilm and sediments. On the sewer wall above the water phase, hydrogen sulfide can be microbially oxidized to sulfuric acid causing sewer pipe corrosion.

The most notable sewer emission is hydrogen sulfide, which is a volatile and poisonous compound with a characteristic odor of rotten eggs. Due to its low odor threshold value (2.3 ppbv) (Feilberg et al. 2010) and significant concentration in sewer systems, the emission of hydrogen sulfide often leads to odor complaints in surrounding areas. If the concentration is high, it poses potential danger to sewer workers due to its toxicity (WHO 2003). In addition, the emission of hydrogen sulfide can induce the corrosion of concrete sewer pipes (Bowlus and Banta 1932, Islander et al. 1991). Hydrogen sulfide emitted into the sewer atmosphere in gravity sewers will be oxidized to sulfuric acid on the sewer wall and consequently corrode the sewer concrete (WERF 2007b).

Another important sewer emission is methane, which is recently found be produced in sewers at significant amounts (Guisasola et al. 2008, Foley et al. 2009, GWRC 2011). Methane is a potent greenhouse gas with a life span of 12 years and a heat retention capability 21-23 times that of carbon dioxide (IPCC 2006). The latest assessment report of the Intergovernmental Panel on Climate Change (IPCC 2013) estimated that anthropogenic activities account for 50 to 65% of total
methane emissions. Due to the absence of data, the IPCC greenhouse gas inventories concluded that wastewater in closed underground sewers is not a significant source of methane (IPCC 2006). However, the Australia Greenhouse Office reported that methane generation in sewers (10-25 mg/L) was estimated to represent an increase of 15-35% to the current total emission for wastewater handling, i.e. 3304.7 CO₂ gas equivalent Gg (AGIES 2008). Therefore further research and field surveys are required to properly include methane from sewers in the global account of greenhouse gas emissions. Apart from the global warming effect, methane emitted to the sewer atmosphere could pose occupational safety risks as it can form explosive mixtures with air. An investigation conducted by an Australian water utility showed methane concentrations in the sewer atmosphere were sometimes higher than the low explosion limits (4.4%) (GWRC 2011). An incident of explosion cause by methane in a sewer system was also reported by Spencer et al (2006).

Recently, volatile organic sulfur compounds (VOSCs), such as methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS), emitted from sewer systems have attracted increasing attention. VOSCs are believed as an important odorant in wastewater due to a combination of malodorous characteristics, high volatility and low odor thresholds which are typically at the level of parts per billion by volume (ppbv) (Hwang et al. 1995, Cheng et al. 2005, Munoz et al. 2010). At higher concentrations, i.e. >0.5–20 parts per million by volume (ppmv), VOSCs could cause health problems (Lomans et al. 2002b, Kastner et al. 2003). The concentration ranges of VOSCs in sewer systems have not been well documented yet. However, several field studies reveal that the VOSC concentrations in sewer systems could be above threshold values and cause odor problems (Muezzinoglu 2003, Cheng et al. 2005, Lasaridi et al. 2010, Sivret et al. 2013a, Sivret et al. 2013b). In particular, Wang et al. (2014) conducted a long term VOSC monitoring program for sewers located at 18 different sites in two major Australian cities (Sydney and Melbourne). In these cities, the average VOSCs concentrations are substantially higher than their odor threshold values and MT appears as a key VOSC causing odor in sewers. They suggested that VOSCs should be considered in the design and assessment of odor abatement systems.

2.2 Sulfide and methane production in sewer systems

2.2.1 Sulfide production

2.2.1.1 Process description

Hydrogen sulfide is generated microbially by the anaerobic reduction of inorganic sulfur and the degradation of organic sulfur compounds such as organic acids, protein and amino acids. The reduction of sulfate by the respiration of sulfate-reducing bacteria (SRB) is considered the dominant process for hydrogen sulfide formation in sewers (Hvitved-Jacobsen 2002).
In wastewater, the average oxidation level of organic carbon is normally close to zero, thus the organic compounds can be regarded as CH2O (Hvitved-Jacobsen 2002). Equation (2-1) shows the typical stoichiometry for sulphate reduction in sewers:

$$\text{SO}_4^{2-} + 2\text{CH}_2\text{O} + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O} + 2\text{CO}_2 + \text{H}_2\text{S}$$

Equation (2-1)

A number of factors can affect sulfate reduction processes and consequently result in different sulfide production in sewers (Hvitved-Jacobsen 2002). Among the key factors identified to influence sulfide production in sewers, high sulfate and COD concentrations and long hydraulic retention times (HRT) will favor higher sulfide production (Hvitved-Jacobsen 2002, Freudenthal et al. 2005, Sharma et al. 2008b, Mohanakrishnan et al. 2009b). In addition, pH also has an important impact on sulfate reduction in the sewer. The optimal pH for sulfate reduction is reported to be in the range of pH 7.0–8.0 and at pH over 8.6 there is inhibition of SRB activity (Gutierrez et al. 2009), with significant inhibition occurring when pH is higher than 10.5 (Gutierrez et al. 2014). pH can also affect fraction of hydrogen sulfide (H2S), bisulfide ion (HS−) and sulfide ion (S2−) in the wastewater (Figure 2-2). However, only H2S can be emitted from wastewater.

![Figure 2-2. The dissociation of H2S at different pH, generated with dissociation constants pKa1=7 and pKa2=14.](image)

There is interest to predict sulfide production rates in sewers. In 1970s and 1980s, a number of empirical equations were established to describe the sulfide production rates in sewers (Thistlewhwayte 1971, Boon and Lister 1975, Pomeroy and Parkhurst 1977, Hvitved-Jacobsen et al. 2009).
According to these equations, for a COD concentration of 200 mg/L at 20 °C, the predicted sulfide production rate is between 0.5 and 2.4 g S/(m²·d). Recently, Hvitved-Jacobsen (2002) suggested that the sulfide production rate in sewers followed the half-order kinetics of easily biodegradable COD. On the other hand, Freudenthal et al. (2005) proposed a combined Monod kinetics using sulfate and different organic compounds as substrates. This kinetic expression implicated that both the sulfate and organic compounds could be the limiting factors for sulfide production. It also recognised that the type of organic compound could influence the sulfide production rate.

2.2.1.2 Physiology and ecology of SRB

(1) Electron-donor metabolism

SRB are known to use hydrogen and a wide range of organic carbon compounds as electron donors (Table 2-1). These organic carbons mainly consist of hydrolysis products (sugar, amino acids, fatty acids) and fermentation intermediates (propionate, butyrate, lactate, ethanol, acetate) (Widdel 1988, Kalyuzhnyi and Fedorovich 1998, Fedorovich et al. 2003, Muyzer and Stams 2008). Some SRB can also grow on aromatic compounds, alkenes and one-carbon compounds such as methanol, carbon monoxide and methanethiol (Widdel 1988, Tanimoto and Bak 1994, Cravo-Laureau et al. 2007, Muyzer and Stams 2008). The organic substrates can be degraded either incompletely to acetate or completely to carbon dioxide. SRB also can grow by the dismutation of thiosulphate, sulfite, and element sulphur, which results in formation of sulfate and sulfide (Gibson 1990, Hao et al. 1996, Muyzer and Stams 2008).

Table 2-1 Examples of metabolic reactions and free energy changes for SRB, compiled according to Widdel (1988) and Muyzer and Stams (2008).

<table>
<thead>
<tr>
<th>Equation</th>
<th>Reaction</th>
<th>Free energy (kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2LA^-+SO_4^{2-}→2HA^-+2HCO_3^-+HS^-+H^+</td>
<td>-160.1</td>
</tr>
<tr>
<td>2</td>
<td>2LA^-+3SO_4^{2-}→6HCO_3^-+3HS^-+H^+</td>
<td>-255.3</td>
</tr>
<tr>
<td>3</td>
<td>2BA^-+ SO_4^{2-}→4HA^-+HS^-+H^+</td>
<td>-55.6</td>
</tr>
<tr>
<td>4</td>
<td>4PA^-+3SO_4^{2-}→4HA^-+4HCO_3^-+3HS^-+H^+</td>
<td>-150.6</td>
</tr>
<tr>
<td>5</td>
<td>HA^-+SO_4^{2-}→2HCO_3^-+HS^-</td>
<td>-47.6</td>
</tr>
<tr>
<td>6</td>
<td>4H_2+SO_4^{2-}+H^+→4H_2O+HS^-</td>
<td>-152.2</td>
</tr>
<tr>
<td>8</td>
<td>S_2O_3^{2-}+H_2O→SO_4^{2-}+HS^-+H^+</td>
<td>-21.9</td>
</tr>
<tr>
<td>9</td>
<td>4SO_3^{2-}+H^+→3SO_4^{2-}+HS^-</td>
<td>-235.6</td>
</tr>
</tbody>
</table>
Note: LA\(^-\), CH\(_2\)CHOHCOO\(^-\) (acetate); BA\(^-\), CH\(_3\)CH\(_2\)CH\(_2\)COO\(^-\) (Butyrate); PA\(^-\), CH\(_3\)CH\(_2\)COO\(^-\) (propionate); HA\(^-\), CH\(_2\)COO\(^-\) (acetate)

In addition to the transformations mentioned above (Table 2-1), the anaerobic oxidation of methane coupled to sulfate reduction was proposed by Reeburgh (1976). This process is believed to be carried by syntrophic communities of archaea which perform reverse methanogenesis, and SRB that oxidize the intermediate formed by the archaea (Boetius et al. 2000). However, successful attempts to enrich these SRB from methane-oxidizing sediments have not yet been reported (Muyzer and Stams 2008).

Typically, polymeric organic compounds, like starch, cellulose, proteins, nucleic acids (DNA and RNA) and fats are not direct substrates for SRB. SRB depend on other microorganisms, which hydrolyse and ferment these polymeric organics to form substrates for them (Muyzer and Stams 2008).

(2) Electron-acceptor metabolism

Principally, SRB use sulfate as the electron acceptor. They can also reduce thiosulfate, sulfate, tetrathionate and elemental sulphur (Gibson 1990, Hao et al. 1996, Muyzer and Stams 2008). When sulfate serves as the electron acceptor, it must be activated by an ATP sulphurylase, resulting in the formation of adenosine-phosphosulphate (APS) before it can be reduced. This is because the standard redox potential of sulfate-sulfite couple \(E_0' = -516 \text{ mV}\) which is too negative to allow reduction by intracellular electron mediators ferredoxin or NADH \(E_0' = -398 \text{ mV}\) and \(E_0' = -314 \text{ mV}\), respectively (Muyzer and Stams 2008). It is also notable that some SRB can use other non-sulfur substances as electron acceptors for maintenance or growth, such as nitrate, nitrite, iron (Fe(III)), chromate (Cr(VI)) and arsenate(As(VI)) (Gibson 1990, Muyzer and Stams 2008). Even oxygen respiration is performed by some SRB (Cypionka 2000).

In the absence of inorganic electron acceptors, some SRB can play a role in the fermentation and anaerobic oxidation of organic compounds to form acetate, hydrogen and carbon dioxide. Furthermore, some SRB are true autotrophs being capable of using CO\(_2\) as a sole carbon source (Gibson 1990, Muyzer and Stams 2008).

(3) Diversity of SRB

Based on comparative analysis of 16S rRNA sequences, the known SRB can be grouped into seven phylogenetic lineages, five within the Bacteria (Deltaproteobacteria, Clostridia, Nitrospirae
Thermodesulfobacteria and Thermodesulfobiaceae) and two within the Archaea (Eurarychaeota and Crenarchaeota) (Muyzer and Stams 2008). Although, most of the sulfate reducers belong to the ~23 genera within *Deltaproteobacteria*.

Ito et al. (2002a) used Microautoradiography-fluorescence in situ hybridization (MAR-FISH) to determine the relative abundance of SRB in sewer biofilm and their substrate-uptake pattern. They found that *Desulfobulbus* and *Desulfovibrio* were the dominant SRB in sewer biofilms. *Desulfobulbus* prefer to utilise propionate and acetate, whereas *Desulfovibrio spp.* were shown to uptake bicarbonate, indicating these sewer SRBs were operating quite differently with regard to carbon requirements.

### 2.2.2 Methanogensis

#### 2.2.2.1 Process description

The products of fermentation provide an optimal environment and substrates for the growth of methanogens in sewers. These microorganisms mainly use either hydrogen and carbon dioxide or acetate as substrates to generate methane.

Recent research carried out on real sewer systems revealed significant methane production in rising mains. The dissolved methane concentration in sewage was measured to be 5-30 mg/L in two rising mains at the Gold Coast, Australia (Guisasola et al. 2008). Another field study investigating multiple locations along a rising main found methane concentrations ranging from 1 to 9 mg/L during a 7.3 hour period (Foley et al. 2009). It has also been seen that the methane concentration in sewage is positivity correlated to the HRT in sewers and to the sewer biofilms area to wastewater volume ratio in the pipe (Guisasola et al. 2008, Foley et al. 2009, Guisasola et al. 2009). Also, trade waste, containing high COD, discharged into the domestic sewer was found to significantly increase the methane production (Jiang 2010, GWRC 2011).

In rising mains, methane may be supersaturated due to the pressure in sewers being higher that atmospheric pressure (Hartley and Lant 2006, Guisasola et al. 2008). The supersaturated methane will escape into the atmosphere at the outlet of the sewer pipes or downstream at gas release valves due to low methane concentrations in the atmosphere (0.000177%), a process that would be exacerbated by high turbulence of the wastewater flow.

#### 2.2.2.2 Physiology and ecology of methanogens

(1) *Physiology of methanogens*
The carbon substrates for methanogenesis are limited to three types that include CO₂, methyl-group containing compounds and acetate. The related reactions, free energy and typical microorganisms are concluded in Table 2-2.

Table 2-2. Free energies and typical microorganisms of methanogenesis reactions, adapted from Liu and Whitman (2008).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Free energy (kJ/mol)</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. CO₂-type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4H₂+CO₂→CH₄+2H₂O</td>
<td>-135</td>
<td>Most methanogens</td>
</tr>
<tr>
<td>4HCOOH→CH₄+3CO₂+2H₂O</td>
<td>-130</td>
<td>Many hydrogenotrophic methanogens</td>
</tr>
<tr>
<td>CO₂+4 isopropanol→CH₄+ 4 acetone+2H₂O</td>
<td>-37</td>
<td>Some hydrogenotrophic methanogens</td>
</tr>
<tr>
<td>4CO+2H₂O→CH₄+3CO₂</td>
<td>-196</td>
<td>Methanothermobacter and Methanosarcina</td>
</tr>
<tr>
<td><strong>II. Methylated C1 compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4CH₃OH→3CH₄+CO₂+2H₂O</td>
<td>-105</td>
<td>Methanosarcina and other methylotrophic methanogens</td>
</tr>
<tr>
<td>CH₃OH+H₂→CH₄+H₂O</td>
<td>-113</td>
<td>Methanomicrococcus blatticola and Methanosphaera</td>
</tr>
<tr>
<td>2(CH₃)₂S+2H₂O→3CH₄+CO₂+2H₂S</td>
<td>-49</td>
<td>Some methylotrophic methanogens</td>
</tr>
<tr>
<td>4CH₃NH₂+2H₂O→3CH₄+CO₂+4NH₃</td>
<td>-75</td>
<td>Some methylotrophic methanogens</td>
</tr>
<tr>
<td>2(CH₃)₂NH+2H₂O→3CH₄+CO₂+2NH₃</td>
<td>-73</td>
<td>Some methylotrophic methanogens</td>
</tr>
<tr>
<td>4(CH₃)₂N+2H₂O→3CH₄+CO₂+4NH₃</td>
<td>-74</td>
<td>Some methylotrophic methanogens</td>
</tr>
<tr>
<td>4CH₃NH₂Cl+2H₂O→3CH₄+CO₂+4NH₄Cl</td>
<td>-74</td>
<td>Some methylotrophic methanogens</td>
</tr>
<tr>
<td><strong>III. Acetate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃COOH→CH₄+CO₂</td>
<td>-33</td>
<td>Methanosarcina and Methanoaeta</td>
</tr>
</tbody>
</table>

For the utilisation of CO₂, hydrogenotrophic methanogens can reduce this to methane with H₂ as the primary electron donor. Formate is another a major electron donor for many hydrogenotrophic methanogens. Hydrogenotrophs may also use secondary alcohols, such as 2-propanol, 2-butanol and cyclopentanol for methanogenesis. However, even in these cases, methane is also derived from the reduction of CO₂, which is generated as an intermediate of the reaction. The second type of substrate is the methyl-group containing compounds. These compounds include methanol, methylated amines (monomethylamine, dimethylamine, trimethylamine and tetramethylammonium) and methylated sulfides (methanethiol and dimethylsulfide). The third type of substrate is acetate, which also serves as an important substrate for methanogenesis. In the acetoelastic pathway, acetate is split with oxidation of the carboxyl-group to CO₂ and reduction of the methyl group to CH₄ (Liu and Whitman 2008).
(2) Diversity

All methanogens strictly belong to the phylum of Euryarchaeota and are classified into five well-established orders: Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales and Methanophyrales based on phylogenetic analysis of 16S rRNA genes. The five orders are further divided into 10 families and 31 genera. Recently, the presence of some novel phylogenetic groups of methanogens are revealed which may represent a new order or family of methanogens (Ralf 2007, Liu and Whitman 2008).

Most methanogen can use CO₂ as substrate for methanogensis. The methyl-group reduction pathway for methane generation is limited to the order Methanosarcinales, except for the genus Methanosphaera, belonging to the order Methanobacteriales, which utilize methyl compounds only in the presence of H₂ (Blaut 1994). The acetoclastic methanogenesis pathway is only observed in two genera: Methanoseata and Methanosarcina. Methanoseata is a specialist, using acetate only, whereas Methanosarcina is a relative generalist that prefers methanol and methylamine to acetate and many species also utilize H₂ (Liu and Whitman 2008). Methanoseata has a low acetate threshold and is able to use acetate at concentrations as low as 5-20 μM while Methanosarcina requires a minimum concentration of about 1 mM. Conversely, Methanosarcina can grow much faster than Methanoseata when the acetate concentration is sufficiently high (Jetten et al. 1992).

The diversity of methanogens in sewer habitats has not been studied systematically. Theoretically, the genera: Methanothermobacter, Methanothermus, methanotorris, methanocaldococcus, and methanopyrus are unable to exist in sewer, as they are extreme thermophiles. Mohannakrishnan et al. (2009b) used FISH to test the existence of Methanosataceae, Methanomicrobiales, Methanosarcinaceae, Methanococcales, Methanocaldococcaceae and Methanobacteriales in laboratory sewer biofilm reactors and found the first five appeared in the tested biofilms whereas the last one did not.

2.2.3 Competition between SRB and methanogens

As both methanogens and SRB use common substrates, i.e. hydrogen and acetate, for methanogenesis and sulfate reduction, they will compete for metabolism of these substrates. The affinity constant of SRB for hydrogen is reported to be five times lower than that for methanogens (Kristjansson et al. 1982, Robertson and Tiedje 1984, Uberoi and Bhattacharya 1997) and much lower in the case of acetate (Schönheit et al. 1982). This implies that SRB would outcompete methanogens in sulfate-rich environments based on substrate utilisation kinetics. This out-competition is also supported by thermodynamic considerations (Table 2-2 and Table 2-3).
Moreover, sulfide generated by SRB is believed to be an inhibitory factor to methanogens (Robertson and Tiedje 1984, Omil et al. 1998). On the other hand, both populations will coexist under sulfate-limiting conditions or even under sulfate non-limiting conditions where the influences of mass transfer limitations (Nielsen 1987), differences in microbial colonization and adhesion properties (Yoda et al. 1987, Santegoeds et al. 1999) or variable sulfide toxicities (Hilton and Oleszkiewicz 1988, Parkin et al. 1990) also come into play.

Guisasola et al. (2008) reported simultaneous sulfate reduction and methanogenesis by sewer biofilms, indicating SRB and MA coexisted in those biofilms. A hypothesis of spatial arrangement of SRB and methanogens is proposed to describe the coexistence (Figure 2-2). In outer layer of the biofilm, the sulfate/COD ratio is high and SRB likely dominates this region due to the different substrate affinities between SRB and methanogens and the possible toxicity of sulfide towards methanogens. However, sulfate only partially penetrates the biofilm whereas the methanogenesis precursors (VFA and H₂) diffuse through the biofilm. As a result, methanogens grow in the inner layer of the biofilm. Supporting this theory, in a laboratory sewer reactor an average of 72% of the total COD (tCOD) loss was measured as due to methanogenic activity (Guisasola et al. 2008).

![Figure 2-4 Schematic representation of an anaerobic sewer biofilm with the partial penetration of sulfate, adapted from Guisasola et al. (2008).](image-url)
2.3 Volatile Organic Sulfur Compounds

2.3.1 General characteristics of VOSCs

Typical volatile organic sulfur compounds (VOSCs) include methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS). These play a potent role in the global sulfur cycle and are therefore of significant environmental interests. DMS is first reported in the ocean by Lovelock et al. (1972) and together with oxidation products are important for “linkage” of the sulfur cycle between the ocean and atmosphere. DMS is also crucial in cloud formation and climate change and is considered to be counteractive to the behavior of greenhouse gases like methane and carbon dioxide (Charlson et al. 1987).

However, VOSCs may also cause environmental problems on local and regional scales (Lomans et al. 2002b). The VOSCs produced by anthropogenic activities may cause the acidification of forests and lakes. As VOSCs have malodorous characteristics and a very low odor threshold value, VOSCs released by composting plants, wastewater treatment plants and paper and textile industry cause regional odor problems. If the concentrations are high enough, they can cause health problems.


<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular formula</th>
<th>Odor description</th>
<th>MW (g/mol)</th>
<th>Relative vapor Density (air=1)</th>
<th>Solubility (g/L 20°C)</th>
<th>Boil point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S</td>
<td>H₂S</td>
<td>rotten eggs</td>
<td>34.1</td>
<td>1.19</td>
<td>5</td>
<td>-60</td>
</tr>
<tr>
<td>MT</td>
<td>CH₃SH</td>
<td>rotten cabbage</td>
<td>48.1</td>
<td>1.66</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>DMS</td>
<td>CH₃SCH₃</td>
<td>decayed cabbage</td>
<td>62.1</td>
<td>2.1</td>
<td>N.A.</td>
<td>37.3</td>
</tr>
<tr>
<td>DMDS</td>
<td>CH₃SSCH₃</td>
<td>decayed vegetable</td>
<td>94.2</td>
<td>1.08</td>
<td>2.5</td>
<td>110</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Explosion limit (%)</th>
<th>Henry’s law constant (mol/kg*bar)</th>
<th>pKa</th>
<th>OTV (ppbv)</th>
<th>TLV (ppmv)</th>
<th>IDLH (ppmv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S</td>
<td>4.3-46</td>
<td>0.10</td>
<td>7.05</td>
<td>2.3</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>MT</td>
<td>3.9-21.8</td>
<td>0.20</td>
<td>10.4</td>
<td>0.07</td>
<td>0.5</td>
<td>150</td>
</tr>
<tr>
<td>DMS</td>
<td>2.2-19.7</td>
<td>0.48</td>
<td>N.A.</td>
<td>5.9</td>
<td>10</td>
<td>N.A.</td>
</tr>
<tr>
<td>DMDS</td>
<td>1.1-16</td>
<td>0.96</td>
<td>N.A.</td>
<td>2.2</td>
<td>0.5</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

a. OTV: odor threshold value: minimal concentration that can be detected by the human nose
b. TLV: Threshold limit value as a time-weighted average concentration for up to a 10-hour workday during a 40-hour workweek.
c. IDLH: the concentration of a chemical which cause Immediately Dangerous to Life and Health. N.A: data not available.
Table 2-3 lists the main physical and chemical properties of typical VOSCs, i.e. MT, DMS and DMDS. In general, the odor threshold values are at ppbv level and the concentration limits for occupational health and safety are not higher than 10 ppm. The occurrences of these compounds in different wastewater systems are compared in Table 2-4. VOSCs concentrations reported in all these studies are higher than the odor threshold, which could potentially be causing odor problems. In some cases, the concentration is even higher than threshold limits for occupational health and safety considerations, which may pose safety issues to workers located in those regions. In addition, the data shows significant regional differences, and even in the same site the concentrations varied widely.
### Table 2-4. Occurrence of VOSCs in different wastewater systems.

<table>
<thead>
<tr>
<th>System description</th>
<th>Country</th>
<th>Sample type</th>
<th>Compounds Unit: gas sample - μg/m³; water sample - μg/L</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H2S</td>
<td>MT</td>
</tr>
<tr>
<td><strong>WWTPs</strong></td>
<td>China</td>
<td>Gas</td>
<td>-</td>
<td>b.d.</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>Gas</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>Gas</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sewers</td>
<td>China</td>
<td>Gas</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>Gas</td>
<td>b.d.-1.4×10⁶</td>
<td>-</td>
</tr>
<tr>
<td><strong>WWTP¹</strong></td>
<td>Turkey</td>
<td>Gas</td>
<td>b.d.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Gas</td>
<td>0.017-446.9</td>
<td>0.008-8.7</td>
</tr>
<tr>
<td>Pumping station</td>
<td>Greece</td>
<td>Gas</td>
<td>2125-40982</td>
<td>344-1046</td>
</tr>
<tr>
<td><strong>WWTPs</strong></td>
<td>Greece</td>
<td>Gas</td>
<td>1.5-36429</td>
<td>b.d.-1001</td>
</tr>
<tr>
<td>Sewers</td>
<td>Australia</td>
<td>Gas</td>
<td>2.5-55318.3</td>
<td>1.8-11380.2</td>
</tr>
<tr>
<td>Pumping station²</td>
<td>Sweden</td>
<td>Gas</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>WWTP</td>
<td>Sweden</td>
<td>Gas</td>
<td>b.d.-12</td>
<td>b.d.-72</td>
</tr>
<tr>
<td>WWTP</td>
<td>USA</td>
<td>Water</td>
<td>&lt;1000-3290</td>
<td>30-66</td>
</tr>
<tr>
<td>WWTP</td>
<td>Japan</td>
<td>Water</td>
<td>0.0011-71</td>
<td>0.0017-332</td>
</tr>
<tr>
<td>WWTP</td>
<td>Spain</td>
<td>Water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a. Creeks were operated as open sewers.
b. Samples were taken from sludge bed and equalization tank.
c. The pumping station was equipped with a compact biofilter to treat the gas streams vented from the station and samples were taken from the effluent of the biofilter.
b.d: below detection limit; -: data not available.
2.3.2 Detection of VOSCs

The detection of VOSCs is challenging due to their highly reactive nature and their low concentrations in environmental systems (Wardencki 1998). After the measurement of VOSCs in different environmental matrices gas chromatography (GC) based analysis is believed to be the most widely accepted approach (Wardencki 1998, Pandey and Kim 2009). Figure 2-3 depicts the frequently used GC-based methods for detection of VOSCs in gas and liquid samples.

Figure 2-4. The most frequently applied methods for determination of volatile sulfur compounds in gases and liquids, adapted from Wardencki (1998) and Pandey and Kim (2009).

Direct analysis of VOSCs is preferred, as opposed to sample pretreatment, as this minimized compound loss and shortens the analysis time (Pandey and Kim 2008). However, due to their low concentrations samples pretreatment is often required (Pandey and Kim 2009). For gas samples, the most frequently used pre-concentration techniques are: (1) sorption onto certain metal surfaces; (2) trapping onto solid sorbents; (3) and cryogenic trapping. These pre-concentration methods are often followed by a thermal desorption process and the desorbed concentrated compounds are then
applied for GC analysis. In recent years, solid-phase microextraction (SPME) is also used as a potential solvent-free sample preparation technique, which allows a single step treatment for isolation and concentrating the compound of interest (Demeestere et al. 2007).

Pretreatment of liquid samples, by liquid or gas extraction techniques, is also required for measurement of VOSCs. The common liquid solvents used for VOSCs extraction are diethyl ether, hexane or mixtures of these compounds. However, liquid extractions are not frequently used due to disadvantages of handling toxic solvents and that they are time consuming to perform (Wardencki 1998). Gas extraction procedures can be divided into static and dynamic methods. The static method detects the VOSCs from liquid or solid samples by analyzing the gas phase that is in thermodynamic equilibrium with the sample in a closed system. This method has been applied successfully for analysis of VOSCs in different matrices (Nedjma and Maujean 1995, Ojala et al. 1997, Mendes et al. 2000). Among the dynamic gas extraction methods, the purge and trap (PT) technique has been extensively applied for pre-concentration of volatile compounds from liquid matrices (Abeel et al. 1994). The PT technique has two steps, the first step is to strip the analytes from the aqueous phase and the second step is to trap the swept compounds to sorbents, cryotraps or to a GC column as per-concentration. As the PT technique can effectively lower the detection limit, this method is extensively used to determine VOSCs in different liquid matrices (Andreae and Barnard 1984, Holdway and Nriagu 1988, Ridgeway et al. 1991, Shooter et al. 1992, Simó et al. 1996).

Table 2-5. Characteristics of gas chromatographic sulfur-sensitive detectors, compiled according to Wardencki (1998) and Firor and Quimby (2001).

<table>
<thead>
<tr>
<th>Detector</th>
<th>Detect limits (pg/s)</th>
<th>Selectivity (S/C)</th>
<th>Linear concentration range</th>
<th>Ease of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPD</td>
<td>20</td>
<td>$10^5$</td>
<td>$10^3$</td>
<td>2</td>
</tr>
<tr>
<td>PFPD</td>
<td>1</td>
<td>$10^6$</td>
<td>$10^3$</td>
<td>2</td>
</tr>
<tr>
<td>MS</td>
<td>10</td>
<td>Specific</td>
<td>$10^5$</td>
<td>4</td>
</tr>
<tr>
<td>AED</td>
<td>2</td>
<td>$10^5$</td>
<td>$10^4$</td>
<td>3</td>
</tr>
<tr>
<td>SCD</td>
<td>0.5</td>
<td>$10^6$</td>
<td>$10^5$</td>
<td>1</td>
</tr>
</tbody>
</table>

The sulfur sensitive detector which is usually used with GC for VOSC measurement includes flame photometric detector (FPD), pulsed flame photometric detector (PFPD), mass spectrometer (MS), atomic emission detector (AED), and sulfur chemiluminescence detector (SCD) (Pandey and Kim 2009). The characteristics of these detectors are compared in Table 2-5.
2.3.3 Microbial transformation of VOSC

Microbial transformation pathways of VOSCs in sewer systems are yet to be studied. This section reviews the microbial production and degradation of VOSCs, mainly MT and DMS, in other reported environmental systems.

2.3.3.1 VOSC production

(1) Cleavage from sulfur-containing amino acids and derivatives

MT and DMS can be generated by the cleavage of sulphur-containing amino acids and their derivatives. Methionine, S-methyl-cysteine and S-methyl-methionine are three typical substrates for MT and DMS production through this pathway. The breakdown of methionine is commonly catalyzed by Methionine γ-lyase forming MT, ammonia and alpha-ketobutyric acid (Equation (2-2)) (Lomans et al. 2002b, Bentley and Chasteen 2004). The degradation of S-methyl-cysteine can be catalyzed by S-alkylcysteine lyase or Methionine γ-lyase and this produces MT, ammonia and pyruvic acid (Equation (2-3)) (Kadota and Ishida 1972, Bentley and Chasteen 2004).

\[
\begin{align*}
\text{CH}_3\text{S-CH}_2\text{CH}_2\text{COOH} & \rightarrow \text{CH}_3\text{S} + \text{CH}_3\text{COOH} + \text{NH}_3 & \text{Equation (2-2)} \\
\text{CH}_3\text{S-CH}_2\text{COOH} & \rightarrow \text{CH}_3\text{SH} + \text{CH}_3\text{COOH} + \text{NH}_3 & \text{Equation (2-3)} 
\end{align*}
\]

S-methyl-methionine (SMM) is another precursor of VOSCs. This can be decomposed to DMS and homoserine or lactone both with and without enzymatic activity. It is suggested that SMM is a significant precursor of DMS in terrestrial regions (Taylor and Kiene 1989). (+)-S-methyl-L-cysteine sulfoxide, another derivative of amino acids, can firstly form the unstable methanesulfeic acid, CH₃-S-OH and then generate MT or DMDS under different conditions (Tulio et al. 2002).

(2) Methylation of sulfide or thiols

Methylation of sulfide and thiols is widespread and has been detected in animals, various microorganisms and plants (Taylor and Kiene 1989). The methyl group of S-adenosyl methionine (AdoMet) is known to transfer to sulfide, MT and other thiols. This is catalyzed by thiol S-methyltransferases producing S-Adenosyl-L-homocysteine (AdoHcy) (Bentley and Chasteen 2004):

\[
\begin{align*}
\text{AdoMet} + \text{SH} & \rightarrow \text{AdoHcy} + \text{MT} & \text{Equation (2-4)} \\
\text{AdoMet} + \text{MT} & \rightarrow \text{AdoHcy} + \text{DMS} & \text{Equation (2-5)} 
\end{align*}
\]

Drotar et al. (1987) demonstrated the widespread occurrence of AdoMet-dependent thiol methyltransferase activities among aerobic bacteria, consequently, this could be a mechanism of
MT production by these bacteria when exposed to H₂S. However, this pathway has not been observed in obligate anaerobic bacteria (Larsen 1985).

Methoxylated aromatics are another possible source of methyl donors for VOSC formation. Various anaerobic bacteria, such as *Holophaga foetida*, *Sporobacter termitidis*, *Sporobacterium olearium*, Strain SA2 and *Parasporobacterium paucivorans*, can perform sulfide-mediated O-demethoxylation where the resulting methyl groups are transferred to H₂S or MT, forming MT or DMS, respectively. (Lomans et al. 2002b):

\[
\text{R-O-CH}_3 + \text{H}_2\text{S} \rightarrow \text{R-OH} + \text{CH}_3\text{-SH} \quad \text{Equation (2-6)}
\]
\[
\text{R-O-CH}_3 + \text{CH}_3\text{-SH} \rightarrow \text{R-OH} + \text{CH}_3\text{-S-CH}_3 \quad \text{Equation (2-7)}
\]

As methoxylated aromatic compounds are readily degraded from lignin, which is a very abundant biopolymer on earth, this mechanism for MT and DMS formation is significant in freshwater systems. As the degradation of lignin is an aerobic process, this pathway is likely to occur at an anaerobic/aerobic interphase (Lomans et al. 2002b).

(3) Conversion from DMSP

Dimethylsulfoniopropionate (DMSP) is an osmolyte common in marine algae, dinoflagellates, coccolithophores and halophilic plants species. It is considered a major precursor of DMS in marine, estuarine and salt marsh systems (Lomans et al. 2002b). However, it is not widely distributed in terrestrial plants, except in sugar cane, in the salt-tolerant, coastal strand plant *Wollastonia biflora* and in the intertidal *Spartina* species (Chasteen and Bentley 2004). DMSP can be degraded directly to DMS and acrylate or be firstly transformed to 3-methylthio-propionate and then eliminated or reduced to MT (Taylor and Visscher 1996).

(4) Conversion from DMSO and DMSO₂

Dimethyl sulfoxide (DMSO) is used industrially as a solvent and a waste product from paper mills (Bentley and Chasteen 2004). It is also found in surface ocean water, where it probably originates from marine phytoplankton (Andreae 1980). DMSO can be reduced to DMS by bacterial DMSO reductases that require a molybdenum cofactor. The DMSO reductase is found in several bacteria including *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* and *E.coli* (Kisker et al. 1998).

Dimethyl sulfone (DMSO₂) is produced in the atmosphere and can also be reduced to DMS by bacteria utilising NADH-dependent dimethylsulfoxide and dimethylsulfone reductase activities.
This pathway has been demonstrated in facultatively methylotrophic strains of *Hyphomictobium* and *Arthrobacter* (Borodina et al. 2000).

### (5) Other pathways

Apart from the major precursors for the formation of MT and DMS mentioned above, there are a few other substances of limited distribution that are potential precursors of DMS. This includes 4-dimethylsulfonio-2-methoxybutyrate and 5-dimethylsulfonio-4-hydroxy-2-aminopentanoate from red algae, and 5-dimethylsulfonio-3-hydroxypentanoate (gonyol) from the marine dinoflagellate *Gonyaulax poledra* (Howard and Russell 1996). Additionally, Lin et al. (2010) reported a possible pathway for the microbial conversion of inorganic carbon to dimethyl sulfide. They observed the formation of DMS through the fixation of bicarbonate, via a reductive pathway in analogy to methanogenesis, causing the methylation of MT. Finally, DMDS is believed be mainly formed by MT chemical oxidation. However, the reduction of DMDS has been observed to cause MT formation (Kiene et al. 1986).

#### 2.3.3.2 VOSC degradation

**1) Oxidation by aerobic microorganisms**

DMS and MT are oxidized by aerobic microorganisms mainly belonging to the genera *Hyphomicrobium* and *Thiobacillus*. (Lomans et al. 2002b, Chasteen and Bentley 2004). These microorganisms are isolated from different environmental systems such as the marine sediments, wastewater treatment plant sludge, soil and biofilters (Lomans et al. 2002b).

The pathway for DMS oxidation by *Hyphomicrobium* is well elucidated by De Bont et al. (1981). In this process, DMS is oxidized by a monoxygenase to MT and formaldehyde. The MT is then subsequently oxidized by MT oxidase to formaldehyde, sulfide and hydrogen peroxide. The sulfide is finally oxidized to sulfuric acid. The formaldehyde is partly assimilated to the cell by the serine pathway and partly oxidized to formic acid and then to carbon dioxide by the catalysis of formaldehyde dehydrogenase. *Hyphomicrobium* is therefore known as a C1-compound-metabolizing microorganism.

*Thiobacilli* are generally known as chemolithoautotrophs and use the Calvin cycle to fix carbon. However, some species are also able to oxidize VOSCs (Smith and Kelly 1988, Cho et al. 1991, Visscher and Taylor 1993b). The oxidation of VOSCs results in the formation of sulfide and formaldehyde, which are further oxidized to sulfuric acid and carbon dioxide.
Methylophaga sulfidovorans is an obligate methylotrophic organism isolated from tidal sediments which is also found to be capable of DMS degradation. In this instance the DMS is oxidized to thiosulfate and carbon dioxide and the carbon is assimilated by Methylophaga sulfidovorans through the formaldehyde assimilation pathway. Consequently, this is also considered a C1-compound-metabolizing microorganism (de Zwart et al. 1996).

(2) Degradation through methanogenesis

Methanogenic conversion of MT and DMS was firstly found by Zinder et al. (1978) in a freshwater sediment. Methanogens can reduce DMS or MT to methane, sulfide and bicarbonate (or carbon dioxide) through the following reactions (Kiene et al. 1986, Finster et al. 1992):

\[
\text{CH}_3\text{SH} + 0.75 \text{H}_2\text{O} \rightarrow 0.75 \text{CH}_4 + 0.25 \text{HCO}_3^- + \text{HS}^- + 1.25 \text{H}^+ \quad \text{Equation (2-8)}
\]

\[
(\text{CH}_3)_2\text{S} + \text{H}_2\text{O} \rightarrow 1.5\text{CH}_4 + 0.5 \text{HCO}_3^- + \text{HS}^- + 1.5 \text{H}^+ \quad \text{Equation (2-9)}
\]

Methanogenic MT degradation has also been detected in other environments, such as marine sediment (Kiene et al. 1986, Finster et al. 1990, Visscher et al. 1995), salt marsh sediments (Kiene et al. 1986, Kiene and Capone 1988), and anaerobically digested biosolids (Zitomer and Speece 1995, Chen et al. 2005, Higgins et al. 2006, van Leerdam et al. 2006). MT-utilizing methanogens isolated from these systems primarily belong to the genera of Methanolobus, Methanosarcina, Methanosalsus and Methanomethylovorans (Lomans et al. 2002b, Jiang et al. 2005, Cha et al. 2013). However, only MT-utilizing methanogens of the genus Methanomethylovorans are isolated from freshwater environments while others are all from saline-water environments. Lomans et al. (2002a) suggest that in anaerobic freshwater conditions, DMS and MT are mainly degraded by methanogenic achaea.

(3) Degradation through sulfate reduction

Many studies reveal the possible role of sulfate-reducing bacteria in the degradation of DMS and MT. These revelations were made on mixed microbial anaerobic communities after the inhibition methanogens (Kiene and Visscher 1987, Lomans et al. 1999c, van Leerdam et al. 2006). However, so far, there are only two studies that report successful isolation of SRB pure cultures that can degrade DMS and MT. Three strains were isolated from a thermophilic digester, and these are likely of the genus of Desulfotomaculum (Tanimoto and Bak 1994). Another strain was isolated from mangrove sediments in the Netherlands, and this is grouped within the genus of Desulfosarcina (Lyimo et al. 2009). Equation (2-10) and Equation (2-11) describe the degradation of MT and DMS by SRB.
Isolation of these degraders is extremely useful for further studies to understand details of VOSC degradation. However, poor success of microbial isolations is typical when attempting to obtain pure cultures from environmental samples. Many factors, such as suboptimal culture conditions, will greatly influence the success of these attempts. However, it is also possible that the contribution of SRB to the MT and DMS degradation occurs by co-metabolism or by a facultative syntrophic metabolism between methanogens and SRB. In the latter case SRB withdraw reducing equivalents, most likely H₂, and this enables the DMS-degrading methanogens to oxidize the MT or DMS to carbon dioxide and H₂S (Lomans et al. 1999c).

(4) Degradation through denitrification

Degradation of VOSCs by denitrification has been detected. Lomans et al. (1999c) observed the complete degradation of DMS in nitrate-amended freshwater sediment slurries after a long incubation period. In that case the responsible organisms were not further studied. So far, only one denitrifier pure culture that can degrade DMS with nitrate or nitrite as the electron acceptor has been isolated from marine sediments (Visscher and Taylor 1993a). Although, a pure culture of SRB that can reduce nitrate to ammonia and degrade DMS to H₂S has been obtained (Tanimoto and Bak 1994).

(5) Degradation by anoxygenic phototrophs

Some anoxygenic phototrophic sulfur bacteria, which normally use sulfide as an electron donor under anaerobic conditions, can also use DMS as an electron donor forming DMSO. This mechanism for DMS degradation is believed very important in microbial mats of intertidal sediments where light is abundant during low tide (Lomans et al. 2002b).

2.4 Changing urban water management practices

The global water crisis is one of the greatest human development challenges in early 21st century (HDR 2006). A large proportion of the world’s population is confronted with water scarcity, a problem that is exacerbating due to climate change and increasing human activities (Vörösmarty et al. 2000, HDR 2006, Jury and Vaux Jr 2007). Climate change results in more frequent and severe drought periods caused by reduced rainfall and enhanced evapotranspiration (Frederick 1997, IPCC 2007). Meanwhile, the increasing water demand caused by population growth, urbanization and
industrialization also poses serious water stress to human beings. Apart from this water shortage problem, water pollution caused by anthropologic activities also aggravates the global water crisis. Due to lack of suitable water treatment or sanitation systems, currently more than one third of the world’s population is facing a lack of safe drinking water (Schwarzenbach et al. 2010). As a result, many countries are changing their water management practices to cope with the crisis. Two commonly used new practices include water demand management and decentralized water management. A brief introduction of these practices follows.

2.4.1 Water demand management
Water demand management is an important strategy to meet water supply and demand balance by reducing the water consumption rate. This is to be brought about through implementation of a series of financial, operational and socio-political policies and regulations (Tate 1990, White and Fane 2002). For instance, penalties or a higher price would be charged for excessive use of water while rewards would be offered to households using high water efficient taps, toilets and appliances. In addition, the detection and repair of water leakage problems would be a priority of water utilities. Moreover, public educational programs would be implemented. As a result of such strategies the residential water consumption in many cities in Australia, France, Canada, Jordan and other countries has been reduced by around 20% to 40% (Kenway et al. 2008, Willis et al. 2009, Marleni et al. 2012). The South East Queensland region (including Brisbane and three other cities/regions) is a good example. Following a long-lasting drought period, starting in 2000, water restrictions were initiated in 2007 which resulted in a regional reduction of domestic water consumption from 282 L per person per day in 2005 to 163 L per person per day in 2011 (QWC 2011).

However, reduced water consumption (RWC) changes both the composition and flow of wastewater discharged to sewer systems. In comparisons of wastewater characteristics before and after RWC, several studies reported increases in total suspended solid (TSS), COD and biological oxygen demand (BOD) (Dezellar and Maier 1980, Parkinson et al. 2005, Sharma et al. 2005, Cook et al. 2010, Min and Yeats 2011). Some of these studies also suggested there could be an increase in sulfate, metal and nitrogen concentrations. This implies that more concentrated wastewater is discharged to sewers following the implementation of RWC. In addition, the reduced wastewater volume would cause reduced flow of sewage thereby resulting in a longer HRT of wastewater in sewers (Zornes et al. 2011).
2.4.2 Decentralized water management

The conventional centralized urban water systems are facing unprecedented challenges from emerging issues that include population growth, aging infrastructure, urbanization and resource constraints. As a result, in many countries like USA, Australia and Japan, decentralized water and wastewater systems are being promoted either in combination with centralized systems; or alone as sustainable solutions for urban water services (Gikas and Tchobanoglous 2009, Sharma et al. 2010). Decentralized systems can be defined as those providing water, wastewater and stormwater services at the allotment, cluster and development scale based on a ‘fit for purpose’ concept (Cook et al. 2009). These systems could potentially increase flexibility and reduce energy consumption for water management and lower the costs of infrastructure replacement as well. With decentralized wastewater treatment, the need to maintain expensive sewer infrastructure can be reduced and non-potable water reuse can be practiced with fewer risks of cross connections (Hering et al. 2013). For drinking water supply, decentralized solutions have usually been considered viable only for small service areas. Further development of reliable real-time monitoring systems and successful demonstration projects are needed before decentralized systems can be expanded to large regions (Hering et al. 2013).

Two major practices that are a part of decentralized water management are water recycling and rainwater harvesting. Water recycling provides an additional water source to satisfy the increasing water demand and consequently decrease the diversion of water from sensitive ecosystems (Anderson 2003). Greywater (wastewater from domestic applications other than the toilet) is another good resource for reuse purposes. After proper treatment greywater is normally suitable for non-potable use, such as gardening, car washing and toilet flushing (Marleni et al. 2012). The recycled wastewater can also be used for industrial purposes. For example, in Australia, a 14000 m³/d dual membrane water reclamation plant has been installed at the Luggage Point sewage treatment plant in Brisbane to supply process water to an oil refinery (Anderson 2003). In addition, rainwater harvesting is another alternative water source that can be utilized to overcome water supply problems. Rainwater harvesting refers to the immediate collection and storage of rainwater from rooftops, and to less extent from ground surface or rock catchments as a water supply source (WaterAid 2013). It is a sustainable practice that supplies water with less cost and energy and is simple in installation and operation (Marleni et al. 2012). Due to recent prolonged droughts, rainwater harvesting recently boomed in many countries.

The operation of decentralized systems could generate some waste products such as waste activated sludge or coagulation sludge. These sludges are generated during practices for the removal of COD
or natural organic material (NOM). Unlike centralized systems, which often contain sludge treatment processes, due to relatively small scale of the decentralized systems, these waste sludges are usually dumped into the sewer systems directly. Since these sludges can be high in organic matter or metals, the placement of the sludges into the sewer may affect in-sewer processes and sewer emissions (Zhang et al. 2011).
Chapter 3 Research Objectives

3.1 Research Objective I

Understanding the effect of reduced water consumption on sulfide and methane production in rising main sewers.

RWC is expected to be increasingly applied in future for the conservation of global water resources. However, as it could lead to more concentrated wastewater with longer HRT in sewers, its unintended impact on sulfide and methane production in sewers needs to be assessed. So far, few studies have been carried out to identify this impact (Marleni et al. 2012). Detailed studies on such effects will not only help the water industry to develop its sewer maintenance strategies in future but also provide information towards evaluating greenhouse gas emissions by sewer systems.

Therefore, the first research objective of this thesis is to understand the effect of RWC on sulfide and methane production in rising main sewers. For this purpose, sulfide and methane concentrations and production rates under normal and RWC conditions are compared. The impacts of RWC on sulfide and methane emissions and the chemical requirements for the mitigation of sulfide are also investigated. In addition, the microbial structure of SRB and MA in the sewer biofilms under RWC conditions was also investigated by multiple approaches, including microelectrode measurements, molecular techniques and mathematical modeling.

3.2 Research Objective II

Understanding the effect of iron-rich coagulation sludge discharged from decentralized systems on sulfide and methane production in sewers.

Coagulation process can be used in decentralized drinking water or water recycling systems to remove natural organic material (NOM), colour and turbidity (Butler and MacCormick 1996). During the coagulation process, a great amount of sludge can be produced. Generally speaking, the sludge treatment would not be included in the decentralized systems since these systems are in relatively small scale. As a result, the coagulation sludge is usually dumped into sewer systems directly. However, the effect of the sludge discharge on sewer processes is unclear.
In particular, iron salts, such as ferric chloride or ferrous chloride, are commonly used coagulants (Henderson et al. 2009). With the dosage of iron salts, the coagulation sludge would contain a high concentration of iron. Previous studies showed that iron salts can be used for sulfide mitigation in sewer systems (Zhang et al. 2008, Zhang et al. 2010). Therefore, the second research objective of this thesis is to understand the effects of iron-rich coagulation sludge on sulfide and methane production in sewer systems. The sulfide and methane concentrations in lab-scale sewer reactors with iron sludge dosing at different dosing rates were compared to the case without dosing. Mathematical modelling is also applied to investigate the reaction between the sludge and sulfide.

3.3 Research Objective III

Understanding the transformation of typical VOSCs in anaerobic sewers under different sewer conditions.

VOSCs are potential odorants in sewer systems but attracted limited attention in the past. With the changing of urban water management practices, the sewer conditions could also be changed accordingly. It might consequently affect the VOSCs transformation in sewer systems. Therefore, the third research objective of this thesis is to understand the transformation of typical VOSCs in anaerobic sewers under different sewer conditions.

To conduct this study, a reliable method for measuring VOSCs in wastewater is necessary. The current methods for VOSCs measurement in wastewater system often require pre-concentration processes before the analysis due to low concentration in the samples and high detection limit of the equipment. However, the pre-concentration processes are time-consuming and can cause potential sample loss. Therefore, the first part of this research objective is to develop an efficient and reliable method to measure VOSCs in wastewater matrices. The error-prone pre-concentration processes can be eliminated in the method and consequently the measurement time be shortened.

Base on this newly developed method, the degradation of MT, a typical VOSC causing sewer odor, were investigated under different sewer conditions. The pathway and kinetics of MT degradation in anaerobic sewer systems are explored through laboratory experimental study and mathematical modeling.
Chapter 4 Impact of reduced water consumption on sulfide and methane production in rising main sewers

4.1 Abstract
Reduced water consumption (RWC), for water conservation purposes, is expected to change the wastewater composition and flow conditions in sewer networks and affect the in-sewer transformation processes. In this study, the impact of reduced water consumption on sulfide and methane production in rising main sewers was investigated. Two lab-scale rising main sewer systems fed with wastewater of different strength and flow rates were operated to mimic sewers under normal and RWC conditions (water consumption reduced by 40%). Sulfide concentration under the RWC condition increased by 0.7 - 8.0 mg S/L, depending on the time of a day. Batch test results showed that the RWC did not change the sulfate-reducing activity of sewer biofilms, the increased sulfide production being mainly due to longer hydraulic retention time (HRT). pH in the RWC system was about 0.2 units lower than that in the normal system, indicating that more sulfide would be in molecular form under the RWC condition, which would result in increased sulfide emission to the atmosphere as confirmed by the model simulation. Model based analysis showed that the cost for chemical dosage for sulfide mitigation would increase significantly per unit volume of sewage, although the total cost would decrease due to a lower sewage flow. The dissolved methane concentration under the RWC condition was over two times higher than that under the normal flow condition and the total methane discharge was about 1.5 times higher, which would potentially result in higher greenhouse gas emissions. Batch tests showed that the methanogenic activity of sewer biofilms increased under the RWC condition, which along with the longer HRT, led to increased methane production.

4.2 Introduction
Nowadays, a large proportion of the world’s population is confronted with water shortage, which would be generally attributed to two main reasons: climate change and increasing human activities (Vörösmarty et al. 2000, HDR 2006, Jury and Vaux Jr 2007). Climate change results in more frequent and severe drought periods caused by reduced rainfall and enhanced evapotranspiration (Frederick 1997, IPCC 2007). Meanwhile, the increasing water demand caused by population growth, urbanization and industrialization also poses serious water stress to human beings. The Human Development Report (2006) estimated that, by 2025, more than 3 billion people would be living in water-stressed countries and the number would further increase to more than 5 billion in 2050.
To cope with this problem, many countries have tried to reduce water consumption by implementing a series of financial, operational and socio-political policies and regulations (Tate 1990, White and Fane 2002). As a result, residential water consumption in many cities in Australia, France, Canada, Jordan and other countries has been reduced by around 20% to 40% (Kenway et al. 2008, Willis et al. 2009, Marleni et al. 2012). The South East Queensland region (including Brisbane and three other cities/regions) is a good example. Following a long-lasting draught period from 2000, water restriction was initiated in 2007, which resulted in the reduction of domestic water consumption in the region from 282 L per person per day in 2005 to 163 L per person per day in 2011 (QWC 2011).

Reduced water consumption (RWC) changes both the composition and flow of wastewater discharged to sewer systems. Comparing wastewater characteristics before and after RWC, several studies (Dezellar and Maier 1980, Parkinson et al. 2005, Sharma et al. 2005, Cook et al. 2010, Min and Yeats 2011) reported an increased total suspended solid (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD) with RWC. Some of these studies also suggested there could be an increase in sulfate, metal (Fe, Cu, Zn) and nitrogen (total Kjeldahl nitrogen (TKN) and ammonium) concentrations. This implies that more concentrated wastewater is discharged to sewers with RWC. In addition, reduced wastewater discharge would cause reduced flow rate thereby resulting in a longer HRT of wastewater in sewers (Zornes et al. 2011). All these changes could have impacts on the in-sewer biotransformation processes and might aggravate the problems related to sewer systems (Zornes et al. 2011, Marleni et al. 2012).

Sulfide production and emission is a well-known problem in sewers, which causes corrosion of sewer pipes, odor nuisance and health hazards (Boon 1995, Hvitved-Jacobsen 2002). In sewers, sulfide is mainly generated anaerobically by the reduction of sulfate in wastewater through the respiration of sulfate-reducing bacteria (SRB). Rising main (also called pressurized mains) sewers are usually operated with full wastewater flows in the absence of oxygenation and thus contribute considerably to sulfide production. Sulfate concentration, COD concentration and HRT are among the key factors identified to influence sulfide concentration in sewers (Hvitved-Jacobsen 2002, Freudenthal et al. 2005, Sharma et al. 2008b, Mohanakrishnan et al. 2009b), with higher sulfate and COD concentrations and longer HRT favoring higher sulfide production.

Methane has also been observed recently generated in rising main sewers with significant amount (Guisasola et al. 2008, Foley et al. 2009). Methane is a potent greenhouse gas with a life span about 12 years and a global warming potential of 21–23 times that of carbon dioxide (IPCC 2006).
Methane production in sewers could contribute considerably to the overall greenhouse gas emissions from wastewater systems (Guisasola et al. 2008). Meanwhile, due to the relatively low explosive limit of methane (down to 5%), the release of methane from sewer systems imposes potential health and safety risks (Spencer et al. 2006, Guisasola et al. 2009, GWRC 2011). In addition, the loss of soluble COD by methanogenesis could cause detrimental impacts on biological nutrient removal at the downstream wastewater treatment plants. Guisasola et al. (2009) revealed that methane production in sewers depends heavily on the soluble COD concentration and the HRT of the wastewater, both of which positively correlate with methane concentration in sewers.

RWC is expected to be increasigly applied in future for the conservation of global water resources. However, as it could lead to more concentrated wastewater with longer HRT in sewers, its unintended potential impact on sulfide and methane production in sewers needs to be assessed. So far, few studies have been carried out to identify this impact (Marleni et al. 2012). Detailed studies on the effect of RWC would not only help the water industry to develop its sewer maintenance strategies in future but also provide information towards evaluating greenhouse gas emissions by sewer systems.

For this purpose, two lab-scale rising main systems were set up to mimic sewers under normal and RWC conditions. Domestic wastewater with different flow rates and strength were fed to the two systems. Sulfide and methane production in the two systems was investigated through both long-term performance monitoring and batch tests. A mathematical sewer model (Sharma et al. 2008a, Sharma et al. 2008b) was set up and run for the two sewer conditions to evaluate the impacts of RWC on sulfide and methane emissions and on the chemical requirements for mitigation of sulfide.

4.3 Materials and Methods
4.3.1 Wastewater composition
Two laboratory-scale rising main sewer systems were set up to mimic two sewer lines operated under normal and RWC conditions. The two systems were fed with domestic wastewater of different strength and at different flow rates. Statistics provided by water commissions revealed that, water consumption in Brisbane was approximately 150L/capita/day at the time of this experimental study (2011), a legacy of 10-year drought (2000 - 2010) and water restrictions. It was much lower than the consumption rates in many other cities and regions all over the world. For example, the water consumption rate in Sydney, Australia was 210L/capita/day and in the USA and Canada, it was around 350 L/cap/day (Marleni et al. 2012). In some cities in China, the water consumption rate could even reach up to about 450L/cap/day (China statistical yearbook 2011). Thus, in this study,
domestic wastewater collected in Brisbane was used to represent wastewater under the RWC condition (referred to as ‘concentrated wastewater’) while wastewater under the normal water consumption condition (referred to as ‘normal wastewater’) was obtained by diluting the Brisbane wastewater with tap water by 40%, mimicking wastewater in Sydney and other cities with a water consumption rate that is 40% higher. Mimicking the ‘normal wastewater’ by diluting ‘concentrated wastewater’ with tap water is a suitable approach as reduced water consumption is realized by decreasing the use of tap water.

4.3.2 Laboratory reactors set-up and operation

Figure 4-1(A) shows the schematic representation of the two laboratory sewer systems. Each consisted of three 1 L gas-tight reactors, made of Perspex™, connected in series. The system under the RWC condition, receiving ‘concentrated wastewater’ was named as the ‘reduced flow line’ (reactors labeled with ‘RL1’, ‘RL2’ and ‘RL3’, respectively). The system under the normal condition, receiving ‘normal wastewater’ was named as ‘normal flow line’ (reactors labeled with ‘NL1’, ‘NL2’ and ‘NL3’, respectively). The arrangement of connecting reactors in series was made to simulate possible spatial variation of sewer biofilms along a sewer line, with each reactor representing a section of a sewer pipe from upstream to downstream (Gutierrez et al. 2008). The inner diameter of each cylindrical reactor was 80 mm and the area to volume ratio (A/V) was calculated to be 55 m⁻¹, with biofilms growing on the wall and top of the reactor considered. Mixing was continuously provided by a magnetic stirrer (Heidolph MR3000) under each reactor, so there was no biofilms growing on the bottom.

‘Concentrated wastewater’ was collected from a local wet well at the Robertson Park pump station (Brisbane, Queensland) on a weekly basis. Dissolved sulfur species (sulfide, sulfate, sulfite and thiosulfate), soluble chemical oxidation demand (COD), volatile fatty acids (VFAs), nitrogen species (nitrate, nitrite and ammonium) in the wastewater were monitored every week using the methods described in Section 4.3.5. The sewage typically contained sulfate at concentrations of 10-25 mg-S/L, sulfide at < 3mg S/L, soluble COD at 200-300 mg/L including 50-120 mg COD/L of VFAs and approximately 50 mg N/L of ammonium. Negligible amounts of sulfite, thiosulfate (<1 mg S/L), nitrate and nitrite (<1 mg N/L) were present. ‘Normal wastewater’ was obtained by diluting the “concentrated wastewater” with 40% of tap water as described in Section 4.3.1. The sulfate concentration of tap water was also monitored weekly, which was in the range of 5-18 mg S/L. The feeding to both lines was stored in a cold room (4°C) to minimize the biological transformation, but was heated to 20 ± 1°C when being pumped into the reactors (Figure 4-1).
Figure 4-1. (A)-Schematic representation of two laboratory-scale rising main sewer systems mimicking sewers under normal and RWC conditions. (B) Pumping pattern and HRT of two systems in an 8-hour period. The vertical solid lines refer to the pumping events in both the reduced flow line and the normal flow line, and dashed lines represent additional pumping events in the normal flow line. The solid and empty dots represent the HRT of the corresponding wastewater slug in the reduced flow line and in the normal line, respectively.

The ‘concentrated’ and ‘normal’ wastewater was pumped into two sewer lines with two intermittently-operated peristaltic pumps (Masterflex 7520-47). For easier reactor monitoring (see Section 4.3.3), each day was divided into three identical 8-hour periods. Figure 4-1(B) shows the pumping patterns applied to the two lines over an 8-hour period and the HRT of sewage in the two
lines, respectively. During each pumping event, wastewater was pumped into the first reactor with a flow rate of 0.5 L/min. The ‘reduced flow line’ had 6 pumping events and each lasted for 2 min in an 8-hour period while the ‘normal flow line’ had 8 pumping events and each lasted for 2.1 min so that the ‘normal flow line’ received 40% more wastewater flow. The duration between pumping events ranged between 15 minutes to 3 hours, and consequently, the HRT in the ‘reduced flow line’ and in the ‘normal flow line’ varied between 2 to 6 hours and 1.19 to 5 hours, respectively. These ranges are similar to those observed in real sewer pipes (Guisasola et al. 2008). Two lines were operated for 6 months to reach pseudo steady-state conditions as indicated by the relatively constant sulfide and methane production rates, before the detailed monitoring described below commenced.

4.3.3 Performance monitoring of the two lines
The long-term effects of RWC on sulfide and methane production in sewers were evaluated by monitoring sulfide and methane profiles in all six reactors fortnightly over a period of 3 months. Sulfide concentration was measured online using the S::CAN VU-VIS spectrolyser (Messtechnik GmbH, Austria), as previously described by Sutherland-Stacey et al. (2008). The liquid in the reactor was continuously diverted to the spectrometer optics of the sensor by a peristaltic pump (Masterflex 7520-47) through a bypass system. The sensor was calibrated regularly (every 7 - 10 days), as previously described in Sutherland-Stacey et al. (2008), by collecting liquid samples from reactors and conducting offline dissolved sulfide analysis using ion chromatography (see Section 4.3.5). Methane concentration was monitored by taking samples from all reactors for measurement with gas chromatography (see Section 4.3.5) at the beginning and the end of each pumping cycle.

4.3.4 Batch tests to determine sulfate-reducing and methanogenic activities
Batch tests were carried out to determine the sulfate-reducing and methanogenic activities of the biofilms in each reactor of both lines every 2 – 3 weeks for 3 month when the reactors reached pseudo steady state. At the beginning of each batch test, fresh sewage was pumped through the reactor for 10 minutes to ensure complete replacement of liquid in the reactor. After feeding, sulfide concentration was measured with the S::CAN VU-VIS spectrolyser continuously for one hour. Wastewater samples were taken at 0, 20, 40, 60 minutes after feeding for the analysis of methane. Sulfate-reducing activity was measured as sulfide production rate (SPR) (sulfate reducing rate and sulfide production rate were found to be very close under anaerobic conditions), and the methanogenic activity was measured as the methane production rate (MPR). SPR and MPR were calculated based on linear regression of sulfide and methane concentrations, respectively.
4.3.5 Analytical methods

For dissolved sulfur species (sulfide, sulfate, sulfite and thiosulfate) analysis, 1.5 ml filtered (0.22 μm membrane) wastewater was immediately preserved in 0.5ml sulfide antioxidant buffer (SAOB) (Keller-Lehmann et al. 2006). Samples were then analyzed in 24 hours on an ion chromatograph (IC) with a UV and conductivity detector (Dionex ICS-2000), as previously described in (Keller-Lehmann et al. 2006). The protocol of methane analysis is as described by Guisosola et al. (2008). Briefly, 5 ml sewage was filtered with 0.22 μm membrane and injected into a 12 ml vacuumed Exetainer® vial with a hypodermic needle attached to a plastic syringe. The tubes were allowed to reach gas-liquid equilibrium overnight. Methane in the gas phase was measured by gas chromatography (Shimadzu GC-9A), equipped with a flame ionization detector (FID). Concentration of methane in the sewage sample was calculated using Henry’s Law by considering both liquid and gas phases. VFA concentration was measured with gas chromatography (PerkinElmer, Inc.). Nitrogen species (nitrate, nitrite and ammonium) were analyzed using a Lachat QuikChem 8000 (Milwaukee) flow injection analyzers (FIA). Total COD and soluble COD was measured using the colorimetric method described in APHA (1998) and pH was monitored using a pH electrode with a TPM-miniCHEM process monitor and controller.

4.3.6 Model-based assessment of sulfide and methane emission and sulfide mitigation strategies

The SeweX model, which describes both sulfide and methane production in sewers as described in Sharma et al. (2008b) and Guisasola et al. (2009), was employed to evaluate the impacts of RWC on sulfide and methane emissions and on chemical dosing as a sulfide mitigation strategy. The model was implemented in MATLAB®/Simulink®. UC09, a rising main of Gold Coast Water with a diameter of 150 mm and a length of 1086 m was used as a test case. Total daily flows under the normal and reduced water consumption conditions were 123 m³/day and 89 m³/day, respectively. Dynamic variation of flow under the two conditions is shown in Supporting Information (SI), Figure 4-S1. The wastewater compositions under normal and reduced water consumption conditions were designed the same as the lab-scale system as mentioned above. The key model parameters such as the areal biofilm sulfide and methane production rates were taken from the activity tests conducted in the laboratory. The activities (g/m³·h) were converted to the areal rates (mg/m²·h) by dividing the former with the A/V ratio (m⁻¹). Values previously calibrated for UC09 (Sharma et al. 2008a) were used for all the other parameters of the model. The sulfide and methane concentrations under normal and RWC conditions were simulated using these parameters and wastewater composition described in Section 4.3.2.
In order to demonstrate the impact of RWC on H$_2$S and methane emissions, profiles of the gas phase H$_2$S and methane concentration in the discharge manhole at the end of UC09 were obtained through simulation. The schematic diagram of the manhole was shown in Figure 4-S1. The manhole diameter and height were 1.05 m and 1.0 m, respectively. The outlet pipe is 0.30 m above the level of inlet pipe. The gas phase concentrations were calculated based on the mass transfer from liquid to gas phase and convective transportation of gaseous compounds by the airflow, as described by Equation 4-1 and Equation 4-2, respectively.

\[
r_{S,\text{release}} = V_w \frac{dS_{aq}}{dt} = -k_{La} \times (S_{aq} - S_{aq}^{*}) \times V_w
\]

\[
V_{\text{air}} \frac{dS_g}{dt} = r_{S,\text{release}} - Q_{V} \times S_g
\]

Where

- \( r_{S,\text{release}} \) is the rate of compound S emitted from water to the headspace;
- \( S_{aq} \) is the concentration of compound S dissolved in water;
- \( S_{aq}^{*} \) is the saturated concentration of compound S in water, calculated based on Henry’s Law;
- \( S_g \) is the concentration of compound S in the headspace;
- \( k_{La} \) is the mass transfer coefficient;
- \( V_w \) is water volume in the manhole;
- \( V_{\text{air}} \) is the headspace volume;
- \( Q_{V} \) is the ventilation rate.

The mass transfer coefficient (\( k_{La} \)) for estimating both H$_2$S and CH$_4$ emission rates was assumed to be 27/day, estimated based on \( k_{La} \) (4/day) for quiescent zone of wastewater tank reported in Foley et al. (2010). Specifically, the \( k_{La} \) of the wastewater tank was firstly converted to \( k_L \) by dividing the water depth in the tank. The \( k_L \) was then used to calculate the \( k_{La} \) of the manhole according to the dimension of the manhole.

To evaluate the effect of RWC on sulfide mitigation strategies, the dosage of four commonly used chemicals, namely oxygen, nitrate, ferric chloride and magnesium hydroxide (Ganigué et al. 2011) were compared in terms of the dosing requirements and the effectiveness on sulfide control by the model analysis. Oxygen and nitrate were dosed at 200 m from the end of the rising main while ferric chloride and magnesium hydroxide were added at the start of the rising main. Previous studies revealed that these are the most suitable dosing locations for the respective chemicals (Gutierrez et al. 2008, Gutierrez et al. 2009, Mohanakrishnan et al. 2009a, Zhang et al. 2009). The chemical and
biological reactions incurred by the addition of these chemicals were also modeled according to Sharma et al. (Sharma et al. 2008a) and de Haas et al. (2008).

4.4 Results

4.4.1 Effect of RWC on H$_2$S production in a rising main sewer

As an example, sulfide profiles of the corresponding reactors in the reduced flow and normal flow lines during an 8-hour cycle are compared in Figure 4-2 (A-C). In all reactors, sulfide concentration in the reduced flow line was higher than that in the normal flow line, with the difference varying from 0.68 to 7.90 mg S/L. Larger differences were observed between the upstream reactors (RL1 vs. NL1) after RL1 experienced extra pumping events, as these pumping events effectively decreased the HRT in RL1. At the downstream locations, the differences became smaller with the increase of HRT. Despite higher sulfide concentrations, the total sulfide discharge from the reduced flow line (329.3 mg S/day) was 14% lower than that from the normal flow line (391.5 mg S/day). This is a result of both low flow rate and high HRT in the reduced flow line. The daily total sulfide production in the reduced flow line (360 mg S/L) was about 20% lower than the normal flow line (450 mg S/L) due to the reduced flow rate. On the other hand, the conversion ratio of sulfate to sulfide in the reduced flow line (91%) was higher than the normal flow line (86%) due to the prolonged HRT.

pH values in the two lines were also monitored in the 8-hour pumping cycle (Figure 4-2, A-C). The average pH value in the discharge from the reduced flow line was $6.84 \pm 0.04$, which was about 0.2 units lower than that from the normal flow line ($7.03 \pm 0.06$). The lower pH would reduce H$_2$S ionization ($\text{H}_2\text{S} \rightarrow \text{H}^+ + \text{HS}^-$). As the first acid ionization value (pKa) of H$_2$S is approximately 7.0 at 25 °C, the molecular H$_2$S concentration at the end of the reduced flow line is estimated be 1.45 times that in the normal flow line.
Figure 4-2. A comparison of sulfide and pH profiles in corresponding reactors in the reduced flow and normal flow lines: (A) -first reactor; (B) -second reactor; and (C) -third reactor.
4.4.2 Effect of RWC on CH₄ production in a rising main sewer

The methane concentrations at the end of each pumping cycle in the two lines are compared in Figure 4-3 (A-C). The average methane concentration in the reduced flow line (5-20 mg/L) was about 2.1 times that in the normal flow line (3-13 mg/L). The concentration difference between the corresponding reactors in the two lines increased gradually from the first reactor to the third reactor due to the accumulation of methane along the line with the increase of HRT. Unlike sulfide, the daily total discharge of methane in the reduced flow line was 1.5 times that in the normal flow line (303 mg/day and 204 mg/day, respectively), despite that the latter had a 1.4 times higher flow rate.

Figure 4-3. A comparison of methane concentrations at the end of each pumping cycle in corresponding reactors of the reduced flow and normal flow lines in an 8-hour period. (A) – first reactor; (B) – second reactor; and (C) – third reactor.
4.4.3 Effect of RWC on sulfate-reducing and methanogenic activities of sewer biofilms

The sulfate-reducing and methanogenic activities of sewer biofilms in the reduced flow line and the normal flow line were investigated through batch tests. The comparison of the corresponding reactors in the two lines indicates that RWC did not have a significant effect on the sulfide production capability of biofilms (T-test for all three reactors: null hypothesis, no significant difference, p>0.05) (Figure 4-4(A)). In contrast, methane production rates of reduced flow line were much higher than the normal flow line in all three reactors (p<0.05) (Figure 4-4(B)). The average methane production rate in the reduced flow line was 8.6 mg/(L·h), whereas in the normal flow line the rate was only about the half of that value (4.4 mg/(L·h)). In both lines, the sulfate-reducing activities decreased gradually at downstream locations whereas methanogenic activities showed the opposite trend. This could be explained by SRB limiting methanogens in sulfate-rich environments (Guisasola et al. 2008). In the first reactor, where sulfate was abundant (> 10 mg S/L), SRB had higher activities and utilised large amounts of carbon substrate, leading to a restriction of methanogenic activities. However, due to decreased availability of sulfate in the following reactors, SRB activities decreased, and thus more carbon substrates could be used for methane production so that more methanogens could develop, resulting in higher methanogenic activities.

Figure 4-4. A comparison of sulfide (A) and methane (B) production rates of six reactors in the reduced flow and normal flow lines.

4.4.4 Model-based analysis of sulfide and methane emissions and chemical dosage under different flow conditions

A simulation study, with the UC09 sewer line as the system investigated, was performed to assess the potential effect of RWC on sulfide and methane emissions, and on mitigation costs, in a
practical system. According to the experimental results, the rate of sulfide production was similar under both flow conditions and hence the same areal hydrogen sulfide production rate (3.5 g S/(m²·day) based on average sulfide production rate of 8.0 mg S/(L·h)) was used in the simulation study in both cases. On the other hand, different areal methane production rates (5.2 and 10.5 g COD/(m²·day), respectively, for the normal and RWC conditions) were used as obtained by the methane production rate measurement in the laboratory study. The comparison of sulfide and methane concentration under normal and RWC conditions (Figure 4-S2 and 4-S3, SI) shows that on average, the sulfide concentration under the RWC condition is about 2.3 mg-S/L higher than under the normal flow condition. For methane, the concentration under the RWC condition was about 2.2 times that under the normal flow condition. These results are consistent with laboratory reactor studies.

Simulation study was performed to compare the effect of RWC on H₂S and methane emissions in the discharge manhole following the UC09 sewer line. As Figure 4-5 shows, both H₂S and CH₄ concentrations under reduced flow were consistently higher (on average 21% and 105%, respectively) compared to the normal flow. The higher methane emission was caused by the higher methane production in the sewer line, while the higher sulfide emission is attributed to both its higher production and lower wastewater pH. As the sewer odor and corrosion problems are directly related to the gas phase H₂S concentration, the reduction in sewer flow is expected to increase severity of these problems. Similarly, the increased emission of methane would increase greenhouse gas emissions from wastewater systems.

Figure 4-5. A comparison of (A) H₂S and (B) CH₄ profiles in a discharge manhole headspace without chemical dosing.
The effect of RWC on chemical dosing strategies for sulfide mitigation was evaluated by comparing the daily dosage rate and the volumetric dosage rate (dosage per volume of wastewater). By the variation of the dosage rates, similar dissolved sulfide concentrations (<1 mg S/L) in the cases of oxygen, nitrate and iron salts addition or pH (>9) in the case of magnesium hydroxide dosage were reached under both RWC and normal conditions (Figure 4-S3). The daily dosage rate and volumetric dosage rate are compared in Table 4-1. The daily chemical dosage of all chemicals in the case of RWC decrease by on average 11%, whereas the volumetric dosing rates increase by an average of 18%.

Table 4-1. A comparison of dosing rates of four typical chemicals under reduced flow and normal flow conditions for controlling hydrogen sulfide in the UC09 sewer system.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dosing Location</th>
<th>Flow Conditions</th>
<th>Dosing Rate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dosing rate&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>200 m upstream of the end of the pipe</td>
<td>Normal Flow</td>
<td>3.00 kg O&lt;sub&gt;2&lt;/sub&gt;/day</td>
<td>24 kg O&lt;sub&gt;2&lt;/sub&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced Flow</td>
<td>2.58 kg O&lt;sub&gt;2&lt;/sub&gt;/day</td>
<td>30 kg O&lt;sub&gt;2&lt;/sub&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>200 m upstream of the end of the pipe</td>
<td>Normal Flow</td>
<td>1.28 kg N/day</td>
<td>10 kg N/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced Flow</td>
<td>1.13 kg N/day</td>
<td>13 kg N/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Start of the pipe</td>
<td>Normal Flow</td>
<td>1.52 kg Fe&lt;sup&gt;3+&lt;/sup&gt;/day</td>
<td>12 kg Fe&lt;sup&gt;3+&lt;/sup&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced Flow</td>
<td>1.39 kg Fe&lt;sup&gt;3+&lt;/sup&gt;/day</td>
<td>16 kg Fe&lt;sup&gt;3+&lt;/sup&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg(OH)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Start of the pipe</td>
<td>Normal Flow</td>
<td>9.06 kg Mg(OH)&lt;sub&gt;2&lt;/sub&gt;/day</td>
<td>73 kg Mg(OH)&lt;sub&gt;2&lt;/sub&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced Flow</td>
<td>7.56 kg Mg(OH)&lt;sub&gt;2&lt;/sub&gt;/day</td>
<td>87 kg Mg(OH)&lt;sub&gt;2&lt;/sub&gt;/1000m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Daily dosing rate

<sup>b</sup> Volumetric dosage rate (dosage per 1000m<sup>3</sup> of wastewater).

4.5 Discussion

4.5.1 The higher sulfide concentration under RWC conditions is mainly because of longer HRT

In the laboratory studies, sulfide concentration in the reduced flow line was on average 2.72 mg S/L higher than that in the normal flow line. The results of batch tests revealed that the sulfate reducing activities were similar in the two lines (Figure 4-4(A)). This suggests that the higher sulfide
concentration under RWC conditions in this study was mainly because of the longer HRT in the reduced flow line.

The above hypothesis is supported by Figure 4-6(A), where the total sulfide production in the reduced flow line is plotted against that in the normal flow line for the same HRT, based on the 8-h profile monitoring results. A good linearity with a slope of 0.98 is observed ($R^2 = 0.9804$), when the sulfide production was less than 9 mg-S corresponding to HRT shorter than 1.5 h. This indicates that the sulfide production was similar in both lines, as revealed in the batch tests (Figure 4-4(A)). However, the above regression line does not describe the relation when sulfide production was higher than 9 mg S, corresponding to HRT longer than 1.5 h. In this case, sulfide production in the normal line was limited by sulfate concentration so that sulfide production became higher in the reduced line. In both lines, sulfide production ceased to occur when sulfate was completely consumed, despite of increases in HRT, resulting in the formation of a cluster at the end of the plot.

The similar sulfate-reducing activities in the two lines were possibly a result of similar sulfate concentrations in the feeding water, i.e. 21.5 ± 1.1 mg S/L ($n=25$) for the reduced flow line and 19.8 ± 1.0 mg S/L ($n=25$) for the normal flow line, respectively. The difference of sulfate concentration in the two lines was insignificant ($p > 0.22$), leading to the development of similar SRB activities in the biofilms. The organic substrates were apparently not a limiting factor for sulfide production even though the normal line had a lower COD concentration. According to the sulfate reduction stoichiometry, 40 mg/L sCOD would be used for the reduction of sulfate at 20 mg S/L. However, in this study, the concentration of sCOD in both lines was higher than 150 mg/L (see Section 4.2).

The underlying reason for the similar sulfate concentrations in the two lines was that the drinking water in the region of this study contained a relatively high level of sulfate (15.7 ± 0.7 mg S/L, $n = 25$), and therefore contributed considerably to the total sulfate in the feeding wastewater for the normal flow line. Currently, there is no strict regulation of sulfate concentration in drinking water, with the limit being 500 mg/L (or 167 mg S/L) in the World Health Organization (WHO) guidelines for drinking-water quality (WHO 2011). In areas with lower sulfate concentration in drinking water, the effect of RWC on sulfide production may be different from what revealed in this study.
Increased methanogenic activity and longer HRT both contributed to higher methane production under the reduced water consumption condition

Methane concentration in the reduced flow line were over 2 times of that in the normal flow line. Among the potential factors affecting methane production in sewers, we suggest that the higher methane concentration under the RWC condition is a result of both increased methanogenic activity and longer HRT.

Batch tests indicated a higher methanogenic activity in the reduced flow line (Figure 4-4(A)). This is consistent with the in-situ methanogenic activity as shown by Figure 4-6(B), which plotted methane production in the reduced flow line against that in normal flow line from the same HRT. The slope of the linear regression is about 1.8, indicating that on average the methane production rate in the reduced flow line is 1.8 times that in the normal flow line, which is consistent with the batch test results (Figure 4-4B). The substrates for methanogenesis mainly consist of acetate and hydrogen, which are mainly derived from the fermentation of organic compounds. The higher sCOD concentration in the feed to reduced flow line (266 ± 28 mg/L) compared with the normal flow line (181 ± 17 mg/L) could result in more acetate and hydrogen produced for methanogenic activity. Different from sulfate, the sCOD concentration in drinking water is negligible, so the ratio of sCOD concentration between the two lines was close to the dilution ratio (1.4:1). The higher sCOD concentration in the reduced flow line likely favors the growth of methanogens by the...
enhanced mass transfer into the inside of biofilms. As a result, higher methanogenic activity in the reduced flow line was achieved.

However, as shown in the 8-hour methane profiles in the two lines (Figure 4 (A-C)), the methane concentration in the reduced flow lines was 2.1 (> 1.8) times that in the normal flow line. This suggests that, apart from the biofilm activity, another factor, most likely HRT, would have also contributed to the higher methane concentration in the reduced flow line. The higher HRT in the reduced flow line provided a longer time for methanogenesis, thus leading to higher methane concentration. The difference in HRT in the two systems is approximately 40%, which would explain the additional 30% (2.1-1.8) increase in methane production in the reduced flow line.

4.5.3 Practical implications
This study revealed that RWC would result in higher dissolved sulfide concentrations and lower pH in wastewater, which would subsequently result in higher gas phase H$_2$S concentrations at places such as manholes, pumping station or inlet of WWTPs, where H$_2$S could be transferred to gas phase. Without mitigation, this effect would worsen the corrosion and odor problems that the water industry is already experiencing. However, the cost for sulfide mitigation through chemical dosing would decrease, despite of increased dosing demand per unit volume of wastewater to be treated, due to decreased sewage flow and hence the amount of wastewater to be treated. Also, sulfide mitigation strategy through chemical dosing would be affected by RWC. The simulation results revealed that the dosing demand per unit volume of wastewater would increase. However, the overall cost of chemical demanding would decrease, due to decreased sewage flow and hence less amount of wastewater to be treated. This indicated that water industry should change the chemical dosing strategy accordingly under RWC conditions. The frequency of the chemical dosing could be reduced according to reduced wastewater flow while the dosage per dosing event needs to increase based on sulfide concentration. In addition, this study found that sulfate in the tap water contributed significantly to sulfate in wastewater and consequently to sulfide in sewers, therefore, measures to reduce sulfate in tap water could also help to reduce sulfide production in sewers.

Methane production for per unit volume of wastewater is expected to increase substantially under RWC conditions, as shown by both laboratory experimental data and the modeling results. The total discharge would also be substantially higher despite of a lower flow rate. As methane would be emitted at downstream locations due to its low solubility, the increased methane production under RWC conditions would contribute significantly to the total GHG inventory of the wastewater system. Calculation based on an empirical model for evaluating methane production in rising mains
(Foley et al. 2009) shows that, for a 300m diameter rising main with a normal flow of 7000 m$^3$/d and HRT of 4h, its annual GHG inventory due to methane emission would increase from 320 tCO$_2$-e to 460 tCO$_2$-e with a 40% RWC is enforced. This value would account for 12%-18% of the total GHG inventory of a typical biological nutrient removal WWTP receiving 7000 m$^3$/d per day (de Haas et al. 2009).

### 4.6 Supporting Information

Figure 4-S1. Pumping pattern of UC09 under normal and reduced flow conditions.

Figure 4-S2. The schematic diagram of the manhole.
Figure 4-S3. Simulation results of total dissolved sulfide (A) and methane (B) concentrations at the end of pipe under normal and reduced flow conditions.

Figure 4-S4. Sulfide concentration or pH value under the normal and reduced flow conditions after chemical dosage of oxygen (A), sodium nitrate (B), ferric chloride (C) and magnesium hydroxide (D). The average sulfide concentrations under both normal and reduced flow conditions after addition of oxygen, sodium nitrate, and ferric chloride were 0.67 mg S/L, 0.57 mg S/L and 0.34 mg S/L, respectively. The average pH after magnesium dosage in both conditions was 9.0.
Chapter 5 Stratified microbial structure and activity in sulfide- and methane-producing anaerobic sewer biofilms

5.1 Abstract
Simultaneous production of sulfide and methane by anaerobic sewer biofilms has recently been observed, suggesting that sulfate-reducing bacteria (SRB) and methanogenic archaea (MA), microorganisms known to compete for the same substrates, can coexist in this environment. This study investigated the community structure and activities of SRB and MA in anaerobic sewer biofilms (average thickness of 800 μm) using a combination of microelectrode measurements, molecular techniques and mathematical modelling. It was seen that sulfide was mainly produced in the outer layer of the biofilm, between 0 - 300 μm, which is in good agreement with the distribution of SRB population as revealed by cryosection - fluorescence in situ hybridization (FISH). SRB have a higher relative abundance of 20% on the surface layer, which decreased gradually to below 3% at a depth of 400 μm. In contrast, MA mainly inhabited the inner layer of the biofilm. Their relative abundances increased from 10% to 75% at depths of 200 μm and 700 μm, respectively, from the biofilm surface layer. High throughput pyrosequencing of 16S rRNA amplicons showed that SRB in the biofilm were mainly affiliated with five genera: Desulfobulbus, Desulfomicrobium, Desulfovibrio, Desulfatiferula and Desulfuregula, while about 90% of the MA population belonged to the genus Methanosaeta. The spatial organization of SRB and MA revealed by pyrosequencing were consistent with the FISH results. A biofilm model was constructed to simulate the SRB and MA distributions in the anaerobic sewer biofilm. The good fit between model predictions and the experimental data indicates that the coexistence and spatial structure of SRB and MA in the biofilm resulted from the microbial types, their metabolic transformations and interactions with substrates.

5.2 Introduction
Sewer biofilms comprise complex multi-species microflora with a typical thickness of only about one millimeter (Hvitved-Jacobsen 2002). According to the electron donors and electron acceptors present in the wastewater, different carbon transformation processes can occur in close proximity in the sewer biofilms. Domestic wastewater normally contains a significant concentration of sulfate (ca. 100–1000 μM) but negligible nitrite and nitrate (Okabe et al. 2003, Sun et al. 2014a). Therefore, under anaerobic conditions (normally occurs in pressure sewers fully filled with wastewater), sulfate reduction carried out by the sulfate-reducing bacteria (SRB) could be an important terminal electron accepting process in the sewer biofilms. The sulfate reduction activity in anaerobic sewers is important as the production of sulfide produced can be transferred to the gas phase of partially-
filled gravity sewers and cause extensive corrosion of concrete sewer pipes (Vollertsen et al. 2008, Jiang et al. 2014). Also, the emission of sulfide from sewers can cause odor problems to the surrounding area and pose health risks to sewer workers (Boon 1995, Ganigué et al. 2011). Apart from sulfate reduction, methanogenesis by the respiration of methanogenic archaea (MA) could also be a key terminal process in anaerobic sewer biofilms (Guisasola et al. 2008, Foley et al. 2009). Guisasola and colleagues (Guisasola et al. 2008) found that methanogenesis accounted for more than 70% of the soluble chemical oxygen demand (sCOD) loss in laboratory anaerobic sewer biofilm reactors. A recent report suggests that methane emissions from sewers contribute significantly to the total greenhouse gas footprint of wastewater systems (GWRC 2011).

Under anaerobic conditions, both sulfate reduction and methanogenesis can potentially occur in the same system while competing for the same electron donors, primarily hydrogen and acetate. In the presence of adequate sulfate concentrations, SRB will typically outcompete MA due to kinetic and thermodynamic advantages (Lovley et al. 1982, Schönheit et al. 1982, Raskin et al. 1996). However, the coexistence of SRB and MA has been observed in anaerobic sewer biofilms in the presence of sulfate. Guisasola et al. (2008) hypothesized the coexistence of SRB and MA in sewer biofilms was due to the penetration limitation of sulfate into the biofilms, resulting in a stratified biofilm structure, with SRB being predominant in the outer zone, nearer to the wastewater, while MA inhabit the inner zone, nearer the sewer pipe. However, to date this hypothesis has not been verified. A few studies have investigated the vertical distribution of SRB in oxic-anoxic sewer biofilms (biofilms attached on gravity sewer pipe with the presence of oxygen or nitrate in wastewater), but studies on the SRB distribution in the anaerobic sewer biofilms is scarce (Ito et al. 2002a). In addition, the distribution of MA in sewer biofilms and their interaction with SRB have not been explored yet. Similarly, the phylogenetic diversity of SRB and MA in the anaerobic sewer biofilms is rarely reported. These fundamental information could provide a better understanding of the sulfate reduction and methanogenesis processes in sewer systems, which would be useful for sewer management. Therefore, the aims of this study are to investigate the community structures of both SRB and MA and to determine their spatial arrangement in anaerobic sewer biofilms.

Both experimental investigations and modeling analyses were conducted to achieve the aims of this study. The experiments were carried out in an annular biofilm reactor mimicking anaerobic sewer conditions, which was fed with real domestic wastewater. Firstly, microelectrodes were applied to determine the spatial distribution of in situ sulfide production activity within the biofilms. Although it would have been ideal to determine the distribution of methane production activity using the same method, this was difficult to perform due to the lack of suitable microelectrodes (Damgaard et al. 2005).
Secondly, the spatial distributions of the SRB and MA in the biofilms and their abundance at different depths were determined by fluorescence in situ hybridization (FISH) after cryosectioning the biofilm samples. This method has been used frequently to determine the spatial distributions of microbial communities in biofilms or granules. However, phylogenetic information is hardly revealed due to the limitation of oligonucleotide probes used in FISH (Lücker et al. 2007). Therefore, 16S rRNA gene amplicon pyrosequencing was applied to further investigate the phylogenetic diversity. In previous studies of sewer biofilms, the phylogenetic analysis is performed on the entire biofilm, and information on the different genera at different biofilm depths is rarely reported (Ito et al. 2002a, Okabe et al. 2003). In this study, we determined the phylogenetic diversity in different layers of the sewer biofilms by innovatively using pyrosequencing combined with cryosectioning. To our knowledge this method has not been applied in any other areas to date. Finally, a mathematical model focusing on the interaction between SRB and MA in the sewer biofilm was developed to evaluate and interpret the experimental results.

5.3 Materials and Methods

5.3.1 Reactor configuration, operation and monitoring
An annular biofilm reactor made of acrylonitrile butadiene styrene (ABS) was set up to mimic an anaerobic sewer pipe section (Figure 5-1). The reactor consisted of an inner cylinder (of height 295 mm, and diameter of 130 mm) enclosed in an outer cylinder (of height 345 mm and inner diameter of 160 mm). Wastewater was filled in the gap between the two cylinders, with a volume of 3 L. Biofilms were grown on the walls of both cylinders in contact with the wastewater, resulting in a biofilm area to reactor volume (A/V) ratio of 119 m⁻¹. Mixing was established by the rotation of the inner cylinder driven by a motor at a speed of 200 rpm. The mixing is expected to create a uniform shear stress on the reactor walls so that biofilms grow relatively evenly on the wall. The average shear stress provided by the mixing was 2.11 N/m², which is typical in sewer systems (Enfinger and Mitchell 2010). Eight removable ABS slides of width and length at 5 mm by 200 mm were mounted in recessed slots on the inside of the outer cylinder. The slides were removable via ports on the top of the reactor for biofilm sampling. The reservoir on the top of the reactor was used to ensure the reactor was full of wastewater during sampling.

Domestic sewage, collected on a weekly basis from a local wet well (Brisbane, Queensland), was used as the feed for the reactor. The sewage compositions varied to a certain extent in terms of sulfate, volatile fatty acids (VFA), and COD concentrations. The sewage typically contained sulfate at concentrations of 10-25 mg S/L, sulfide at < 3 mg S/L, soluble COD at 200-300 mg/L, 50-120 mg COD/L of VFAs and approximately 50 mg N/L of ammonium. Negligible amounts of sulfite,
thiosulfate (<1 mg S/L), nitrate and nitrite (<1 mg N/L) were present. The sewage was stored in a cold room (4°C) to minimize biological transformations, and was heated to 20±1°C prior to being pumped into the reactors (Figure 5-1). The sewage was fed to the reactor intermittently by a peristaltic pump (Masterflex 7520-47) to simulate the typical flow patterns of rising main sewers. For easier reactor monitoring, each day was divided into three identical 8-hour periods. Figure 5-S1 in Supporting Information (SI) shows the pumping patterns applied to the reactor for an 8-hour period and the hydraulic retention time (HRT) of sewage in the reactor. Every pumping event lasted for 3 min, delivering one reactor volume (3 L) of wastewater into the reactor. The HRT of the wastewater ranged between 30 minutes to 4 hours, which are in the range of HTR observed in a typical real sewer pipe (Guisasola et al. 2008).

Monitoring of the reactor performance was carried out during the eight-hour cycle periods every two weeks. Sulfide concentrations during the eight hour cycle were continuously monitored using the S::CAN VU-VIS sepcitro::lyser (Messtechnik GmbH, Austria), as previously described by Sutherland-Stacey et al. (2008). In addition, samples were taken from the reactor before and after each pumping event and also at 2.5h, 5h, and 6.5h for the analysis of dissolved methane, sulfate, total COD (tCOD), soluble COD (sCOD) and VFAs, using methods as described by Sun et al. (2014a). Detailed studies of the biofilm were carried out when the reactor reached pseudo steady-state conditions as indicated by the relatively constant sulfide and methane profiles.

![Figure 5-1. Schematic of the laboratory-scale anaerobic, annular biofilm reactor.](image-url)
5.3.2 Microelectrode measurement

Hydrogen sulfide (i.e. molecular H$_2$S), pH, and dissolved oxygen in the biofilm were measured using microelectrodes (Unisense A/S, Denmark) with tip diameters of 10 μm, 25 μm and 100 μm, respectively. The sensors were calibrated according to the manufacturer’s instructions. Hydrogen sulfide and pH profiles were measured to determine the total dissolved sulfide concentration as described in Kuhl et al. (1992). Oxygen profiles were measured to confirm anaerobic conditions.

Before the microelectrode analyses, about 5 cm of the biofilm slide was removed from the reactor and mounted in a flow cell (as described in Gutierrez et al. (2008)) containing 140 mL of 0.22 μm filtered wastewater and 20 mL of 300 mM phosphate buffer (added to ensure a stable pH of 7.0–7.5). Nitrogen gas (99.99% purity) was bubbled through the flow cell to ensure anaerobic conditions and to provide mixing. Microelectrodes were mounted on a micromanipulator and positioned on the surface of the biofilm using a dissecting microscope. The concentration gradients through the biofilm were obtained by moving the microelectrodes in increments of 25–100 mm. Steady-state profiles were obtained by incubating the biofilm for 1 h in the medium before measurements were made.

The local sulfide production rates were calculated from the total sulfide profiles based on Fick’s second law of diffusion. The calculation was carried out by a stepwise procedure as described by Gieseke and de Beer (Gieseke and de Beer 2004). Briefly, the production rate at point n can be calculated using following equations.

\[
\eta_n = \frac{J_{n-1,n} - J_{n,n+1}}{0.5(x_{n-1} + x_n) - 0.5(x_n + x_{n+1})}
\]

\[
J_{n-1,n} = D_{eff} \frac{C_{n-1} - C_n}{x_{n-1} - x_n}, J_{n,n+1} = D_{eff} \frac{C_n - C_{n+1}}{x_n - x_{n+1}}.
\]

Where

- $J_{n-1,n}$ and $J_{n,n+1}$ are flux between point n-1 and n, and point n and n+1, (mol/m$^2$/s);
- $x_{n-1}, x_n$ and $x_{n+1}$ are depths of point n-1, n and n+1, (m);
- $C_{n-1}, C_n$ and $C_{n+1}$ are sulfide concentrations at point n-1, n and n+1, (mol/m$^2$)
- $D_{eff}$ is effective diffusion coefficient of sulfide in the biofilm (m$^2$/s). The value used in this study was 1.39×10$^{-9}$m$^2$/s (Kuhl and Jorgensen 1992) and this was based on the assumption that the diffusion coefficients within the biofilm were equal to the molecular diffusion coefficients.
5.3.3 Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was carried out to determine the distribution of SRB and MA in the biofilm. The sequences of all oligonucleotide probes used in this study are summarized in SI Table 5-S1 and further detailed information is in probeBase (Loy et al. 2007) Due to a drawback of SRB probes which can detect other bacteria that are not SRB, in this study the SRB were determined by the overlapping fluorescence signal of the probes DELTA495a (CY3), DELTA495b (CY3) and DELTA495c (CY3) with probes SRB385 (CY5), SRB385Db (CY5) and DABAC 357 (CY5). Using this approach, most SRB in the phylum of the Deltaproteobacteria were detected, while discriminating the non-SRB targeted by these probes (Lücker et al. 2007). SRB in other phyla were not detected by 16S rRNA pyrosequencing results, so probes for those phyla were not used. For FISH detection of MA, a combination of probes MSMX860 (CY5), MG1200b (CY5), MB1175 (CY5), MC1109 (CY5) and MC504 (CY5) were used to determine the total MA population in the biofilm. This combination of probes covers a wide range of MA in these ecosystems (Crocetti et al. 2006). SRB and MA were determined using different samples due to the different formamide concentrations required (35% for the SRB detection and 45% for the MA detection). The probes EUB338mix (FITC) and ARC915 (CY3) were used to determine all bacteria and archaea in the biofilm respectively.

To conduct FISH analysis, the biofilm sampling slides were removed from reactor and cut to approximately 10 mm × 5mm. The biofilm samples on the small pieces were fixed with freshly prepared 4% paraformaldehyde solution for 2 h at 4°C. The fixed biofilm sample was then embedded in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan) following the procedures described by Batstone et al. (2004). The biofilm samples were then allowed to settle on the base of the OCT moulds and frozen at -20 °C. The frozen samples were then sectioned using a Research Cryostat (Leica CM3050 S) with a knife temperature of -20 °C, a cabinet temperature of -18 °C and a section size of 10 μm. The samples were divided into two groups and cryosectioned in two different directions. One group of samples was sectioned perpendicular to the substratum, to provide sections to visualize the arrangement of SRB and MA distributed through the depth of the biofilm. The other group of samples were sectioned parallel to substratum successively from the surface to the bottom of the biofilm. These samples were used to determine the relative abundance of SRB and MA at different depths within the biofilm. The cryosectioned samples were placed on Poly-L-Lysine coated microscope slides (Polysciences Asia Pacific, Inc.) and air dried for 6 – 10 h. The slides were then dehydrated for 3 min each in a 50%, 80% and 100% aqueous ethanol solution.
All in situ hybridizations were performed according to the protocol (Amann et al. 1990b) in hybridization buffer at 46°C for 2–3 h. The buffer contained 0.9 M NaCl, 20 mM Tris hydrochloride (pH 7.2), 0.01% sodium dodecyl sulfate and formamide concentrations as previously mentioned. Subsequently, a stringent washing step was performed at 48°C for 15 min in 50 ml of washing solution comprising NaCl at a concentration dependent on the formamide concentration, and 20 mM Tris hydrochloride at pH 7.2. The slides were examined and recorded using a Zeiss LSM 510 confocal laser scanning microscope (CLSM) (Carl Zeiss, Jena, Germany) using three excitation channels (488 nm, green emission; 545 nm, red emission; and 633 nm, blue emission). The biofilm thickness was estimated by measuring the width of the biofilm sections cut perpendicular to the substratum. FISH images at different depth of the biofilm (0-10 μm, 100-110 μm, 200-210 μm, 300-310 μm, 400-410 μm, 500-610 μm and 700-710 μm) were analysed using DAIME version 1.3 (Daims et al. 2006) to determine the biovolume fractions of SRB and MA. About 20 confocal images of the biofilm sections were analyzed for each sample. The quantification results were calculated based on the average of two separately analyzed samples.

5.3.4 16S rRNA gene amplicon pyrosequencing

16S rRNA gene amplicon pyrosequencing was conducted to investigate the phylogenetic diversity of SRB and MA at different layers in the biofilm. Biofilms on a 10 mm × 5 mm piece of slide were quickly removed from the reactor and embedded in OCT compound and then frozen at -20 °C in a OCT mould. The frozen samples were then cryosectioned successively from the surface to the bottom of the biofilm with a section size of 150 μm, using the cryostat as described above. The sectioned biofilm samples were then placed separately in 1 ml eppendorf tubes containing 0.5 ml of phosphate buffered saline (PBS, containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 2 mM KH₂PO₄) for DNA extraction.

Genomic DNA was extracted using the FastDNA SPIN Kit for soil according to the manufacturer’s instructions (Q-Bio gene, Australia). The quantity and quality of the extracted DNA was measured using a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technology, Rockland, DE) and agarose gel (0.8%, wt/vol) electrophoresis. The primers 926f (5’-AAACTYAAAKGAATTGACGG-3’) and 1392r (5’-ACGGGCAGGTGTGAC-3’) (Amann et al. 1995) containing multiplex identifiers and LibL adaptor sequences (not shown) were used to generate amplicons. The pyrosequencing of amplicons was carried out according to Roche 454 protocols using a Roche 454 GS FLX sequencer (Roche, Switzerland). The sequence data was analysed through the ACE Pyrosequencing Pipeline (https://github.com/minillinim/APP) in a local implementation. Firstly, The sequencing reads were split according to the barcode in QIIME v1.8.0
(Caporaso et al. 2010b). Then, De-multiplexed sequences were trimmed to 250 bp length and de-noised using ACACIA (Bragg et al. 2012). Sequences with 97% similarity were assigned to one operational taxonomic unit (OTUs) by CD-HIT-OTU (Wu et al. 2011) and aligned by Pynast (Caporaso et al. 2010a). Each sequence was then assigned to the taxonomy with the BlastTaxonAssigner in QIIME through the greengenes database (2013 Aug release). Sequences that were assigned to the classes of Clostridia and Deltaproteobacteria (containing most of the mesophilic SRB) and those assigned to the domain Archaea (containing the methanogens) were also compared with other sequences previously deposited in GenBank (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) and genus level classification were assigned (if > 98% identity were obtained). Finally a non-normalized OTU table was generated by QIIME. Then, Normaliser (https://github.com/minillinim/Normaliser) was used to construct a centroid normalized OTU table.

5.3.5 Biofilm modelling

A multispecies one-dimensional biofilm model was constructed to simulate the microbial structure and biological reactions in the anaerobic sewer biofilm, employing the software AQUASIM V2.1d (Reichert 1994). The biofilm reactor compartment of AQUASIM described a reactor with a completely mixed bulk water volume and with a biofilm growing on a substratum surface in the reactor. The equations solved in the biofilm reactor compartment followed the one-dimensional conservation laws as show in the following equation:

$$\frac{\partial \rho}{\partial t} + \frac{\partial j}{\partial z} = \dot{r}$$

where:
- $\rho$ is amount of conserved quantity per unit of compartment length;
- $j$ is amount of conserved quantity transported per unit of time;
- $\dot{r}$ is amount produced per unit of compartment length;
- $t$ is time;
- $z$ is the distance from the substratum.

Diffusive mass transport of soluble compounds in the biofilm matrix was considered while no diffusion of particular compounds is assumed. The detachment of biomass from the biofilm was described by a global detachment velocity, with particulate compounds being detached from the biofilm surface. The detached biomass were assumed to be removed from the system, and therefore re-attachment of detached biomass was not considered in the model. The steady state biofilm
thickness was set according to the experimental observation. The biofilm model was developed to evaluate the experimental results according to Sharma et al. (2008b) and Guisasola et al. (2009). The biological reaction model is schematically summarized in SI Figure 5-S2 with definition of model components summarized in SI Table S5-2.

Briefly, the biological model consisted of four types of microbial processes: hydrolysis, fermentation, sulfate reductions and methanogenesis. Glucose is used in the reaction to represent fermentable substrates (e.g. sugars and/or other carbohydrates), in the same way as used previously (Guisasola et al. 2009). Three fermentation products were considered in the model, namely hydrogen, acetate and propionate. Sulfate reductions were carried out with the three electron donors, i.e. hydrogen, acetate and propionate. Given the fact that SRB tend to outcompete acetogenic bacteria for propionate utilisation and that propionate concentrations in real sewage were always lower than 10 mg COD/L, propionate was considered as an electron donor only for sulfate reduction (Guisasola et al. 2009). While sulfate reduction using fermentable substrates (e.g. sugars or other carbohydrates) is also possible, it was not considered in the model (Guisasola et al. 2009). The use of these substrates by SRB would otherwise be accounted for by the use of the fermentation products from these substrates. Both hydrogenotrophic and acetoclastic pathways for methanogenesis were included in the model. The stoichiometric matrix for microbial processes and the kinetic expressions of processes were shown in Table 5-S3 and Table 5-S4, respectively. All model parameters were obtained from the literature and are presented in Table 5-S5.

5.4 Results

5.4.1 Performance of the anaerobic sewer reactor

The reactor was operated for 10 months to reach pseudo steady state. The typical sulfide and methane profiles in the sewer biofilm reactor during an 8-hour operation cycle is shown in Figure 5-2A and B. Sulfide and methane were produced simultaneously in the reactor and concentrations of sulfide (13.0-18.6 mg S/L) and methane (9.3-14.9 mg/L) at the end of each pumping cycle varied according to the hydraulic retention time (HRT). During the 8-h cycle, the total chemical oxygen demand (tCOD) was decreased by 17% and nearly 86% of the sulfate was reduced. Table 1 shows the average daily transformation of COD, VFAs, sulfur species and methane at pseudo steady state, calculated based on the concentration differences at the beginning and end of each pumping cycle. The tCOD was consumed by 688.2 ± 29.2 mg/day, with productions of sulfide and methane at 123.9 ± 11.1 mg S/day and 103.4 ± 3.2 mg/day, respectively. Similar daily sulfate consumption and sulfide production indicated that sulfide was the major product of sulfate reduction. The sCOD and propionate were also consumed in the reactor while acetate accumulated. The COD balance was
calculated assuming that all hydrogen produced due to fermentation was consumed during the experiment. The COD utilization per gram of sulfide and methane formed is assumed to be 2 g COD/gH₂S-S and 4 g COD/gCH₄, respectively (Guisasola et al. 2008). Therefore, sulfidogenesis accounted for 36.0 ± 2.4 % of the tCOD loss in the wastewater while methanogenesis accounted for 60.0 ± 4.3 % (Table 5-1).

Figure 5-2. Sulfide (A) and methane (B) profiles in the sewer biofilm reactors during a typical 8-hour cycle. For convenience, each day was divided into three identical 8-hour cycles, with four pumping events in each cycle with intervals mimicking real pumping stations (Guisasola et al. 2008). The vertical solid lines at the bottom of the graphs indicate the pumping events in the 8-hour cycle. During each pumping event, one reactor volume of fresh wastewater was fed into the reactor, resulting in the sulfide and methane concentration dynamics.

Table 5-1. Daily transformation of COD, VFA, sulfur species and methane in the sewer biofilm reactor.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Unit</th>
<th>Daily transformation¹</th>
<th>ΔCOD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total COD</td>
<td>mg COD</td>
<td>-688.2 ± 29.2</td>
<td>-100.0 ± 0.0 %</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>mg COD</td>
<td>-362.5 ± 12.7</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>mg COD</td>
<td>+49.4 ± 17.2</td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>mg COD</td>
<td>-76.5 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Sulfate</td>
<td>mg S</td>
<td>-123.5 ± 12.8</td>
<td></td>
</tr>
<tr>
<td>Sulfide</td>
<td>mg S</td>
<td>+123.9 ± 11.1</td>
<td>36.0 ± 2.4 %</td>
</tr>
<tr>
<td>Dissolved methane</td>
<td>mg</td>
<td>+103.4 ± 3.2</td>
<td>60.0 ± 4.3 %</td>
</tr>
<tr>
<td>COD balance</td>
<td></td>
<td></td>
<td>-4.0 ± 2.0 %</td>
</tr>
</tbody>
</table>

¹ “+” refers to production and “-” refers to consumption.
5.4.2 Distribution of sulfide production within the biofilm

The micro-scale sulfide, pH and oxygen levels were measured throughout the depth of the biofilm (Figure 5-3). A significant increase of sulfide concentration is seen from the biofilm surface to ca. 250 μm into the biofilm. The pH remained constant throughout the depth of the biofilm, due to the buffering capacity of the system. Negligible levels of oxygen were detected within the biofilm. The in situ sulfide production rates were calculated based on the sulfide profiles (Figure 5-3), which indicated that sulfide was mainly produced in the region that extended from the biofilm surface to a depth of about 300 μm into the biofilm.

Figure 5-3. Profiles of measured total dissolved sulfide, oxygen, pH and calculated sulfide production rate in the biofilm. Negative depths in the profile represent the distance from the biofilm surface into the wastewater.
5.4.3 Spatial distributions of SRB and MA populations determined by FISH

FISH of the biofilm sections cut perpendicular to the substratum show the localization of SRB and MA (Figure 5-4 (A) and (B)). SRB (white in Figure 5-4(A)) were mainly situated at the outer layer (0-300 μm) of the biofilm while MA (purple in Figure 5-4(B)) were mainly located in the inner layer (below 250 μm). Figure 5-4 (C-F) shows typical FISH images of the biofilm sections cut parallel to the substratum at depths of 100 μm and 700 μm. Accordingly, SRB were detected in much higher abundance in the biofilm section at the depth of 100 μm in comparison to the 700 μm deep section (Figure 5-4(C) vs. Figure 5-4(D)). In contrast, there was hardly any MA at the depth of 100 μm whereas MA were dominant at the depth of 700 μm (Figure 5-4(E) vs. Figure 5-4(F)). The relative abundances of SRB and MA at different depths show that SRB accounted for about 20% of the total population at the surface and at 100-μm into the biofilm and the percentage decreased continuously to lower than 3% at the depth of 400 μm (Figure 5-5). This distribution of SRB is consistent with the profile of the in situ sulfide production rate (Figure 5-3). In contrast to the SRB distribution, the MA were detected at below 3% abundance at the surface and at the depth of 100 μm, and increased to 10% at 200 μm, 60% at 500 μm and then 75% at the depth of 700 μm (Figure 5-5).
Figure 5-4. FISH images of different sections of the sewer reactor biofilm. (A) and (B) are images of the biofilm sections cut perpendicular to the substratum with SRB in white (in A) and MA in purple (in B). Arrows indicate the biofilm surface. (C) and (D) are images of biofilm sections cut parallel to the substratum at the depth of 100 μm and 700 μm, respectively, with SRB in white, archaea in red and other bacteria in green, blue and yellow. (E) and (F) are images of biofilm sections cut parallel to the substratum at the depth of 100μm and 700 μm, respectively, with MA in purple, other archaea in red and bacteria in green. The scale bar is 50 μm.
biofilm community structure as determined by 16S rRNA sequence analysis

The 16S rRNA gene sequence analysis was applied to five layers of the biofilm, successively from the surface to the bottom of the biofilm (Layer 1 to Layer 5). The thickness of each layer was 150 μm. The results revealed that the SRB detected in the biofilm were mainly affiliated with five genera and their proportions of the total SRB detected were: *Desulfobulbus* at 33%, *Desulfomicrobium* at 19%, *Desulfovibrio* at 24%, *Desulfitotferula* at 7% and *Desulforegula* at 16%. The heatmap (Figure 5-6(A)) displaying the distribution of the predominant SRB in the different layers (Layer 1 to Layer 5) confirmed that SRB were mainly situated in the outer layer. SRB of the genera *Desulfobulbus, Desulfomicrobium* and *Desulfovibrio* were also observed in the inner layers 4 and 5, but SRB in these inner layers only accounted for less than 10% of the total SRB detected in the biofilm. About 90% of the MA population belonged to the genus of *Methanosaeta*, which use acetate as substrate rather than hydrogen. The other 10% of the MA population mainly belonged to five genera: *Methanospirillum, Methanomethylovorans, Methanobrevibacter, Methanobacterium, Candidatus Methanomethylophilus*. The heatmap (Figure 5-6(B)) also demonstrates that MA was mainly located in the inner layer of the biofilm. Interestingly, *Methanobrevibacter, Methanomethylovorans*, and *Methanospirillum* actually showed higher abundance in the outer layer than in the inner layer. However, these accounted for about 5% of the total MA population detected in the biofilm, thus having only a minor effect on the overall MA distribution in the biofilm.
5.4.5 Mathematical modeling

Mathematical modeling was performed to describe the microbial distribution and the sulfide concentration profiles within the biofilms. The model-predicted relative abundances of SRB and MA fit well with the experimentally results as determined by FISH (Figure 5-7 (A) and (B)). The SRB abundance was 19% at the surface and decreased gradually to below 5% at the depth of 400 μm. The abundance of MA was lower than 5% at the surface and at 100 μm, increased to 65% at the depth of 500 μm and then gradually rose up to 80% at 700 μm. These results are consistent with the experimental data. The model-predicted sulfide concentration profiles within the biofilms also matched well with the data measured by microelectrode. The good agreement between the model-predicted results and the experimental data indicated that the spatial structure of SRB and MA in the anaerobic sewer biofilms resulted from their metabolic transformations, kinetics and interactions with substrates.

Figure 5-6. Heatmap displaying the distribution of the predominant SRB (A) and MA (B) in different biofilm layers from the biofilm surface to the bottom (Layer 1 to Layer 5).
Figure 5-7 Comparison of model-predicted results with the experimentally measured data: (A) Relative abundance of SRB and MA, (B) sulfide concentration profiles in the biofilm.

5.5 Discussion

5.5.1 Distribution of SRB and MA in anaerobic sewer biofilms

This study investigated the distribution of SRB and MA in the sewer biofilms through both experimental and simulation analysis. The results show that SRB were mainly located in the outer layer of the biofilm while MA was mainly situated in the inner layer. The distribution of in situ sulfide production activity was consistent with the distribution of the SRB population. The high sulfide concentration in the inner layer of the biofilm is mainly due to the diffusion transport mechanism. While the sulfide production activity in the inner layer of the biofilm is much lower than that in the outer layer, in the absence of a sulfide sink in this layer, any sulfide produced will accumulate to a level higher than that in the outer layer, providing a concentration gradient for the sulfide produced to be transferred out of the biofilm.

Under anaerobic conditions, SRB and MA are known to compete for the same substrates (primarily acetate and hydrogen) for metabolism. In sulfate-rich environments SRB can normally out-compete MA and this is commonly attributed to the different affinities for substrates of the two populations. The affinity constant for hydrogen of SRB is considered to be around five times lower than that of MA (Kristjansson et al. 1982, Uberto and Bhattacharya 1997). The difference is even stronger in the case of acetate (Schönheit et al. 1982, Kalyuzhnyi and Fedorovich 1998). However, the coexistence of SRB and MA are observed in some systems under sulfate-limiting conditions or even in sulfate
non-limiting conditions where other factors play a role. These included mass transfer limitations (Nielsen 1987), differences in microbial colonization and adhesion properties (Yoda et al. 1987, Santegoeds et al. 1999) or variable sulfide toxicities (Hilton and Oleszkiewicz 1988, Parkin et al. 1990).

In anaerobic sewers, sulfate is normally not depleted, particularly in networks with relatively short HRT. The stratified distribution of SRB and MA suggests that mass transfer limitation plays an important role for the coexistence of SRB and MA in sewer biofilms. We used model simulation to determine the average concentrations of sulfate and fermentable COD in the sewer biofilm (Figure 7). Sulfate could penetrate into the outer layer of the biofilm. In these conditions SRB outcompeted MA due to their higher affinity to acetate and hydrogen, resulting in a relatively higher abundance of SRB in the outer layer. However, sulfate was almost consumed in the outer layer due to the sulfate reduction activity, and could not reach the inner layer (Figure 5-8). As a result, SRB activity and growth was limited in the deeper layers of the biofilm. On the other hand, fermentable COD was not totally consumed by SRB in the outer layer of the biofilm and it was able to penetrate into the inner layers, providing substrate for methanogenesis. Consequently, the co-existence and stratification of these populations is largely a result of the mass transfer of substrates into the biofilm.

The domination of MA in cores of anaerobic granules or at the inner layers of anaerobic biofilms has previously been attributed in some studies to better attachment characteristics of MA (Harmsen et al. 1996, Santegoeds et al. 1999). However, this cannot be a main reason in the case of anaerobic sewer biofilms. During the startup of the sewer reactor the sulfate reducing activity increased much faster than the methanogenic activity in the first several weeks (data not shown), indicating that at the beginning more SRB were attached on the substratum than MA, and that these were the pioneering colonizers of the biofilm. Variations of sulfide toxicities to SRB and MA are also considered as a reason for the coexistence of SRB and MA in some studies (Hilton and Oleszkiewicz 1988, Parkin et al. 1990). However, in our system, the sulfide concentration is far below toxic threshold levels to either group of microorganisms. It has been reported that sulfide concentrations of above 300 ppm are required to induce 50% inhibition of the growth of most SRB and MA (O'Flaherty et al. 1998).

The spatial arrangement of SRB and MA in sewer biofilms revealed in this study is of practical importance. Chemicals such as nitrate, oxygen, magnesium hydroxide and sodium hydroxide are often added to sewers to control the emission of hydrogen sulfide in sewers (Ganigué et al. 2011).
As MA mainly inhabit in the inner layer of the biofilms, they are likely to be protected from being exposed to chemicals added for in-sewer sulfide and methane mitigation. Jiang et al. (2013) found that sewer biofilms were capable of methanogenesis after nitrate dosing for four weeks. To explain this they suggested that nitrate was not able to fully penetrate into the biofilm and it failed to reach the MA in the deeper layer. This is supported by a complete suppression of methane production after they increased the nitrate-dosing rate. Similar results were also observed by Ganigué et al. (2014), where they found methane was produced by the sewer biofilms after oxygen treatment and attributed it to the partial penetration of oxygen. Consequently, given the spatial distribution of MA in sewer biofilms, full penetration of chemicals into biofilms is required to completely control methane production. This should be an important consideration for methane abatement strategies in sewers.

![Figure 5-8. Model predicted sulfate and fermentable COD profiles in the biofilm.](image)

5.5.2 Phylogenetic diversities of SRB and MA in anaerobic sewer biofilms

This study innovatively used pyrosequencing coupled with cryosection to investigate the phylogenetic diversity of SRB and MA in anaerobic sewer biofilms. Pyrosequencing can provide more detailed phylogenetic information than FISH. Together with cryosectioning, the phylogenetic information at different depths in the biofilms was investigated. However, it is worthwhile to note, due to a significant quantity of biomass required for pyrosequencing analysis, the biofilm sections
needed for this purpose was much thicker than those for FISH (150 μm vs. 10 μm in this study). Consequently, the spatial resolution of the method was limited to layers of this size. However, this approach was successful and revealed the microbial diversity of both SRB and MA at two depths of the biofilm, allowing us to attempt to reconstruct the possible metabolic transformations in different regions of the sewer biofilm.

SRB detected in this anaerobic sewer biofilm were mainly affiliated with five genera: *Desulfobulbus, Desulfovibrio, Desulfomicrobium, Desulforegula* and *Desulfatiferula*. The first four genera have also been found in aerobic/anoxic wastewater biofilms, with *Desulfobulbus, Desulfovibrio, Desulfomicrobium* appearing in higher abundances (Okabe et al. 1999, Ito et al. 2002a, Ito et al. 2002b, Okabe et al. 2003). Also, *Desulfobulbus* and *Desulfovibrio* are reported to be numerically important in anaerobic methanogenic-sulfidogenic aggregates (Santegoeds et al. 1999). *Desulfatiferula* is a newly defined genus by Cravo-Laureau et al. (2007) and members are mesophilic, Gram-negative sulfate-reducing bacteria.

SRB can use many different compounds as electron acceptors besides acetate and hydrogen (Kalyuzhnyi and Fedorovich 1998, Fedorovich et al. 2003, Muyzer and Stams 2008). In the studied sewer biofilm, *Desulfobulbus* spp., with 98.4% sequence identity to *Desulfobulbus propionicus*, are a well-known propionate-utilizing SRB (Okabe et al. 2003, Muyzer and Stams 2008, Pagani et al. 2011). As SRB tend to outcompete acetogenic bacteria for propionate utilisation due to their stronger affinity for this carbon substrate (Rinzema et al. 1988, Uberoi and Bhattacharya 1997), the high fraction of *Desulfobulbus* in the SRB population explained the low propionate concentration in the effluent (data not shown). *Desulfovibrio* spp. can use hydrogen, formate, lactate, pyruvate and many other organic compounds to reduce sulfate (Voordouw 1995). It has been suggested that *Desulfovibrio* is an important member of the hydrogen-utilizing bacteria in wastewater biofilms (Ito et al. 2002b, Okabe et al. 2003). *Desulfomicrobium* spp. are also able to use various substrates such as hydrogen, acetate and lactate (Dias et al. 2008, Barton and Fauque 2009). It is recognized that hydrogen, acetate and propionate are important electron donors for sulfate reduction in sewer systems (Sharma et al. 2008b, Guisasola et al. 2009). However, in this study, we also observed the proliferation of SRB which normally grow on large molecular organic substrates rather than hydrogen, acetate and propionate. *Desulforegula* and *Desulfatiferula* are known to use long-chain fatty acids and long-chain alkenes to reduce sulfate (Rees and Patel 2001, Cravo-Laureau et al. 2007). Also, some *Desulfovibrio* spp. are known to use amino acids and many other organic compounds as electron donors (Voordouw 1995, Hernandez-Eugenio et al. 2000). It is thought that SRB can be outcompeted by very fast growing fermentative (acidogenic) bacteria for the large
molecular organic substrates (Widdel 1988, Fedorovich et al. 2003). However, since fermentable COD or sCOD is abundant in sewer systems and they would not be totally used by fermentative bacteria (See Table 5-1 and Figure 5-8), the coexistence of SRB using large molecular organic substrates with fast growing fermentative bacteria is possible. From an ecological viewpoint, it is interesting to understand how different SRB, which use different electron donors, compete for sulfate when it is limiting. However, to date, only a few studies have addressed this competition for sulfate (Muyzer and Stams 2008). The coexistence of different SRB in our biofilm seems to indicate that their affinities to sulfate are similar.

Though SRB mainly inhabited the outer layer of the sewer biofilm, small amounts of Desulfobulbus, Desulfomicrobium, Desulfovibrio were also observed in the inner layers (Figure 5-7). Since sulfate did not penetrate here, the SRB in the inner layers probably grew by fermenting organic matter. Desulfobulbus species can ferment lactate and ethanol (plus carbon dioxide) to acetate and propionate in the absence of sulfate, and many Desulfovibrio and Desulfomicrobium species grow by fermenting pyruvate to form acetate, carbon dioxide and hydrogen as products (Voordouw 1995, Muyzer and Stams 2008, Barton and Fauque 2009). In comparison, Desulforegula and Desulfatiferula were not detected in the inner layers, as these SRB can hardly ferment organic matter (Rees and Patel 2001, Cravo-Laureau et al. 2007).

Of the MA, about 90% of the population was Methanosaeta, which is an obligate acetoclastic methanogen. Therefore, acetate was likely the main substrate for methanogenesis in anaerobic sewer systems. Currently, the only genera known to use acetate for methanogenesis are Methanosarcina and Methanosaeta. However, Methanosarcina failed to inhabit in the anaerobic sewer biofilms, which is consistent with the finding that usually only one acetoclastic methanogen dominates such anaerobic environments (Leclerc et al. 2004). It is likely Methanosaeta outcompeted Methanosarcina due to differences in their affinities for acetate. Methanosaeta is a superior acetate utilizer in that it can use acetate at concentrations as low as 5–20 μM, while Methanosarcina requires a minimum concentration of about 1 mM (Jetten et al. 1992). The acetate concentration in the wastewater was about 0.6 mM and would therefore not favor the growth of Methanosarcina.

MA that use other substrates such as hydrogen or methylated compounds only accounted for less than 10% of the total MA population in the sewer biofilm. The hydrogen-utilizing MA mainly belonged to genera of Methanobrevibacter, Methanospirillum and Methanobacterium. Theoretically, the relative contribution of acetate and hydrogen in methanogenesis is close to 2:1, given the fact that the fermentation of hexose yields 4 H₂, 2 acetate, and 2 CO₂ and that 4 H₂ are required to
reduce CO$_2$ to methane (Liu and Whitman 2008). One possible reason for the low abundance of hydrogenotrophic MA in the sewer biofilms was the low hydrogen concentration in the system. This can be explained in that hydrogenotrophic MA was out-competed by the hydrogen-utilizing SRB, which have higher affinity and lower threshold values for hydrogen (Kristjansson et al. 1982, Uberoi and Bhattacharya 1997). In addition, at 20°C, homoacetogenesis might occur, which could also outcompete methanogenesis for hydrogen (Conrad et al. 1989). It is interesting to note that although there were more SRB in the outer layer of the biofilm, the hydrogen-utilizing MA were more abundant in the outer layer as opposed to the inner layers (Figure 5). Though hydrogen was largely consumed by SRB, still more hydrogen was available in the outer layer, due to H$_2$-producing bacteria having a higher abundance in the outer layer (data not shown). *Methanomethylovorans* and *Candidatus Methanomethylophilus* are known to use methylated compounds such as methanol, methanethiol and dimethyl sulfide for methanogenesis (Lomans et al. 1999b, Borrel et al. 2012, Cha et al. 2013). Their low abundance could be explained by the relatively low concentrations of these substrates in the wastewater (Hwang et al. 1995, Sun et al. 2014b).

### 5.6 Supporting Information

![Figure 5-S1](image.png)

Figure 5-S1. Pumping pattern applied to the sewer reactor in an 8-hour period, and the resulting hydraulic retention time (HRT) of sewage in the reactor. The vertical solid lines refer to the pumping events and dashed lines represent the HRT of wastewater in the reactor.
Table 5-S1 oligonucleotide probes used for fluorescence *in situ* hybridization (FISH) in this study.

<table>
<thead>
<tr>
<th>Probe-label</th>
<th>Probe target</th>
<th>Sequence (5’→3’)</th>
<th>FA(%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB338mix- FITC</td>
<td>Bacteria</td>
<td>GCTGCCCTCCCGTAGGAGT&lt;br&gt;GCAGCACCCTCAGGGTAG &lt;br&gt;GCTGCCACCCCTAGGGTAG</td>
<td>-</td>
<td>(Daims et al. 1999)</td>
</tr>
<tr>
<td>ARC915 - CY3</td>
<td>Archaea</td>
<td>GTGCTCCCCCGCCCAATTCCT</td>
<td>-</td>
<td>(Raskin et al. 1994)</td>
</tr>
<tr>
<td>DELTA495a - CY3</td>
<td>Most Deltaproteobacteria &lt;br&gt;most Gemmatimonadetes</td>
<td>AGTTAGCCGCGTCTCCT</td>
<td>35</td>
<td>(Löy et al. 2002)</td>
</tr>
<tr>
<td>cDELTA495a</td>
<td>Competitor for DELTA495a</td>
<td>AGTTAGCCGCGTCTCCT</td>
<td>35</td>
<td>(Macalady et al. 2006)</td>
</tr>
<tr>
<td>DELTA495b - CY3</td>
<td>Some Deltaproteobacteria</td>
<td>AGT TAG CGGCGCTCCT</td>
<td>35</td>
<td>(Löy et al. 2002)</td>
</tr>
<tr>
<td>cDELTA495b</td>
<td>Competitor for DELTA495b</td>
<td>AGTTAGCCGCGTCTC(T/G)T</td>
<td>35</td>
<td>(Lücke et al. 2007)</td>
</tr>
<tr>
<td>DELTA495c - CY3</td>
<td>Some Deltaproteobacteria</td>
<td>AAT TAGCGGCTGTCCTCCT</td>
<td>35</td>
<td>(Löy et al. 2002)</td>
</tr>
<tr>
<td>cDELTA495c</td>
<td>Competitor for DELTA495c</td>
<td>AATTAGCCGCGTCTCCT</td>
<td>35</td>
<td>(Lücke et al. 2007)</td>
</tr>
<tr>
<td>SRB385 – CY5</td>
<td>Most Desulfovibrio &lt;br&gt;and other Bacteria</td>
<td>CGGCCTCGCTGCGTACGG</td>
<td>35</td>
<td>(Amann et al. 1990a)</td>
</tr>
<tr>
<td>SRB385Db – CY5</td>
<td>Desulfobacteriales, &lt;br&gt;Desulfuromonales, &lt;br&gt;Syntrophobacteriales, &lt;br&gt;Myxococcales, &lt;br&gt;and other Bacteria</td>
<td>CGGCCTGCTGCTGTCAGG</td>
<td>35</td>
<td>(Rabus et al. 1996)</td>
</tr>
<tr>
<td>DSBAC357 – CY5</td>
<td>Desulfobacteriales, &lt;br&gt;Desulfuromonales, &lt;br&gt;Syntrophobacteriales, &lt;br&gt;Myxococcales, &lt;br&gt;and other Bacteria</td>
<td>CCATGCCTACGAGTCACAC</td>
<td>35</td>
<td>(Lücke et al. 2007)</td>
</tr>
<tr>
<td>c1DSBAC357</td>
<td>Competitor for DSBAC357</td>
<td>CCATGCCTACGAGTCACAC</td>
<td>35</td>
<td>(Lücke et al. 2007)</td>
</tr>
<tr>
<td>c2DSBAC357</td>
<td>Competitor for DSBAC357</td>
<td>CCATGCCTACGAGTCACAC</td>
<td>35</td>
<td>(Lücke et al. 2007)</td>
</tr>
<tr>
<td>MSMX860– CY5</td>
<td>Methanosarcinales</td>
<td>GGCTCGCTCCCAGGGCTCCTCCT</td>
<td>45</td>
<td>(Raskin et al. 1994)</td>
</tr>
<tr>
<td>MG1200b – CY5</td>
<td>most Methanomicrobiales</td>
<td>CCGTAAATTCGGGCGATGCTG</td>
<td>20</td>
<td>(Crocetti et al. 2006)</td>
</tr>
<tr>
<td>MB1175– CY5</td>
<td>most Methanobacteriales</td>
<td>TACCGTGCTGACTGTTCCTCCTC</td>
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<td>(Raskin et al. 1994)</td>
</tr>
<tr>
<td>MC1109– CY5</td>
<td>Methanococcales</td>
<td>GCAACATAGGGCACGGGTCTC</td>
<td>45</td>
<td>(Raskin et al. 1994)</td>
</tr>
<tr>
<td>MC504– CY5</td>
<td>Methanocaldococcaceae</td>
<td>GGCTGCTGCACCAGACTGTCACC</td>
<td>55</td>
<td>(Crocetti et al. 2006)</td>
</tr>
<tr>
<td>cMC504</td>
<td>Competitor for MC504</td>
<td>GGCTGCTGCTGACTGTCACC</td>
<td>55</td>
<td>(Crocetti et al. 2006)</td>
</tr>
</tbody>
</table>

1. FA: Formamide concentration
Figure 5-S2. Schematic of the biofilm model. Hydrolysis (dotted line), Fermentation (dash–dotted line), Sulfate reduction (solid line), and methanogenesis (dashed line); adapted from Guisasola et al. (2009).

Table 5-S2. The definition and units of model components.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xs</td>
<td>Readily biodegradable COD</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>Xs₂</td>
<td>Hardly biodegradable COD</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>SF</td>
<td>Fermentable COD</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>SPROP</td>
<td>Propionate</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>SAC</td>
<td>Acetate</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>SH₂</td>
<td>Hydrogen</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>S CH₄</td>
<td>Methane</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>S SO₄</td>
<td>Sulfate</td>
<td>kg S/m³</td>
</tr>
<tr>
<td>SH₂S</td>
<td>Total dissolved sulfide</td>
<td>kg S/m³</td>
</tr>
<tr>
<td>XH,AC</td>
<td>Heterotopic bacteria (acetogenesis)</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XH,PROP</td>
<td>Heterotopic bacteria (acidogenesis)</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XSRB,PROP</td>
<td>SRB grown on propionate</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XSRB,AC</td>
<td>SRB grown on acetate</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XSRB,H₂</td>
<td>SRB grown on H₂</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XMA,AC</td>
<td>MA grown on acetate</td>
<td>kg COD/m³</td>
</tr>
<tr>
<td>XMA,H₂</td>
<td>MA grown on H₂</td>
<td>kg COD/m³</td>
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<tr>
<td>X₁</td>
<td>Inert particular COD</td>
<td>kg COD/m³</td>
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Table 5-S3. Stoichiometry of the biofilm model.

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<tr>
<th>Processes</th>
<th>$S_1$</th>
<th>$S_{HF,P}$</th>
<th>$S_{AC}$</th>
<th>$S_{HAC}$</th>
<th>$S_{HR}$</th>
<th>$S_{AC}$</th>
<th>$X_{HAC}$</th>
<th>$X_{PROP}$</th>
<th>$X_{H2,AC}$</th>
<th>$X_{H2,PROP}$</th>
<th>$X_{H2,HR}$</th>
<th>$X_{H2,HR}$</th>
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<tr>
<td>Hydrolysis of readily biodegradable COD</td>
<td>1</td>
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<td></td>
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<tr>
<td>Hydrolysis of hardly biodegradable COD</td>
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<td>-1</td>
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</tr>
<tr>
<td>Fermentation</td>
<td>$\frac{1}{Y_{H,AC}}$</td>
<td>$\frac{2 \times (1 - Y_{H,AC})}{3 \times Y_{H,AC}}$</td>
<td>$\frac{1 - Y_{H,AC}}{3 \times Y_{H,AC}}$</td>
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<tr>
<td>Acetogenesis</td>
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<tr>
<td>Fermentation (Acetoclastic)</td>
<td>$\frac{1}{Y_{H,PROP}}$</td>
<td>$\frac{7 \times (1 - Y_{H,PROP})}{9 \times Y_{H,PROP}}$</td>
<td>$\frac{2 \times (1 - Y_{H,PROP})}{9 \times Y_{H,PROP}}$</td>
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<td>Methane production (Acetoclastic)</td>
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<tr>
<td>Methane production (Hydrogentrophic)</td>
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<tr>
<td>H$_2$S production (using propionate)</td>
<td>$\frac{1}{Y_{H,PROP}}$</td>
<td>$\frac{4 \times (1 - Y_{H,PROP})}{7 \times Y_{H,PROP}}$</td>
<td>$\frac{3 \times (1 - Y_{H,PROP})}{14 \times Y_{H,PROP}}$</td>
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<td>H$_2$S production (using acetate)</td>
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<tr>
<td>H$_2$S production (using hydrogen)</td>
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<td>0.1</td>
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<tr>
<td>Decay of $X_{H2,PROP}$</td>
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<td>0.1</td>
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<tr>
<td>Decay of $X_{H2,AC}$</td>
<td>0.9</td>
<td>0.1</td>
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<tr>
<td>Decay of $X_{H2,HR}$</td>
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<td>0.1</td>
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86
<table>
<thead>
<tr>
<th>No.</th>
<th>Processes</th>
<th>Kinetic expressions</th>
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<tbody>
<tr>
<td>1</td>
<td>Hydrolysis of readily biodegradable COD</td>
<td>$k_{S1} \cdot X_{S1}$</td>
</tr>
<tr>
<td>2</td>
<td>Hydrolysis of hardly biodegradable COD</td>
<td>$k_{S2} \cdot X_{S1}$</td>
</tr>
<tr>
<td>3</td>
<td>Fermentation</td>
<td>$m_{XH,AC} \cdot \frac{S_F}{K_{XH,AC,F} + S_F} \cdot X_{H,AC}$</td>
</tr>
<tr>
<td>4</td>
<td>Fermentation</td>
<td>$m_{XH,PROP} \cdot \frac{S_F}{K_{XH,PROP,F} + S_F} \cdot X_{H,PROP}$</td>
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<tr>
<td>5</td>
<td>Methane production (Acetoclastic)</td>
<td>$m_{XMA,AC} \cdot \frac{S_{AC}}{K_{XMA,AC} + S_{AC}} \cdot X_{MA,AC}$</td>
</tr>
<tr>
<td>6</td>
<td>Methane production (Hydrogentrophic)</td>
<td>$m_{XMA,H2} \cdot \frac{S_{H2}}{K_{XMA,H2} + S_{H2}} \cdot X_{MA,H2}$</td>
</tr>
<tr>
<td>7</td>
<td>H$_2$S production (using propionate)</td>
<td>$m_{XSRB,PROP} \cdot \frac{S_{PROP}}{K_{XSRB,PROP} + S_{PROP}} \cdot \frac{S_{SO4}}{K_{XSRB,PROP,SO4} + S_{SO4}} \cdot X_{SRB,PROP}$</td>
</tr>
<tr>
<td>8</td>
<td>H$_2$S production (using acetate)</td>
<td>$m_{XSRB,AC} \cdot \frac{S_{AC}}{K_{XSRB,AC} + S_{AC}} \cdot \frac{S_{SO4}}{K_{XSRB,AC,SO4} + S_{SO4}} \cdot X_{SRB,AC}$</td>
</tr>
<tr>
<td>9</td>
<td>H$_2$S production (using hydrogen)</td>
<td>$m_{XSRB,H2} \cdot \frac{S_{H2}}{K_{XSRB,H2} + S_{H2}} \cdot \frac{S_{SO4}}{K_{XSRB,H2,SO4} + S_{SO4}} \cdot X_{SRB,H2}$</td>
</tr>
<tr>
<td>10</td>
<td>Decay of X$_{H,AC}$</td>
<td>$k_{dec} c_{XH,AC} \cdot X_{H,AC}$</td>
</tr>
<tr>
<td>11</td>
<td>Decay of X$_{H,PROP}$</td>
<td>$k_{dec} c_{XH,PROP} \cdot X_{H,PROP}$</td>
</tr>
<tr>
<td>12</td>
<td>Decay of X$_{MA,AC}$</td>
<td>$k_{dec} c_{XMA,AC} \cdot X_{MA,AC}$</td>
</tr>
<tr>
<td>13</td>
<td>Decay of X$_{MA,H2}$</td>
<td>$k_{dec} c_{XMA,H2} \cdot X_{MA,H2}$</td>
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<tr>
<td>14</td>
<td>Decay of X$_{SRB,PROP}$</td>
<td>$k_{dec} c_{XSRB,PROP} \cdot X_{SRB,PROP}$</td>
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<tr>
<td>15</td>
<td>Decay of X$_{SRB,AC}$</td>
<td>$k_{dec} c_{XSRB,AC} \cdot X_{SRB,AC}$</td>
</tr>
<tr>
<td>16</td>
<td>Decay of X$_{SRB,H2}$</td>
<td>$k_{dec} c_{XSRB,H2} \cdot X_{SRB,H2}$</td>
</tr>
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</table>
Table S5. Kinetic parameters of the biofilm model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{S1}$</td>
<td>First order hydrolysis rate for readily biodegradable COD</td>
<td>h$^{-1}$</td>
<td>0.210</td>
<td>(Sharma et al. 2008b)</td>
</tr>
<tr>
<td>$k_{S2}$</td>
<td>First order hydrolysis rate for hardly biodegradable COD</td>
<td>h$^{-1}$</td>
<td>2.10×10$^{-2}$</td>
<td>(Sharma et al. 2008b)</td>
</tr>
<tr>
<td>$m_{X_{HAC}}$</td>
<td>Maximum growth rate of $X_{HAC}$</td>
<td>h$^{-1}$</td>
<td>0.125</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$K_{X_{HAC},F}$</td>
<td>Half saturation value of $X_{HAC}$ for $S_F$</td>
<td>kg COD/m$^3$</td>
<td>0.500</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$m_{X_{HPROP}}$</td>
<td>Maximum growth rate of $X_{HPROP}$</td>
<td>h$^{-1}$</td>
<td>0.125</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$K_{X_{HPROP},F}$</td>
<td>Half saturation value of $X_{HPROP}$ for $S_F$</td>
<td>kg COD/m$^3$</td>
<td>0.500</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$m_{X_{MA,AC}}$</td>
<td>Maximum growth rate of $X_{MA,AC}$</td>
<td>h$^{-1}$</td>
<td>0.010</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$K_{X_{MA,AC}}$</td>
<td>Half saturation value of $X_{MA,AC}$ for $S_{AC}$</td>
<td>kg COD/m$^3$</td>
<td>0.409</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$m_{X_{MA,H2}}$</td>
<td>Maximum growth rate of $X_{MA,H2}$</td>
<td>h$^{-1}$</td>
<td>5.00×10$^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$K_{X_{MA,H2}}$</td>
<td>Half saturation value of $X_{MA,H2}$ for $S_{H2}$</td>
<td>kg COD/m$^3$</td>
<td>1.10×10$^{-4}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$m_{X_{SRB,PROP}}$</td>
<td>Maximum growth rate of $X_{SRB,PROP}$</td>
<td>h$^{-1}$</td>
<td>3.71×10$^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$K_{X_{SRB,PROP}}$</td>
<td>Half saturation value of $X_{SRB,PROP}$ for $S_F$</td>
<td>kg COD/m$^3$</td>
<td>5.62×10$^{-3}$</td>
<td>(Uberoi and Bhattacharya 1997)</td>
</tr>
<tr>
<td>$K_{X_{SRB,PROP},SO4}$</td>
<td>Half saturation value of $X_{SRB,PROP}$ for $S_{SO4}$</td>
<td>kg S/m$^3$</td>
<td>3.40×10$^{-3}$</td>
<td>(Uberoi and Bhattacharya 1997)</td>
</tr>
<tr>
<td>$m_{X_{SRB,AC}}$</td>
<td>Maximum growth rate of $X_{SRB,AC}$</td>
<td>h$^{-1}$</td>
<td>2.30×10$^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$K_{X_{SRB,AC}}$</td>
<td>Half saturation value of $X_{SRB,AC}$ for $S_{AC}$</td>
<td>kg COD/m$^3$</td>
<td>4.10×10$^{-3}$</td>
<td>(Harada et al. 1994)</td>
</tr>
<tr>
<td>$K_{X_{SRB,AC},SO4}$</td>
<td>Half saturation value of $X_{SRB,AC}$ for $S_{SO4}$</td>
<td>kg S/m$^3$</td>
<td>3.20×10$^{-3}$</td>
<td>(Fedorovich et al. 2003)</td>
</tr>
<tr>
<td>$m_{X_{SRB,H2}}$</td>
<td>Maximum growth rate of $X_{SRB,H2}$</td>
<td>h$^{-1}$</td>
<td>5.70×10$^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$K_{X_{SRB,H2}}$</td>
<td>Half saturation value of $X_{SRB,H2}$ for $S_{H2}$</td>
<td>kg COD/m$^3$</td>
<td>2.90×10$^{-5}$</td>
<td>(Harada et al. 1994)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Unit</td>
<td>Value</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------------------------------------------</td>
<td>-----------------------</td>
<td>------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>$K_{\text{XSRB,H2,SO}_4}$</td>
<td>Half saturation value of $X_{\text{SRB,H2}}$ for $S_{\text{SO}_4}$</td>
<td>kg S/m$^3$</td>
<td>$3.30 \times 10^{-3}$</td>
<td>(Fedorovich et al. 2003)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XHLAC}}}$</td>
<td>First order decay rate of $X_{\text{HLAC}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XLPROP}}}$</td>
<td>First order decay rate of $X_{\text{LPROP}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XMA,AC}}}$</td>
<td>First order decay rate of $X_{\text{MA,AC}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XMA,H2}}}$</td>
<td>First order decay rate of $X_{\text{MA,H2}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XSRB,F}}}$</td>
<td>First order decay rate of $X_{\text{SRB,F}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XSRB,AC}}}$</td>
<td>First order decay rate of $X_{\text{SRB,AC}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$k_{\text{dec}_{\text{XSRB,H2}}}$</td>
<td>First order decay rate of $X_{\text{SRB,H2}}$</td>
<td>h$^{-1}$</td>
<td>$8.33 \times 10^{-4}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$Y_{\text{XHLAC}}$</td>
<td>Yield of $X_{\text{HLAC}}$</td>
<td>kg COD/kg COD</td>
<td>0.15</td>
<td>(Siegrist et al. 2002)</td>
</tr>
<tr>
<td>$Y_{\text{XLPROP}}$</td>
<td>Yield of $X_{\text{LPROP}}$</td>
<td>kg COD/kg COD</td>
<td>0.10</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$Y_{\text{XMA,AC}}$</td>
<td>Yield of $X_{\text{MA,AC}}$</td>
<td>kg COD/kg COD</td>
<td>$5.00 \times 10^{-2}$</td>
<td>(Batstone et al. 2002)</td>
</tr>
<tr>
<td>$Y_{\text{XMA,H2}}$</td>
<td>Yield of $X_{\text{MA,H2}}$</td>
<td>kg COD/kg COD</td>
<td>$1.78 \times 10^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$Y_{\text{XSRB,AC}}$</td>
<td>Yield of $X_{\text{SRB,AC}}$</td>
<td>kg COD/kg COD</td>
<td>$5.68 \times 10^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$Y_{\text{XSRB,H2}}$</td>
<td>Yield of $X_{\text{SRB,H2}}$</td>
<td>kg COD/kg COD</td>
<td>$7.53 \times 10^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
<tr>
<td>$Y_{\text{XSRB,PROP}}$</td>
<td>Yield of $X_{\text{SRB,PROP}}$</td>
<td>kg COD/kg COD</td>
<td>$5.00 \times 10^{-2}$</td>
<td>(Kalyuzhnyi et al. 1998)</td>
</tr>
</tbody>
</table>

Note: The parameter values are applied under mesophilic conditions.
Chapter 6 Feasibility of sulfide control in sewers by reuse of iron rich coagulation sludge

6.1 Abstract

Sewer odour and corrosion caused by hydrogen sulfide is a major issue in sewer management. Dosage of iron salt is the most commonly used method for sulfide control in sewer networks but incurs high chemical costs. In this study, we experimentally investigate the feasibility of the use of iron rich drink water treatment sludge (iron sludge) for sulfide control in sewer networks. A lab-scale rising main sewer biofilm reactor was operated and the sulfide concentrations with iron sludge dosing at different dosing rates were compared to the case without dosing. The sulfide concentration in the effluent decreased from 15.5-19.8 mgS/L (without dosing) to below 0.7 - 2.3 mgS/L at a sludge dosing rate of iron to total dissolved sulfur ratio (Fe:S) of 1:1 with a further removal of sulfide possible with prolonged reaction time. In fact, batch tests revealed an Fe consumption to sulfide removal ratio of 0.5±0.02 (mole:mole), suggesting the possible occurrence of other reactions involving the removal of sulfide. Modelling revealed that the reaction between iron in sludge and sulfide has reaction orders of 0.65±0.01 and 0.77±0.02 with respect of Fe and sulfide concentrations, respectively. The addition of sludge slightly increased the total chemical oxidation demand (tCOD) concentration (by approximately 12%) as expected, but slightly decreased the soluble chemical oxidation demand (sCOD) and methane formation by 7% and 20%, respectively. Same phosphate removal (13%) was also observed at the sludge dosing rate of 1:1 (Fe:S), which can be beneficial to nutrient removal from the wastewater. Overall, this study showed that dosing iron sludge to sewers is an effective strategy for sulfide removal in sewer systems, which would also reduce the sludge disposal costs from drinking water treatment works. However, its potential side-effects on sewer sedimentation and on the wastewater treatment plant effluent remained to be investigated.

6.2 Introduction

Hydrogen sulfide generation is a major problem in sewer management. It causes sewer corrosion, the release of obnoxious odours and health risks sewer workers (WERF 2007a). It has an enormous economic impact due to the need for rehabilitation or replacement of corroded sewer pipes and the need for hydrogen sulfide control strategies (Sydney et al. 1996, Brongers et al. 2002, WERF 2007a). Methods to control hydrogen sulfide in sewer networks normally involve the addition of large amounts of chemicals for either the mitigation of hydrogen sulfide after its formation or by controlling hydrogen sulfide generation through suppressing sulfate reduction, as described in detail.
by Zhang et al. (2008) and Ganigue et al. (2011). Iron salts are commonly used chemicals for sulfide control by oxidizing and/or precipitating sulfide. A recent industry survey showed that iron salts comprise ~66% of the total amount of chemicals dosed for sulfide control in Australia (Ganigüé et al. 2011). Although iron dosage is an effective sulfide control method, it requires continuous addition, which incurs high chemical costs (Ganigüé et al. 2011, Jiang et al. 2011a). Therefore, a cheaper source of iron is highly desirable for the water industry.

Iron salts are also used in large amounts and play an essential role in the production of drinking water, for the removal of natural organic material (NOM), colour and turbidity (Henderson et al. 2009). Its use results in the production of large amounts of iron rich sludge, which requires handling and ultimately disposal through e.g. landfill (Dentel 1991). If coagulants could be successfully recovered and reused this would enable a significant reduction in chemical usage during water treatment processes. Therefore, several studies investigated the feasibility of recovery and direct reuse at drinking water treatment plants, as reviewed by Babatunde and Zhao (2007) and Keeley et al. (2012). Various studies showed that it is feasible to recover coagulants, although the obtained quality of the recovered coagulant (e.g. the presence of NOM and Heavy Metals) in most cases did not allow for direct reuse in the drinking water treatment process (Keeley et al. 2012). Therefore, several studies aimed to increase the product quality of the recovered coagulant to enable direct reuse in the drinking water treatment process, using different approaches including Donnan dialysis (Prakash and Sengupta 2003, Prakash et al. 2004, Prakash and Sengupta 2005), liquid ion exchange (Sthapak et al. 2008) and ion exchange with a cation resin (Petruzzelli et al. 2000). Although these studies achieved a sufficient product quality for direct re-use, their practical implementation remains restricted due to their unfavorable process economics compared to the use of fresh coagulants (Keeley et al. 2012).

Considering the high iron concentration in coagulation sludge (in case iron salts are used as coagulants), it has the potential to be beneficially reused in sewer networks for sulfide control. In comparison to direct reuse for drinking water production, the product quality in terms of the presence of organics and trace amount of metals is far less restrictive. Surprisingly, to the author’s best knowledge, the feasibility of coagulation sludge for sulfide control in sewer networks has not been studied in detail yet. Therefore, this study aims to experimentally evaluate the potential of iron rich drinking water treatment sludge (hereinafter refer as to “iron sludge”) for sulfide control in sewer networks. To do so, iron sludge was added to a simulated rising main. Online measurement was used to enable continuous monitoring of the dissolved sulfide concentrations. Subsequently, batch tests were performed to determine the stoichiometry and kinetics of the reaction between
sulfide and the iron in sludge. A kinetic expression of the reaction was proposed and calibrated using the batch tests results.

### 6.3 Materials and methods

#### 6.3.1 Sludge source and characteristics

The iron sludge was obtained from a local drinking water treatment plant (Australia), where FeCl₃ was dosed as coagulant. The main characteristics of the sludge are shown in Table 6-1. Iron was the predominant component of metals in the sludge with a concentration of 155±3.4 g/kg dry mass (DM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (g/L)</td>
<td>64.20±1.31</td>
<td>Ni (mg/g DM)</td>
<td>0.04±0.001</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>22.10±1.23</td>
<td>Pb (mg/g DM)</td>
<td>0.09±0.002</td>
</tr>
<tr>
<td>Fe (mg/g DM)</td>
<td>155.0±3.40</td>
<td>Zn (mg/g DM)</td>
<td>0.14±0.003</td>
</tr>
<tr>
<td>Al (mg/g DM)</td>
<td>9.03±0.28</td>
<td>TKN (mg/g DM)</td>
<td>11.87±0.20</td>
</tr>
<tr>
<td>Mn (mg/g DM)</td>
<td>3.58±0.08</td>
<td>TKP (mg/g DM)</td>
<td>2.24±0.05</td>
</tr>
<tr>
<td>Cu (mg/g DM)</td>
<td>0.03±0.001</td>
<td>tCOD (mg/g DM)</td>
<td>352.0±9.0</td>
</tr>
<tr>
<td>Cd (mg/g DM)</td>
<td>0.01±0.0002</td>
<td>sCOD (mg/g DM)</td>
<td>3.08±0.09</td>
</tr>
</tbody>
</table>

DM: dry mass

#### 6.3.2 Lab-scale sewer system set up and operation

A 0.75 L gas-tight cylindrical reactor, made of Perspex™, was set up to mimic a pressure sewer pipe (Figure 6-1A). The inner diameter of the reactor was 80 mm with a height of 149 mm, resulting in an area to volume ratio (A/V) of 70.9 m⁻¹. Biofilms developed on the wall and the inner surface of the reactor lids. Mixing was continuously provided by a magnetic stirrer (Heidolph MR3000) at 250 rpm under the reactor, so there was no biofilm growing on the bottom in the reactor. Previous studies showed that the biofilms on the wall and lid were primarily responsible for sulfide and methane formation in the reactor, with the suspended biomass in sewage playing a negligible role (Guisasola et al. 2008).

Domestic sewage, collected on a weekly basis from a local wet well (Brisbane, Queensland), was used as the feed of the reactor. The sewage compositions varied to a certain extent in terms of sulfate, volatile fatty acids (VFA), and chemical oxygen demand (COD) concentrations. The sewage typically contained sulfate at concentrations of 10-25 mg S/L, sulfide at < 3mg S/L, soluble
COD (sCOD) at 200-300 mg/L, VFA at 50-120 mg COD/L and approximately 50 mg N/L of ammonium. Negligible amounts of sulfite, thiosulfate (<1 mg S/L), nitrate and nitrite (<1 mg N/L) were present. The sewage was stored in a cold room (4°C) to minimize biological transformation, and was heated up to 20±1°C prior to being pumped into the reactors (Figure 6-1A).

Figure 6-1. (A) Schematic representation of a lab-scale sewer reactor; (B) The pumping pattern and hydraulic retention time (HRT) of the system in an 8-hour period. The vertical solid lines refer to the pumping events and dashed lines represent HRT of wastewater in the reactor.

The sewage was fed to the reactor intermittently by a peristaltic pump (Masterflex 7520-47) to simulate the typical flow pattern of rising main sewers. For easier reactor monitoring (see Section 6.3.3), each day was divided into three identical 8-hour periods. Figure 6-1B shows the pumping patterns applied to the reactor for an 8-hour period and the hydraulic retention time (HRT) of sewage in the reactor, respectively. Every pumping event lasted for 2 min, delivering one reactor volume (0.75L) of wastewater into the reactor. The HRT of the wastewater ranged between 15 minutes to 3 hours. Consequently, the reactor mimicks a upstream section of a real sewer pipe (Guisasola et al. 2008). The reactor operated for 6 months to reach pseudo steady-state conditions,
prior to sludge dosing tests (see section 6.3.3), as evidenced by the stable sulfide profiles in the reactor over cycles.

6.3.3 Sludge dosing tests
One series of sludge dosing tests lasted for four successive days to evaluate the effectiveness of iron sludge on sulfide control in the reactors. On the first day, the reactor was operated without sludge dosing, representing the control condition. In the following three days, the iron sludge was dosed into the reactor at various dosing rates. The sludge dosing rate was defined based on the molar ratio (Fe:S) of total iron concentration resulting from sludge dosing versus the total dissolved sulfur (sulfide + sulfate + sulfite + thiosulfate) concentration in the sewage. In total, three different Fe:S dosing ratios were used, which are 1:1, 1:1.2 and 1:1.5 Fe:S. On each day, the amount of sludge was added into the reactor manually immediately after each pumping event in the first 8-h cycle. The sludge was manually added by a syringe through a small port on the reactor, thereby maintaining oxygen-free conditions in the reactor at all time. After each 8-h cycle with sludge dosing, the dosing was stopped in the following two 8-h cycles (16 h) and the reactor was allowed to recover, before another 8-h cycle of sludge dosing was applied. To ensure that all sewage in the reactor was completely replaced with fresh sewage, 3 L of wastewater were transferred to the reactor by each pumping event (the volume of the reactor is 0.75L). The above tests were carried out in duplicate.

The dissolved sulfide concentration was monitored online using the S::CAN VU-VIS spectrolyser (Messtechnik GmbH, Austria), as previously described by Sutherland-Stacey et al. (2008). The sewage in the reactor was continuously diverted to the spectrometer optics of the sensor by a peristaltic pump (Masterflex 7520-47) through a bypass system (Figure 1A), as described by Sun et al. (2014a). The sensor was calibrated before and during the tests by offline dissolved sulfide analysis using ion chromatography (see Section 6.3.5).

To assess the impact of sludge dosing on wastewater characteristics and methane emissions in the reactor, the total COD (tCOD), sCOD, phosphate and methane concentrations were monitored in the control test (without dosing) and the test with a Fe:S dosing rate of 1:1. Samples were taken before and after each pumping event (representing effluent and influent of the reactor) during the first 8-h cycle of each day and analyzed using methods described in Section 6.3.5.

6.3.4 Batch tests to determine stoichiometry and kinetics of the reaction between iron sludge and dissolved sulfide
Batch tests were carried out in a reactor identified to that use in the sludge test but without biofilm. At the beginning of each test, the reactor was completely filled with fresh sewage (no head-space). Sulfide was spiked into the reactor to achieve a total dissolved sulfide concentration of ~15 mg/L. Two series of experiments were performed to (i) determine the stoichiometry of the reaction (n=3) and (ii) determine the kinetics of the reaction between the sludge and sulfide (six tests with Fe:S ratios from 1:3 to 1:0.375). An overview of the experimental design is presented in Table 2. During the course of each experiment, the change in dissolved sulfide concentrations was monitored online using a S::CAN sensor. In all tests, pH was controlled at 7.5 ± 0.02 using a 0.01 M HCl solution via a programmable logic controller (PLC). A further test was also conducted during which iron sludge was not added. The sulfide concentration, monitored with the S::CAN sensor, remained constant, indicating that sulfide production in the tests was negligible in the absence of sewer biofilms (data not shown).

Table 6-2. Purpose, set points and duration of the batch tests.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Purpose</th>
<th>Test No.</th>
<th>Fe:S</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>To identify the stoichiometry of the reaction</td>
<td>1</td>
<td>1:3</td>
<td>4 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1:3</td>
<td>4 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1:3</td>
<td>4 h</td>
</tr>
<tr>
<td>Type II</td>
<td>To determine the kinetics of the reaction</td>
<td>1</td>
<td>1:3</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1:2</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1:1.5</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1:1</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1:0.75</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1:0.375</td>
<td>2 h</td>
</tr>
</tbody>
</table>

The stoichiometry of the reaction between iron sludge and dissolved sulfide was determined as the molar ratio of the total iron in the sludge and the total dissolved sulfide removed (expressed as $\alpha$). To identify this stoichiometric ratio ($\alpha$), the Fe:S ratio for the batch tests was set at 1:3 (Table 6-2). Preliminary tests showed that sulfide in the wastewater could not be totally removed under this ratio (data not shown). Each batch test was conducted over a 4 h to ensure that stable dissolved sulfide concentrations were reached. The stoichiometric parameter $\alpha$ was calculated as the molar ratio of iron added into the reactor to the removed dissolved sulfide throughout the whole test period. The test was performed in triplicate.
To determine the kinetics of the reaction, experiments using 6 different molar ratios of Fe:S (see Table). Each test was performed for a period of 2 hours. The kinetics of the reaction was modeled by the following expression:

\[
\dot{r}_{H_2S,\text{removal}} = \frac{d(TDS)}{dt} = k[Fe]^x[TDS]^y
\]  
(Equation 6-1)

Where:
TDS is total dissolved sulfide concentration in the reactor (mgS/L);
Fe is the total Fe concentration (mgFe/L);
k is reaction rate constant ((mgFe/L)^x (mgS/L)^1-y h^-1);
x is the reaction order with respect to total Fe (-);
y is the reaction order with respect to total dissolved sulfide (-).

Parameters were estimated by fitting the model-predicted total dissolved sulfide concentrations with the measured profiles to the kinetics expression using a modified version of Aquasim 2.1d with the sum of squared errors as an objective function and k, x and y as calibrated parameters(Batstone et al. 2009). The uncertainty evaluation was carried out according to Batstone et al. (2003), with a 95% confidence level for significance testing and parameter uncertainty analysis. The standard errors and 95% confidence intervals of individual parameter estimates were calculated from the mean square fitting errors and the sensitivity of the model to the parameters. The determined F-values were used for number of parameters and degrees of freedom in all cases.

6.3.5 Chemical analysis
Dissolved sulfur species (i.e. sulfide, sulfate, sulfite and thiosulfate) were measured using ion chromatograph (IC) with a UV and conductivity detector (Dionex ICS-2000), as described elsewhere (Keller-Lehmann et al. 2006). The iron concentration was analyzed by means of Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Perkin Elmer Optima 7300DV, Waltham, MA, USA). Total kjeldahl nitrogen (TKN), Total kjeldahl phosphate (TKP) and phosphate was analyzed using a Lachat QuikChem 8000 (Lachat Instrument, Milwaukee,Wisconsin) flow injection analyzers (FIA). Chemical Oxygen Demand (tCOD and sCOD) was determined by means of COD cuvette tests (Merck, range 25–1500 mg L^-1). The protocol of methane analysis is as described by Guisososola et al. (2008). Briefly, 5 ml sewage was filtered with 0.22 μm membrane and injected into a 12 ml vacuumed Exetainer® vial with a hypodermic needle attached to a plastic syringe. The tubes were allowed to reach gas-liquid equilibrium overnight. Methane in the gas phase was measured by gas chromatography (Shimadzu GC-9A), equipped with a flame ionization
detector (FID). Concentration of methane in the sewage sample was calculated using Henry’s Law by considering both liquid and gas phases. The pH was monitored online using a pH sensor with a TPM-mini CHEM process monitor and controller.

6.4 Results

6.4.1 Effect of iron sludge on sulfide control and wastewater characteristics the sewer reactor

The effect of iron sludge dosage on the dissolved sulfide concentration in the reactor is shown in Figure 6-2. The dissolved sulfide concentration in the reactor during the sludge dosing periods was significantly lower than that during control period at all the three tested dosing rates. The range of sulfide concentration in the effluent at the three different dosing rates, i.e. 1:1.5, 1:1.2 and 1:1 (Fe:S) were 4.9 - 6.0 mg S/L, 2.0 - 4.3 mg S/L, 0.7 - 2.3 mg S/L, respectively, compared to 15.5 - 19.8 mgS/L without sludge addition. The lowest sulfide concentration in the effluent was 0.7 mg S/L at the dosing rate of 1:1 (Fe:S) with HRT of 3h. It is clear that the sulfide concentrations in all cases would continue to decrease should the reaction time was prolonged. The results clearly show that sulfide in the sewer reactor can be successfully controlled by the addition of iron sludge and that the sulfide removal effect is improved with the increase of sludge dosing rates. Under the highest dosing rate used (i.e. Fe:S = 1:1), the sulfide concentration decreased continuously with the increase of HRT (except for the first pumping cycle), while under the other two dosing rates (i.e. 1:1.2 and 1:1.5), a slight increase of the sulfide concentration was first observed before a decrease. This indicated sulfide production happened simultaneously with sulfide removal under the sludge dosing conditions, with sulfide concentration in the sewage dependent on both the sulfide production rate of sewer biofilms and the sulfide removal rate of the sludge. This is also supported by high sulfide production in the recovery period. In fact, the sulfide production largely recovered in the following 8-h cycle, and fully recovered in the second 8-h cycle, indicating that the sludge addition did not have inhibition effect on sulfate reducing bacteria (SRB) in the sewer biofilm. The relatively lower sulfide concentration in the first recovery cycle was probably due to that the retention of some sludge still remained in the reactor, most likely attached on the biofilm.
Figure 6-2. Sulfide profiles in the sewer reactor during a 4-day sludge dosing test. (A) without sludge dosing; (B) sludge dosing rate at Fe:S = 1:1.5, (C) sludge dosing rate at Fe:S = 1:1.2, (D) sludge dosing rate at Fe:S = 1:1 and (E) sludge dosing rate at Fe:S = 1:1.

Figure 6-3A shows the COD concentrations (total and soluble) in the effluent of the sewer reactor at a sludge dosing rate of Fe:S = 1:1 as well as the COD concentrations without sludge dosing. The average tCOD concentration increased (from 324±20 mg/L to 364±23 mg/L) by sludge addition, representing a 12% increase of tCOD in the raw sewage. However, the sCOD concentration decreased slightly (from 195±9 mg/L to 181±12 mg/L), equivalent to 7% of sCOD in the raw sewage.
sewage. The decreased of sCOD is probably due to the adsorption of organic matter (e.g. colloidal organic) on the iron sludge, as previously reported (Basibuyuk and Kalat 2004). Despite the increase in tCOD concentration, methane production decreased by ~20% (1.64 mg/L) (Figure 6-3B), which could be related to the loss of sCOD. The addition of iron sludge would not aggravate the greenhouse gas emissions from sewers. In addition to sulfide removal, a slight decrease in phosphate concentration was also observed with the phosphate concentrations being reduced by 13% (0.89±0.21 mg-P/L) (Figure 6-3B). This is probably due to phosphate precipitation with the iron in the sludge.

Figure 6-3. tCOD (A), sCOD (A), methane (B), and phosphate (B) concentrations in the sewer reactor at the end of each pumping cycle without sludge dosing and with a sludge dosing rate of Fe:S = 1:1.
6.4.2 Stoichiometry and kinetics of the reaction between iron sludge and dissolved sulfide

Table 6-3 presents the results of the type I batch tests aimed to determine the reaction stoichiometry between the sludge and the sulfide. The obtained α (the molar ratio of Fe:S required to achieve complete sulfide removal) was 0.50±0.02. This is lower than the theoretical stoichiometric value of 0.67 for the reaction between ferric and sulfide: 2Fe$^{3+}$ + 3S$^{2-}$ $\rightarrow$ 2FeS + S$^0$, (Zhang et al. 2009). The low ratio suggests that some other physiochemical processes likely took place simultaneously between the sludge and sulfide leading to enhanced sulfide removal. Given the low levels of other metals in the sludge (Table 6-1), it is unclear which reactions were responsible for the additional sulfide removal. Further research is needed to identify the exact reasons. However, the reaction between ferric and sulfide is believed to be the dominant mechanism for sulfide removal as the ratio of 0.5 to 1 is still close to the theoretical value. This is also supported by the change of the color of wastewater to black, a typical color of FeS, after sludge dosing.

<table>
<thead>
<tr>
<th>No.</th>
<th>TDS removed (mmol/L)</th>
<th>Total Fe added (mmol/L)</th>
<th>Fe:Sulfide (α)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.37</td>
<td>0.18</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>0.33</td>
<td>0.16</td>
<td>0.48</td>
</tr>
<tr>
<td>3</td>
<td>0.38</td>
<td>0.20</td>
<td>0.52</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.50±0.02</td>
</tr>
</tbody>
</table>

Figure 6-4 shows the experimentally obtained sulfide removal rates under different iron to sulfide ratios. In all experiments, the sulfide concentration decreased rapidly at the beginning, which was followed by a relative slower decrease. The iron sludge typically contains ferric hydroxides bound with other organic or inorganic compounds (Bratby 2006). The removal of sulfide by ferric hydroxide required approach of sulfide to the surface of the hydroxide and the approaching rate would depend on the net surface charge on the solid, physical adsorption and chemisorption (Pyzik and Sommer 1981). At a higher sludge dosing rate, more surface areas were available, and as a result, a higher removal rate was achieved. The decreasing of the reaction rate over time could be explained by the decrease of the reactive sites with the continuous precipitation reactions.
Figure 6-4. (A) Measured and simulated sulfide concentrations under different Fe:sulfide ratios. The symbols represent the experimental measurements and the lines represent the model. (B) 95% confidence regions for the parameter combinations of y and k with the best fits in the center as well as 95% confidence intervals.

Although the reaction between iron sludge and sulfide may involve adsorption, oxidation, precipitation and some other processes, we propose an empirical rate expression (Equation (6-1)) to describe the kinetics of the sulfide removal process. The parameters in the model were estimated using batch tests results described above and the estimated parameter values are presented in Table 6-4. Figure 6-4 shows that with the estimated parameters, the model could satisfactorily reproduce all sets of experimental results, indicating that the lumped rate expression (Equation (6-1)) can reasonable describe the kinetics of reactions between the iron sludge and sulfide.

Table 6-4. Estimated parameters for the reaction and correlation matrix resulting from parameter estimation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard error</th>
<th>Correlation matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>x</td>
<td>0.65</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>y</td>
<td>0.77</td>
<td>0.02</td>
<td>0.078</td>
</tr>
<tr>
<td>k</td>
<td>0.69</td>
<td>0.03</td>
<td>-0.564</td>
</tr>
</tbody>
</table>
The Correlation Matrix (CM) of all the parameters in Equation (6-1) is also shown in Table 6-4. The correlations between the parameters are acceptable, indicating that these parameters generally have good identifiability. The highest correlation coefficient in the CM is found between parameters k and y. However, the 95% confidence region for these two variables is reasonable small, with the estimates approximately sitting in the center. This suggests that the estimates of these two parameters have reasonably high-levels of certainty.

6.5 Discussion

This study clearly demonstrates that dissolved sulfide in sewers can be effectively controlled by the addition of iron rich drinking water treatment sludge. The experimentally obtained results showed that, with an adequate sludge dosing rate, sulfide concentrations below <1 mg S/L can be achieved. The required sludge dosing rates (i.e. based on a Fe to sulfide ratio) were found to be very similar compared to conventional ferric chloride dosing in sewers (Zhang et al. 2009). When the sulfide removal rate is higher than the sulfide production rate by the sewer biofilms, sulfide production can be completed controlled. Our results indicated that to completely control sulfide production, a sludge dosing rate of Fe:S = 1:1 was required (Figure 6-2A). It is worthwhile to note that this rate could vary according to the sulfide production rate of sewer biofilms as well as the area to volume ratio of the sewer pipe. Considering that the A/V ratio of our reactor is higher that of the real sewer pipe (26.7 - 6.7 m⁻¹ for 150 - 600 mm diameter pipe compared to 70.9 m⁻¹ in this study), the volumetric sulfide production rate is also higher. As a result, the required dosing rate in practical application is expected to be lower. The reaction stoichiometry revealed indicates a ratio of Fe:S = 0.5:1 could be adequate, provided that enough reaction time is given. Therefore, the preferred dosing location of the sludge is the upstream of the rising main sewer (either the wet well or the pumping station), which will allow a long retention time for the sludge to react with sulfide. In addition, the dosing of the sludge should avoid small sewer pipes with low flow rate, in case that the sludge would be settled in the upstream of the pipe and could reach the downstream location.

The removal of sulfide was mainly achieved by physiochemical reaction of the sludge with sulfide. Sludge addition did not result in inhibition of the sulfide production by the sewer biofilm, as shown by the recovery of the sulfide production between dosage cycles (See Figure 6-2). The reaction between ferric and sulfide forming elemental sulfur and FeS is believed as the main reason for dissolved sulfide removal. However, some other reactions might have also contributed to the decrease of dissolved sulfide concentration, as indicated by the Fe to sulfide reaction ratio that is lower than the theoretical ratio. Possible processes included oxidation of sulfide by other compounds like high redox potential NOM in the sludge, formation of organic sulfur compound,
adsorption of sulfide on the sludge (Perlinger et al. 2002, Heitmann and Blodau 2006). It is worth noting that the elemental sulfur formed with the sludge dosing might be reduced to sulfide again. Therefore, overdosing of the iron sludge may be required to control sulfide reduced by elemental sulfur. However, further study is needed to investigate the percentage of elemental sulfur being reduced to sulfide to determine a proper dosing rate.

The dosing of iron sludge is expected not to significantly affect the sewage characteristics. Our results showed the increase in tCOD caused by sludge doing only account for 12% total COD loading. The increase of heavy metals in the sewage was negligible, which can be explained by their very low concentrations in the sludge itself (Table 1). The dosing of iron sludge is not expected to aggravate the methane production in sewers despite the slight increase in COD. Instead, the methane production decreased after dosing. This implies that the iron sludge might even reduce methane in sewers to some extent. The results also showed that in addition to sulfide phosphate concentrations in sewage decreased after sludge dosing, depending on the Fe:S dosing ratio used. This is in agreement with literature, which showed that iron sludge could be used for phosphate removal in the wastewater water treatment plants (Makris et al. 2004, Leader et al. 2008)

Iron salt (typically as ferric chloride) is a widely used coagulant for drink water treatment (Matilainen et al. 2010). Considering that a typical coagulant dosing rate for drinking water production was at 5-20 mg Fe /L (Pikaar et al. 2014) and that about 60% of the drinking water normally ends up in the sewage (Kenway et al. 2011), it can be estimated that the iron sludge produced by drinking water treatment could achieve up to 94% of sulfide removal in sewer networks (assuming the sludge dose rate of Fe:S = 1:1 and the wastewater total dissolved sulfur concentration at 20mgS/L, the same condition as this study). This indicated the cost for purchase of chemicals for sulfide control in sewer could largely decreased by dosing iron sludge to sewers in case drinking water providers use iron chloride as coagulant. It has been estimated daily sludge produced by drinking water treatment plant is around 10000 tons on global scale (Dharmappa et al. 1997). Often the costs of handling the enormous quantities of waterworks sludge can account for a significant part of the overall operating costs of water treatment works (Babatunde and Zhao 2007). For example, in the Netherlands, the total cost of disposing waterworks sludge stands at £30–40 million per year (Horth et al. 1994). Hence, the results obtained in this study reveal a unique opportunity for drinking water providers to turn drinking water ‘waste’ sludge into a valuable resource, while the costs for sludge disposal for themselves and the costs for sulfide control in sewers for wastewater service providers could be significantly reduced at the same time.
In addition, Gutierrez et al. (2010) recently found that the addition of ferric chloride to sewers for sulfide control enhances phosphorus removal at the downstream wastewater treatment plant, where iron sulfide precipitates are oxidised in aeration tanks, regenerating iron phosphate precipitates. A very recent study further showed that iron used for phosphate removal in WWTPs could achieve sulfide control in sludge digesters (Ge et al. 2013). This indicates the coagulation sludge, currently regarded as a nuisance, can potentially be re-used multiple times for (i) sulfide control in sewer networks, (ii) phosphate removal and (iii) sulfide control during sludge digestion at downstream WWTPs.

Overall, this study showed that dosing iron sludge to sewers is an effective strategy for sulfide removal in sewer systems, which would also reduce the sludge disposal costs from drinking water treatment works. However, this method might have some side effects on sewer sedimentation due to the increase of solids or on the WWTPs due to the presence of NOM. These potential issues need to be investigated in future. Also, testing on real sewers could be useful to fully demonstrate the effectiveness of this method.
Chapter 7 An efficient method for measuring dissolved VOSCs in wastewater using GC-SCD with static headspace technique

7.1 Abstract
Volatile organic sulfur compounds (VOSCs) are important sources of unpleasant odor in wastewater systems. However, the study of VOSCs is usually hindered by their complicated measurement method and highly reactive nature. In this work, a static headspace method utilising gas chromatography (GC) with a sulfur chemiluminescence detector (SCD) was developed to quantitatively analyze VOSCs in wastewater matrices. The method has low detection limits and requires no pre-concentration treatment. Three typical VOSCs, namely methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS), were chosen as examples for this study. The calibration curves of all three compounds covering a wide range from 0.5 ppb to 500 ppb showed good linearity ($R^2 > 0.999$). The method detection limits (MDL) were 0.08, 0.12 and 0.21 ppb for MT, DMS and DMDS, respectively. The reproducibility (relative standard deviation) was approximately 2%. The recovery ratio of MT, DMS and DMDS in spiked wastewater samples were 83±4%, 103±4% and 102±3%, respectively. Sample preservation tests showed that VOSCs in wastewater samples could be preserved in vials without headspace under acidified conditions (pH ~1.1) for at least 24 h without significant changes (<1.8 ppb). The analysis of real wastewater samples from both a laboratory-scale sewer system and a full-scale sewer pipe demonstrated the suitability of this method for routine wastewater VOSC measurement.

7.2 Introduction
Odor problems in wastewater collection and treatment systems have become critical issues to water industry (Stuetz and Frechen 2001). In addition to hydrogen sulfide, volatile organic sulfur compounds (VOSCs), such as methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) are believed to be important sources of unpleasant odor in municipal and industrial wastewater (Devai and DeLaune 1999, Hvitved-Jacobsen 2002, Cheng et al. 2005, Sekyiamah et al. 2008, Catalan et al. 2009, Marleni et al. 2012). Because of their malodorous characteristics and low odor thresholds (0.07 - 5.9 ppbv) (van Gemert 2011), even a small amount of VOSCs can contribute to significant odor pollution. At higher concentrations (> 0.5 - 20 ppmv), they could cause health problems (Lomans et al. 2002b). Some recent studies have focused on VOSC measurement in the air around wastewater treatment plants (WWTPs) (Ras et al. 2008, Sekyiamah et al. 2008, Sheng et al. 2008, Lasaridi et al. 2010). However, it is also worthwhile to monitor VOSC concentrations in the wastewater itself as it can help understand the conversion of VOSCs in wastewater and thus
solve the odor problem at the root. Therefore, it is important to have a reliable and efficient method to measure VOSCs in wastewater.

The analyses of VOSCs in wastewater have been mainly carried out by using gas chromatography (GC) with flame photometric detector (FPD) or mass spectrometry (MS) (Van Langenhove et al. 1985, Hwang et al. 1995, Abalos et al. 2002, Cheng et al. 2007, Sheng et al. 2008, Godayol et al. 2011). Since the detection limits of these two detectors are relatively high ($10^{-11}$ g S/s), pre-concentration of VOSCs in wastewater samples is often required before the measurement. One commonly used pre-concentration method is purge-and-trap (Van Langenhove et al. 1985, Hwang et al. 1995, Cheng et al. 2007, Sheng et al. 2008). VOSCs are firstly stripped from the aqueous phase and adsorbed on a sorbent. During the injection, the analytes on sorbent are desorbed thermally and flushed to GC column with an inert gas. However, major disadvantages of this method include expensive equipment, tedious procedure and potential loss of VOSCs from the trap if excessive purge time or flow rates are used (Wylie 1988). Solid phase microextraction (SPME) was an alternative pre-concentration method recently used in wastewater VOSC analysis (Abalos et al. 2002, Godayol et al. 2011). This method involves the use of a thin polymer-coated silica fiber to adsorb VOSCs from the headspace of the wastewater sample. The fiber is then inserted directly into the GC injection port for thermal desorption and analysis. Compared with the purge-and-trap process, SPME is relatively simple and inexpensive. However, the extraction process is time-consuming, normally taking more than half an hour for a sample. Moreover, Lestremau et al. (2004) showed that a large proportion of MT was dimerized to DMDS during the SPME process, resulting in errors in MT and DMDS measurements.

Sulfur chemiluminescence detector (SCD) is a relatively new gas chromatographic sulfur-selective detector. It converts the sulfur compounds to sulfur chemiluminescent species and detects the chemiluminescence from the reactions between ozone and sulfur chemiluminescent species (Yan 2002). This detector, coupled with GC, has been applied for detection of sulfur containing compounds in petroleum, atmosphere and food (Di Sanzo et al. 1994, Steely Jeffrey 1994, Galán et al. 1997, López García et al. 2002, Rouseff Russell 2002, Nylén et al. 2004). Compared to FPD and MS, SCD is superior on the following aspects:

1. Excellent sensitivity. The detection limit of SCD can reach $10^{-13}$ g S/s, which is about 2 orders of magnitude lower than FPD and MS (Wardencki and Zygmunt 1991);
2. High selectivity. The sulfur-selective characteristic of SCD makes it superior to MS, as it can eliminate the signals of many other compounds that may interfere with the detection. Though it is
also sulfur selective, FPD has a selectivity (C/S) of about 1 to 4 orders of magnitude lower than SCD (Wardencki 1998);

(3) Easy operation. The operation of SCD is much easier than MS and also simpler than FPD.

The prominent advantages and successful application of SCD in other fields suggest its promising potential for measuring VOSCs in wastewater matrices. Especially for its high sensitivity, the use of SCD might make it possible to eliminate the complicated, time-consuming and error-prone pre-concentration processes. However, to our knowledge, no studies have been reported to date on the use of SCD to detect VOSCs in wastewater.

The purpose of this paper is to develop a method for the measurement of VOSC compounds in wastewater using GC-SCD. The static headspace technique, rather than a pre-concentration process, was used for the transfer of VOSCs from water to the gas phase, which made the measurement fast and simple. Also, it would avoid errors caused by sample loss or contamination during the pre-concentration. The GC was operated above room temperature (28°C), so the cooling system of GC column, which is usually applied to enhance separation of volatile compounds, is not required. The linear ranges, detection limits, reproducibility, and recovery ratios of this method were examined and compared with other VOSC detection methods. Given the highly reactive nature of VOSCs, different sample preservation methods were assessed and an effective method was selected. Finally, this method was applied to measure VOSC concentrations in real wastewater samples collected from laboratory and real sewer systems.

**7.3 Material and Methods**

**7.3.1 The GC-SCD method with static headspace technique**

The whole procedure of the VOSC analysis using GC-SCD includes 6 steps as illustrated in Figure 7-1. The details of all these steps are described in following sections.
7.3.2 Standard solution
Methanethiol (MT), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were chosen as examples of VOSCs in this work, which are VOSCs typically found in wastewater (Wu et al. 2006, Sheng et al. 2008, Lasaridi et al. 2010). Analytical reagent grade of CH$_3$SNa, DMS and DMDS (Sigma-Aldrich, Australia) were used to prepare the standard solutions using MilliQ water (Merck Millipore, Germany). As these compounds can be easily oxidized, the MilliQ water was deoxygenated before making the solution by purging it with nitrogen gas (99.99%, BOC, Australia) for at least 1 h. A concentrated stock solution (50 ppm) was firstly prepared, which was further diluted to 5 different levels (0.5-500 ppb) for calibration purpose. All the standard solutions were prepared without headspace to avoid loss of compounds through volatilization.

7.3.3 Sample preparation
A 12 ml glass headspace vial (Labco Limited, United Kingdom) was used to prepare samples for GC-SCD analysis. The vial was firstly purged with nitrogen gas for 10 min to remove oxygen. Subsequently, 3ml of standard solution or filtered wastewater sample (0.22 μm membrane) was injected into the vial. The possible adsorption of VOSCs on the membrane filter was investigated by comparing response areas with and without sample filtration, and the results showed insignificant difference (Figure 7-S1, Supporting Information (SI)). To further reduce the risk of adsorption, the filter was used to filter an initial 3 ml of the same wastewater without collecting the filtrate. If there was any adsorption, the VOSCs on membrane surface would be saturated.
Wastewater usually contains a high concentration of H$_2$S. Its peak could create a large tail on the chromatogram, which could affect the detection of MT as the MT peak would appear on the tail of the H$_2$S peak. In order to solve this problem, two different buffers, namely a boric buffer (pH=8.1 ± 0.1) and a phosphate buffer (pH=7.6 ± 0.1), each with two different strengths at 0.05 M and 0.15 M, were tested. Three milliliters of buffer was added to the headspace vial and their effect on reducing the spread of the H$_2$S peak were investigated.

As the vial was sealed and gas inside would not be released when injecting sample or buffer, it resulted in overpressure in the vial. The overpressure would not change the partial pressure of the VOSCs in the headspace, which is determined by the amount of VOSCs in the liquid sample at equilibrium conditions (according to Henry’s Law). However, the relative concentration of VOSCs (ppmv) in the headspace would vary with the overall pressure in the vial headspace, which could affect the detection limits of the method. The addition of 6 ml liquid into the vial would result in relatively high concentrations of VOSCs (Figure 7-S2) so that relatively low detection limits could be achieved.

The vial was then mixed using a vortex mixer for 2 min to ensure that the gas-liquid equilibrium was reached (There were no increase of GC response areas of all three compounds for mixing time longer than 2 min). At last, 300 μL of headspace gas was drawn with a gas-tight syringe (SGE Analytical Science, Australia) and injected into the GC for analysis.

7.3.4 Instrumentation
The analysis was performed on an Agilent 7890A GC (Agilent Technologies, Santa Clara, California) coupled with an Agilent 355 SCD. The GC uses a capillary column (30 m × 320 μm × 5 μm, Zebron™, Phenomenex) for VOSC separation and helium as a carrier gas. The injection was operated in pulsed splitless mode. In order to optimize GC separation of targeted compounds in both standard solutions and wastewater samples, the injection temperatures ranging from 80°C to 120°C were tested. Also different GC oven temperature programs were performed (temperature starting at 28°C, 40°C and 50°C respectively; total retention time varying from 8.5 min to 11.6 min). The SCD was operated according to the manufacturer’s guidelines. The burner was operated at 800°C. The hydrogen and air flow rates were maintained at 42 ml/min and 62 ml/min, respectively, and the pressure in the reaction cell was at ~8 Torr.
7.3.5 Sample preservation method

As GC-SCD is normally unavailable in field and VOSCs are highly reactive, it is critical to preserve wastewater samples prior to their analysis for VOSCs. In this study, two different preservation methods were evaluated. One method was to store the headspace of the wastewater sample in a separate glass vial (hereinafter referred to as “separated headspace method”). 4ml gas was drawn from the aforementioned 12ml headspace vial containing wastewater sample and injected into a separate 4ml glass vial containing CaCl$_2$ (0.5 g) and ascorbic acid (0.3 g). These two compounds were used to remove moisture and oxygen in the VOSCs-containing air and prevent the oxidation of VOSCs (Tangerman 1986, Inomata et al. 1999). The vial with gas only was covered with aluminium foil to avoid light and then stored at ~4°C.

The second method was to acidify the wastewater samples (hereinafter referred to as “acidification method”) since VOSCs were found more stable in acidified wastewater (Cheng et al. 2007). This method was carried out in the following steps. A 40 ml glass vial, capped with butyl rubber septa, was firstly flush by nitrogen gas for 10 min to remove oxygen. The vial was then filled to the top with 37.5 ml wastewater sample filtered through a 0.22 μm membrane, and 2.5 ml HCl (3 M) so that the pH was adjusted to ~1.1. The vial was covered with aluminium foil to avoid exposure to light and stored at ~4°C. Before doing the analysis, the sample was heated in a water bath (20°C) for 10 min and the pH of sample was raised to ~7.0 by adding 2.4 ml NaOH (3M) into the bottle, with an equivalent volume of the HCl and wastewater mixture withdrawn. The dilution effects of HCl and NaOH addition were considered while calculating the VOSC concentrations in wastewater. Then, 3 ml of sample was taken from the bottle and the normal static headspace technique and GC-SCD analysis was performed as previously described (Section 7.3.1).

The capabilities of sample preservation by these two methods were evaluated by monitoring the change of MT, DMS and DMDS concentrations in wastewater after different time intervals. The wastewater used for the test was obtained from an anaerobic sewer reactor mimicking a rising main sewer as will be further described in Section 2.6. In each test, several samples were taken at the same time and one of them was measured immediately. Then, samples stored directly in headspace vials and preserved by separated headspace method were measured after 8 h, while samples preserved by acidification method were analysed after 24 h and 48 h. Spiked wastewater samples were also tested for the effect of acidification method at a high concentration range using the same method as described before.
7.3.6 Real wastewater sample analysis

Real wastewater samples from both a laboratory-scale sewer system and a real sewer pipe were tested to evaluate the application potential of the method developed in this study. The laboratory sewer reactor used was a cylindrical gas-tight reactor, which mimicked a section of a rising main sewer pipe under anaerobic conditions (Guisasola et al. 2008). The reactor was fed intermittently (6 pumping events per day) with municipal wastewater collected weekly from a local sewage pump station in Brisbane (Queensland, Australia). The wastewater was stored in a cold room (4°C) to minimize the biotransformation and was heated up to 20°C before being pumped to the reactor. Further details of the reactor and its operation can be found in Zhang et al. (2009). The reactor was under the steady state at the time of conducting the tests described below. Batch tests were applied to investigate the change of VOSC concentrations in the reactor. At the beginning of each test, the reactor was filled with fresh wastewater. Then samples were collected every 30 min for VOSC measurement during 6-hour experiments.

Field samples were obtained from a rising main sewer pipe (C016) in the Gold Coast area (Queensland, Australia). The C016 rising main had an internal pipe diameter of 300 mm (surface area to volume ratio, A/V = 13.3 m⁻¹), a total daily flow of ~700 m³, with 33 pump events (typically 4–6 min in duration) per day. Samples were collected at two locations: (1) wet well of the C016 pump station; (2) a sampling point at 1100m downstream of the pump station. Hourly samples were taken from 10:00 am until 2:00 pm and preserved using the acidification method described in Section 2.5. All samples were measured immediately after being delivered to the laboratory. Inorganic sulfide and soluble methane concentrations were also measured using ion chromatography (IC) with UV and conductivity detector (Dionex ICS-2000) (Jiang et al. 2009) and GC with a flame ionization detector (FID) (PerkinElmer, Inc.) (Guisasola et al. 2008), respectively.

7.4 Results and Discussion

7.4.1 Optimizing analytical conditions

The boric buffer (pH=8.1 ± 0.1) with the strength of 0.15 M was proven to achieve the best effect of reducing H₂S peak on the chromatogram (Figure 7-2). Since the acid disassociation constant (pKₐ) of H₂S is around 7.0 (20°C), pH 8.1 would ensure over 90% of the total dissolved sulfide being in the form of HS⁻. This would greatly decrease the H₂S concentration in the headspace of the vial and thus improves separation of the H₂S and MT peaks. While the addition of 3 ml boric buffer of 0.15 M to a 3 ml sample is effective in separating the H₂S and MT peaks for the municipal wastewater we tested, specific tests may be needed to determine a suitable buffer concentration for wastewater
samples with different sulfide and MT concentrations or pH levels, to achieve satisfactory separation of H2S and MT peaks.

Figure 7-2. The effect of different boric buffers on the separation of H2S and MT peaks on the chromatogram.

For GC parameters, the GC injector temperature was finalized to 120 °C. The oven temperature was programmed at 28°C for 5 min then increased at a rate of 20 °C/min to 160°C with the total retention time of 11.6 min. Under the analytical conditions described above, optimized GC-SCD performance could be achieved, judged based on the separation and magnitudes of the peaks. Figure 7-3 shows examples of chromatograms of both standard solutions and wastewater samples. The peaks of all three targeted compounds (MT, DMS and DMDS) were in good sharp shapes. They were well separated in the wastewater samples and were not interfered by other compounds. As shown in Figure 7-3B, the small peak next to the DMS peak is an ethanethiol peak. Though these two peaks are very close, there was no overlapping between the two peaks in all wastewater samples tested. The DMS concentration measured would thus not be affected by the presence of ethanethiol in municipal wastewater.
7.4.2 Calibration curve

The calibration curves for MT, DMS and DMDS were constructed in the concentration range of 0.5 - 500 ppb (Figure 7-4). This range covered the possible concentration range of these substances in wastewater (see Section 7.4.5). All the three calibration curves presented good linearity with correlation coefficients over 0.999. The calibration results indicate that this method covers a broad linear dynamic range (4 orders of magnitude).
7.4.3 Method detection limits

Method detection limit (MDL) is defined as the lowest concentration of a substance that can be determined by a given method with 99% confidence that the concentration is higher than zero (US EPA 2010). In this study, the MDL is determined based on analyzing 8 samples at the concentration of 0.5 ppb. The MDL was calculated as follows (US EPA 2003):

$$\text{MDL} = S \times t$$

(Equation 7-1)

where $S$ is the standard deviation of the 8 samples at the concentration of 0.5 ppb; $t$ is the one-sided student’s $t$ value (2.998) for a 99% confidence interval with 7 degrees of freedom. The method detection limits of MT, DMS and DMDS of this method were determined as 0.08, 0.12 and 0.21 ppb, respectively. The detection limits of this method may be further decreased by optimizing the liquid volume injected into the vial or reducing the buffer solution volume by for example increasing the buffer solution concentration.

7.4.4 Reproducibility

The reproducibility was determined by repetitive measurement of 5 separately prepared spiked wastewater samples at the concentration of 50 ppb. The relative standard deviations (RSD) of MT, DMS and DMDS calculated based on the 5 measurements were 2.3%, 2.2% and 2.1%, respectively.

7.4.5 Recovery ratios

Figure 7-4. Calibration curves of MT, DMS and DMDS (0.5 - 500ppb)
The recovery ratios of MT, DMS and DMDS in wastewater were tested by spiking a pre-known amount of these compounds into a VOSC-free wastewater matrix and calculating the relative difference between measured concentrations and real concentrations. The VOSC-free wastewater was obtained by purging with nitrogen for 20 min to remove any pre-existing VOSCs. The result was obtained based on 5 tests for each compound with concentration ranging from 5 ppb to 500 ppb. The recovery ratios of MT, DMS and DMDS were 83±4%, 103±4% and 102±3%, respectively. The recovery ratio for MT is relatively low, but still reasonable. The underlying reason for this recovery is not clear, which may be due to wastewater matrix effect. Further research is needed to identify the reason and to improve the recovery.

7.4.6 Sample preservation
The effect of two sample preservation methods, i.e. the separated headspace method and the acidification method, are shown in Figure 7-5. The initial concentrations of VOSCs in different tests varied to a certain extent since these experiments were carried out using different batches of real wastewater. The MT concentration in wastewater samples stored directly in headspace vials or preserved by the separated headspace method decreased 11.9 - 13.5 ppb after 8h. DMS and DMDS concentrations decreased by 0.2 - 0.5 ppb during the same period. With the acidification method, wastewater samples could be preserved for 24 h without significant changes in composition (MT concentration decreased by 1.8 ppb, DMS by 0.4 ppb and DMDS by 0.2 ppb). After 48h, MT concentration decreased by 7.2 ppb. In addition, there were no significant variations of DMS and DMDS concentrations after 48 h. In the high concentration range (spiked wastewater tests), with the acidification method, the concentration of three compounds decreased slightly (<1%) after 48h preservation. These results suggest that MT in the wastewater could be preserved using the acidification method for at least 24h while DMS and DMDS could be preserved for at least 48 h.

7.4.7 Comparison with other methods
A comparison of this method and other reported methods for wastewater VOSC measurement is listed in Table 7-1. As this method does not require the pre-concentration processes, the analytical time is reduced by at least 40 min for the measurement of each sample. In addition, the complication of sample handling is avoided. The calibration range of this method covers 4 orders of magnitude, which is comparable to results of other methods. The higher correlation coefficients ($R^2$) and relatively lower RDS values obtained indicate a better precision of measurement. The detection limits of this method are lower than or comparable to those obtained using purge-and-trap pre-concentration, although they are about 10 times higher than those achieved by the SPME pre-
concentration method. The recovery ratios are also comparable to results obtained using GC system with pre-concentration processes.

Figure 7-5. Variation of MT (A), DMS (B) and DMDS (C) in the wastewater samples with different preservation methods. “Headspace vial”, “Separated headspace” and “Acidification I” refer to real wastewater samples preserved in a headspace vial directly, by the separated headspace method and by the acidification method, respectively. “Acidification II” refers to the spiked wastewater sample preserved by the acidification method.
Table 7-1. A comparison of different methods for wastewater VOSC measurement.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds measured</th>
<th>Apparatus</th>
<th>Pre-concentration</th>
<th>Analytical time per sample (min)</th>
<th>Calibration range (ppb)</th>
<th>( R^2 )</th>
<th>RSD (%)</th>
<th>Detection limits (ppb)</th>
<th>Recovery (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MT; DMS; DMDS</td>
<td>GC-SCD</td>
<td>No</td>
<td>17</td>
<td>0.5 - 500</td>
<td>0.9995 - 0.9998</td>
<td>2.1 - 2.3</td>
<td>0.08 - 0.21</td>
<td>83-103</td>
<td>This study</td>
</tr>
<tr>
<td>2</td>
<td>MT; DMS; DMDS</td>
<td>GC-MS</td>
<td>Purge-and-trap</td>
<td>58</td>
<td>5 - 500</td>
<td>0.993 - 0.998</td>
<td>0 - 8</td>
<td>1.2 - 4.8</td>
<td>81-100</td>
<td>(Cheng et al. 2007)</td>
</tr>
<tr>
<td>3</td>
<td>DMS; EMS(^a); THIO(^b); DES(^c); DMDS</td>
<td>GC-MS</td>
<td>HS-SPME</td>
<td>70</td>
<td>0.0044 - 10.6</td>
<td>0.995 - 0.997</td>
<td>4.08 - 6.12</td>
<td>0.006-0.035</td>
<td>N.A.(^d)</td>
<td>(Abalos et al. 2002)</td>
</tr>
<tr>
<td>4</td>
<td>DMDS</td>
<td>GC-MS</td>
<td>HS-SPME</td>
<td>72</td>
<td>0.1 - 100</td>
<td>0.9719</td>
<td>14</td>
<td>0.03</td>
<td>86</td>
<td>(Godayol et al. 2011)</td>
</tr>
<tr>
<td>5</td>
<td>H(_2)S; CS(_2); MT; DMS; DMDS</td>
<td>GC-FPD</td>
<td>Purge-and-trap</td>
<td>&gt;72</td>
<td>N.A.(^d)</td>
<td>N.A.(^d)</td>
<td>15</td>
<td>ppt level</td>
<td>N.A.(^d)</td>
<td>(Hwang et al. 1995)</td>
</tr>
</tbody>
</table>

\(^a\) EMS: ethylmethyl sulfide; \(^b\) THIO: thiophene; \(^c\) DES: diethyl sulphide; \(^d\) N.A: Data not available;
7.4.8 Application of the method to real wastewater samples

(1) Laboratory reactor study

Time series of MT, DMS and DMDS concentrations in the lab-scale anaerobic sewer reactor obtained in two separate batch tests are presented in Figure 7-6. The MT concentration increased from about 45 ppb to a peak value of 77 - 103 ppb in the first hour and then decreased gradually to around 10 ppb after five hours. In contrast, DMS and DMDS concentrations were at relatively low levels (0.5 - 2 ppb) during the entire test period in both cases. The results indicate that MT could be produced and subsequently degraded under anaerobic sewer conditions. This trend of MT transformation was also observed in other anaerobic systems such as anaerobic digestion and fresh water sediments (Lomans et al. 1999c, Du and Parker 2012). The production might be due to the cleavage of sulfur containing amino acids or methylation of sulfide, while the degradation likely resulted from the activity of methanogens and/or sulfide reducing bacteria (Lomans et al. 2001, Higgins et al. 2006).

![Figure 7-6](image)

Figure 7-6. Time series of MT, DMS and DMDS concentrations in the lab-scale anaerobic sewer reactor obtained in two separated tests (A) and (B).

(2) Field study

The concentration profiles of VOSCs, dissolved sulfide and methane concentrations measured in the field study are shown in Figure 7-7. In the pump station, concentrations of all the three VOSCs remained at low levels. Most values were lower than 2 ppb, with MT concentrations being the
exception, which increased from below 2 ppb slightly to 5-6 ppb after 12:00 pm. The MT concentration at the pump station in this study is similar to what reported by Lasaridi et al. (2010). They measured the MT concentration in the air above the sewage in a pump station in the range of 160 – 487 μg/m³, which indicated that the concentration in the sewage at that pump station could be around 0.8 – 2.4 ppb (calculated by Henry’s Law assuming gas-liquid equilibrium). To our knowledge, the DMS and DMDS concentrations at wastewater pump stations have not been reported yet. In agreement with previous studies (Guisasola et al. 2008, Foley et al. 2009), the dissolved sulfide and methane concentrations were low, constant below 1 ppm.

![Figure 7-7. The presence of VOSCs, H₂S and CH₄ in the CO16 rising main sewer: in the pump station (A, B) and at 1100 m downstream (C, D).](image)

At the sampling point in the rising main sewer (1100 m downstream of the pump station), the MT concentration varied between 18.6 to 72.8 ppb, which was much higher than DMS and DMDS concentrations between 0.7 - 3 ppb. The MT concentration is in the range of 11 - 322 ppb reported by Hwang et al. (1995), who measured the concentration in the influent of a WWTP. DMS and DMDS concentrations in this study are lower than Hwang’s results with 3 - 27 ppb for DMS and 30 - 79 ppb for DMDS, respectively. However, our result of DMDS concentration is close to what reported by Godayol et al. (2011), who measured the DMDS concentration in the influent of a
WWTP with concentrations in the range of 0 - 5 ppb. The VOSC concentrations are indeed expected to be dependent of wastewater composition and the sewage retention time in sewers.

![Graphs showing correlation analysis](image)

Figure 7-8. Correlation analysis between MT and sulfide concentrations (A), MT and methane concentrations (B), DMS and sulfide concentrations (C), DMS and Methane concentrations (D), DMDS and sulfide concentrations (E) and DMDS and Methane concentrations (F).

The concentrations of MT and DMS in the wastewater samples obtained in the main at 1100 m downstream of the pump station were constantly higher than those obtained from the pump station. This suggests MT and DMS were produced in this anaerobic sewer line. We hypothesize that the increase is dependent of the hydraulic retention time (HRT) of the sewage in the pipe. From the pump operation data, we calculated that the HRT at 10:00 am to 11:00 am was about 1.5 h while the HRT at 12:00 pm to 2:00 pm was around 3 h. The longer HRT around the midday was likely responsible for the higher increase in MT and DMS concentrations in this period. Figure 7-8A-D plotted the correlation between MT and DMS concentration and sulfide or methane concentration based on linear regression. Both MT and DMS concentrations showed high correlation with sulfide
and methane concentrations ($R^2 = 0.84-0.94$). This could also support that HRT plays important role for MT and DMS concentrations in rising main sewers, since sulfide and methane concentrations in rising main sewer are known to be highly correlated with HRT (Sharma et al. 2008b, Guisasola et al. 2009).

In contrast to the cases of MT and DMS, the DMDS concentration did not vary significantly between the two locations. The correlation between DMDS and sulfide or methane concentration was low ($R^2 = 0.04-0.21$, Figure 7-8 (E) and (F)). So the production of DMDS in rising main sewers might follow a mechanism different from that of MT and DMS. More research needs to be conducted before clearly understanding the transformation of VOSCs in sewer systems.

The VOSCs concentrations measured in real wastewater samples from both our laboratory sewer reactor and field sites were in the detection range (0.5-500 ppb) of this GC-SCD method. This range also covered the VOSC concentrations in sewage sampled from WWTPs, pump stations and drainage systems reported by other researchers (Hwang et al. 1995, Cheng et al. 2005, Sheng et al. 2008, Godayol et al. 2011). Therefore, we suggest this GC-SCD method with static headspace technique is suitable for routine wastewater VOSCs measurement.

### 7.5 Supporting Information

![Figure 7-S1](image) Relative response areas of MT, DMS and DMDS with and without filtration (The average response area of the unfiltered sample is 100%). These tests were designed to verify if VOSCs would be adsorbed onto the 0.22 μm membrane filter. The response areas of each compound at 50 ppb in a standard solution with and without filtration were compared.
Figure 7-S2. Calculated dependency of VOSC concentrations in the vial headspace on the total liquid volume injected, after the gas and liquid equilibrium was reached. In the calculation, we assumed the liquid volume consisted of wastewater and a buffer solution at a volumetric ratio of 1:1, as used in our experimental studies. The wastewater sample was assumed to have dissolved MT, DMS and DMDS concentrations of 50 ppb for each compound. The formulae used for the calculation include Henry’s law, ideal gas equation of state and the law of conversion of mass.
Chapter 8 Degradation of methanethiol in anaerobic sewers and its correlation with methanogenic activities

8.1 Abstract
Methanethiol (MT) is considered one of the predominant odorants in sewer systems. Therefore, understanding MT transformation in sewers is essential to sewer odor assessment and abatement. In this study, we investigated the degradation of MT in laboratory anaerobic sewers. Experiments were carried out in seven anaerobic sewer reactors with biofilms at different stages of development. MT degradation was found to be strongly dependent on the methanogenic activity of sewer biofilms. The MT degradation rate accelerated with the increase of methanogenic activity of sewer biofilms, resulting in MT accumulation (i.e. net production) in sewer reactors with relatively low methanogenic activities, and MT removal in reactors with higher methanogenic activities. A Monod-type kinetic expression was developed to describe MT degradation kinetics in anaerobic sewers, in which the maximum degradation rate was modeled as a function of the maximum methane production rate through a power function. It was also found that MT concentration had a linear relationship with acetate concentration, which may be used for preliminary assessment of MT presence in anaerobic sewers.

8.2 Introduction
For decades, the unpleasant odors emitted from sewer systems have been a major issue for water utilities (Hvitved-Jacobsen et al. 1988, Boon 1995, Sharma et al. 2008b). Studies and practices dealing with this problem were, for decades, focused on hydrogen sulfide, a well-known odorant in wastewater. However, odors in wastewater can be caused by many other compounds, such as free ammonia, volatile fatty acids (VFAs), volatile organic compounds (VOCs) and volatile organic sulfur compounds (VOSCs) (Hvitved-Jacobsen 2002, Zarra et al. 2008, van Leerdam et al. 2011). Among these compounds, VOSCs are believed to be of particular importance due to a combination of malodorous characteristics, high volatility and low odor thresholds which are typically at the level of parts per billion by volume (ppbv) (Cheng et al. 2005, Munoz et al. 2010, Sivret et al. 2013a). At higher concentrations, i.e. >0.5–20 parts per million by volume (ppmv), VOSCs could cause health problems (Lomans et al. 2002b, Kastner et al. 2003). As a result, VOSCs should be considered in the design and assessment of odor abatement systems (Sivret et al. 2013a).

Methanethiol (MT) is a typical VOSC and has a putrid smell like rotten cabbage. The odor threshold value of MT (0.07 ppbv) is one of the lowest in the VOSC category (Feilberg et al. 2010).
In addition, the volatility of MT (interpreted by the Henry's law constant which is equal to 5 kg·bar/mol for solubility in water at 298.15K) is one of the highest (Debruyn et al. 1995). Thus, MT could have a higher potential to contribute to malodor than other VOSCs, if all these compounds were present at similar concentrations. To our knowledge, the concentration ranges of VOSCs in sewer systems are not well-documented. However, some case studies have revealed that MT can be a predominant VOSC in wastewater and sewer gases. Hwang et al. (1995) measured that the average MT concentration in the influent of a wastewater treatment plant (WWTP) was about 3–200 times higher than other VOSCs like dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and carbon disulfide (CS2). Lasaridi et al. (2010) also found that MT was the dominant VOSC at a pump station and seven WWTPs in Greece. More recently, Wang et al. (2014) conducted a long term VOSC monitoring program for sewers located at 18 different sites in two major Australian cities (Sydney and Melbourne). In both cities, the MT concentration (675.3–1421.1 ug/m³) in the sewer air was substantially higher than the concentrations of four other tested VOSCs (7.8 ug/m³–94.0 ug/m³). From these results, MT is likely a key odor-causing VOSC in domestic wastewater. Therefore, understanding the transformation of MT in sewer systems is critical for solving odor problems caused by VOSCs.

Our recent study (Sun et al. 2014b) found that MT concentration in anaerobic sewer wastewater changed dynamically with the hydraulic retention time (HRT). The concentration initially increased with the increase of HRT and then decreased. This indicated that MT was being both generated and degraded under anaerobic sewer conditions. The production of MT under anaerobic conditions is mainly attributed to the cleavage of sulfur containing amino acids or methylation of sulfide during the degradation of methoxylated aromatic compounds (Kadota and Ishida 1972, Lomans et al. 2002b, Chasteen and Bentley 2004, Higgins et al. 2006). However, the mechanism for MT degradation in anaerobic sewer systems is unclear. In the past few decades the degradation of MT has been studied in anaerobic/anoxic aquatic environments e.g. marine sediment (Kiene et al. 1986, Visscher et al. 1995), salt marsh sediment (Kiene et al. 1986, Kiene and Capone 1988), freshwater sediment (Zinder and Brock 1978, Kiene et al. 1986, Lomans et al. 1999a, Lomans et al. 1999c), and anaerobically digested biosolids (Chen et al. 2005, Higgins et al. 2006, van Leerdam et al. 2006). Methanogens and/or sulfate reducing bacteria (SRB) were found to be responsible for MT degradation in these environments (Kiene et al. 1986, Kiene and Capone 1988, Tanimoto and Bak 1994, Visscher et al. 1995, Lomans et al. 1999b, Lomans et al. 1999c, Chen et al. 2005, Higgins et al. 2006). These microorganisms also exist in anaerobic sewers (Guisasola et al. 2008, Sharma et al. 2008b). Since MT degradation could alleviate odor emission from sewers, an understanding of the
process is essential for identification of MT emission hot spots along the sewer pipes, which would then provide guidance for odor abatement strategies in the water industry.

Therefore, the aim of this study is to understand the degradation of MT in anaerobic sewers. The study was carried out in biofilm reactors simulating anaerobic sewers at different biofilm development stages. Batch tests were conducted to investigate the degradation pathways and kinetics. A kinetics expression for MT degradation in anaerobic sewers is proposed based on the results of the batch test. To our knowledge, the present study describes for the first time the pathways and kinetics of MT degradation in anaerobic sewer systems.

8.3 Materials and Methods

8.3.1 Operation of anaerobic sewer reactors

Seven cylindrical reactors (R1–R7), each with a volume of 1 L, were used in this study to grow anaerobic wastewater biofilms mimicking those in sewers (Guisasola et al. 2008). The seven reactors were operated in parallel, fed with actual domestic wastewater (Supporting Information, Figure 8-S1(A)). Biofilms were developed on the walls and inner surface of reactor lids. The wastewater was pumped into each reactor intermittently (Figure 8-S1(B)) to simulate the typical dynamic flow patterns of rising main sewers, where anaerobic wastewater biofilms grow (Sharma et al. 2008b). The HRT of the wastewater in each reactor varied from 15 min to 3 h. Previous studies demonstrated that the biotransformation processes in these biofilm reactors mimic well those in real sewers (Guisasola et al. 2008, Gutierrez et al. 2009, Jiang et al. 2011b, Jiang et al. 2013, Sun et al. 2014a).

Based on the reactor performance, the seven reactors (R1–R7) were divided into two groups. R4–R7 had been operated for a minimum of two years and the biofilms had reached pseudo-steady states, indicated by stable methane and sulfide production rates (Figure 8-S2, SI). On the other hand, R1–R3 had been previously treated by FNA at various times for sulfide and methane control (Jiang et al. 2011b). These three reactors were in the ‘recovery’ stage when experiments were conducted. The methane and sulfide production rates measured before the experiment are also shown in Figure 8-S2 (SI).

8.3.2 Batch test I to correlate MT profile with methane, sulfide, DMS and acetate profiles

MT profiles in the seven reactors were monitored in 2–4 h batch tests. At the beginning of each test, fresh sewage was pumped through the reactor for 10 minutes to ensure complete replacement of liquid in the reactor. MT, methane, inorganic sulfide, DMS and VFA concentrations in the reactors
were measured every 30 min. The methanogenic and sulfidogenic activities were designated as the maximum methane production rate (MPR) and maximum sulfide production rate (SPR), respectively. These were calculated through linear regression of methane and sulfide concentration based on the methane and sulfide data in the first hour, during which there was no substrate limitation.

### 8.3.3 Batch test II to identify MT degradation pathways

Studies in other anaerobic environments suggest that methanogens and SRB may be responsible for the consumption of MT in anaerobic sewers. The proposed reactions of MT degradation by methanogen and SRB are shown in Equation (8-1) and Equation (8-2), respectively (Zinder and Brock 1978, Finster et al. 1992).

**By methanogen:** $4\text{CH}_3\text{SH} + 3\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + 4\text{HS}^- + \text{HCO}_3^- + 5\text{H}^+$ \hspace{1cm} Equation (8-1)

**By SRB:** $\text{CH}_3\text{SH} + 0.75\text{SO}_4^{2-} \rightarrow 1.75\text{HS}^- + \text{HCO}_3^- + 1.25\text{H}^+$ \hspace{1cm} Equation (8-2)

Based on this hypothesis, the role of methanogens and SRB on MT degradation in anaerobic sewers was investigated by using selective inhibitors to block the potential pathways. Here, 2-bromoethanesulfonic acid (BES, 70 mM) was used to inhibit methanogens and molybdate (sodium salt, 7 mM) was used to inhibit SRB. These two chemicals have been used as exclusive inhibitors for methanogen and SRB in previous studies (Kiene et al. 1986, Kiene and Capone 1988, Lomans et al. 1999a, Lomans et al. 1999c, Higgins et al. 2006). The inhibition tests were carried out in reactors R4–R7, which had very similar methanogenic and sulfidogenic activities. Different combinations of inhibitors were used to inhibit either methanogens or SRB, or both, as listed in Table 8-1. Since the inhibition of methanogens by BES takes place after a lag time (Lomans et al. 2002b), the reactors for the methanogen inhibition (R5 and R7) were pretreated with 70 mM BES for 10 hours before the batch test.

During the batch test, the reactors were firstly filled with MT-free and MT-substrate-free wastewater. The wastewater was obtained by collecting effluent from R4–R7 with HRT of ~24 h to allow both MT and MT-substrate be consumed. Preliminary tests (data not shown) revealed the reactor fed with this wastewater showed no MT production, and the MT concentration remained very low (<5 μg S/L). Then different types of inhibitors were added into each reactor according to Table 8-1. Sodium sulfate was also added to the reactors to a concentration of 10 mg S/L to provide an electron acceptor for SRB (Equation (8-2)). Subsequently, 7 ml of MT stock solution (50 mg S/L)
was spiked into the reactor to achieve MT concentration in the reactor at 350 μg S/L. The MT concentration in the reactor was measured every 30 min over 3 h. The abiotic loss of MT was tested in a similar reactor which did not contain biofilm. The reactor was filled with filtered wastewater (0.22 μm) and the MT concentration was spiked to 350 μg S/L. MT concentration was also monitored every 30 min over 3 h.

Table 8-1. Inhibition tests in R4–R7 — inhibitors and targeted microbial groups.

<table>
<thead>
<tr>
<th>Test</th>
<th>Reactor</th>
<th>Inhibitor added</th>
<th>Target microbial group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R4</td>
<td>70 mM BES</td>
<td>Methanogens</td>
</tr>
<tr>
<td>2</td>
<td>R5</td>
<td>7 mM molybdate</td>
<td>SRB</td>
</tr>
<tr>
<td>3</td>
<td>R6</td>
<td>7 mM molybdate + 70 mM BES</td>
<td>SRB, methanogens</td>
</tr>
<tr>
<td>4</td>
<td>R7</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

8.3.4 Batch test III to investigate stoichiometry of MT degradation

To further elucidate the MT degradation pathway and the stoichiometry of MT degradation, the molar ratios between CH₄ and H₂S produced and MT consumed was determined in this set of tests. For this purpose, two types of batch tests were conducted in R7 without inhibitors. In the first test, the reactor was initially filled with fresh wastewater, followed by the addition of 6 ml MT stock solution (500 mg S/L) to achieve an MT concentration of 3 mg S/L in the reactor. The MT, CH₄ and H₂S concentrations were measured immediately after MT addition, and also 24 h post MT addition. Preliminary tests showed that after 24 h, H₂S and CH₄ concentrations were stable, and MT concentration was negligible, indicating that the added MT and other substrates in the original wastewater for sulfide and methane production were consumed. The second test was carried out in a similar way but without MT addition. MT, CH₄ and H₂S concentrations were measured after the reactor was fully replaced by the same fresh wastewater and measured again after 24 hours. The CH₄ and H₂S produced by the degradation of spiked MT were calculated by the increased CH₄ and H₂S production in the first test compared with the second test. The tests are duplicated.

8.3.5 Batch test III to investigate the MT degradation kinetics and correlation with methanogenic activity

This set of batch tests, aimed to investigate MT degradation kinetics, was conducted in reactors R1–R4, each of which displayed different methanogenic activities. The methanogenic activity in each reactor was tested prior to the kinetics measurement using the same method as per Batch tests I. Briefly, at the beginning of each test, fresh sewage was pumped through the reactor for 10 min to ensure complete replacement of the liquid inside. Then wastewater samples were taken at 0, 20, 40,
60 min for the analysis of methane concentration. Methanogenic activity was designated as the maximum MPR, which was calculated based on linear regression of methane concentrations.

For MT degradation kinetic measurements, batch tests were conducted at the initial MT concentration of 1000 μg S/L. At the beginning of the tests, the reactor (R1–R4) was filled with MT-free and MT-substrate-free wastewater. MT stock solution (20 ml, 50 mg/L) was then added to the reactor. During the tests, wastewater samples were taken for MT analysis, until the MT concentration became negligible.

8.3.6 Kinetic modelling
Monod kinetics (Equation (1)) was applied to describe MT degradation. This expression has been widely used to describe kinetics related to biofilms (Hvitved-Jacobsen 2002, Sharma et al. 2008b).

\[
\frac{dS_{MT}}{dt} = -k \times \frac{S_{MT}}{K_{MT} + S_{MT}}
\]

Equation (8-3)

Where:
- \(k\) is the maximum MT degradation rate (μg S/(L·h));
- \(t\) is the reaction time (h);
- \(S_{MT}\) is the MT concentration (μg S/L) in the reactor;
- \(K\) is half-saturate concentration of MT (μg S/L).

The parameter estimation and uncertainty evaluation were carried out according to Batstone et al. (2003), with a 95% confidence level for significance testing and parameter uncertainty analysis. The standard errors and 95% confidence intervals of individual parameter estimates were calculated from the mean square fitting errors and the sensitivity of the model to the parameters. The determined F-values were used for number of parameters and degrees of freedom in all cases. A modified version of AQUASIM 2.1d was used to determine the parameter surfaces (Batstone et al. 2009).

8.3.7 Chemical analysis
The VOSCs (MT and DMS) were measured immediately after the samples were taken, using gas chromatography (Agilent 7890A), equipped with a sulfur chemiluminescence detector (SCD), using the method described by Sun et al. (2014b). Dissolved methane was analyzed by gas chromatography (Agilent 7890A), equipped with a flame ionization detector (FID) using the method described by Guisasola et al. (2008). VFAs were determined by gas chromatography
according to standard methods (APHA, 1998). Dissolved inorganic sulfide was analyzed on an ion chromatograph (IC) with a UV and conductivity detector (Dionex ICS-2000) (Sun et al. 2014a).

8.4 Results

8.4.1 MT profiles in anaerobic sewer reactors with biofilms at different stages of development

The MT profiles in three anaerobic sewer reactors (R1–R3) with biofilms in different stages of development followed distinct trends, as shown in Figure 8-1(A). MT concentration in R1 increased substantially in 4 h, reaching a maximum concentration of 495.3 μg S/L at the end of the test. However, the MT production rate decreased gradually as indicated by the slope of the concentration profile. In R2, the MT concentration increased gradually in the first 3.5 h demonstrating a lower rate as compared with R1. Subsequently, the concentration decreased only slightly during the last half hour. The highest MT concentration in R2 was 344.0 μg S/L achieved at 3.5 h. In contrast, the MT concentration in R3 increased slowly in the first hour, reaching a relatively stable level at about 140 μg S/L in the second hour. The concentration then decreased continuously to 57.0 μg S/L in the following 2 h. In R3, MT concentration peaked at 141.5 μg S/L in the first hour, which was much lower than that in R1 and R2.

The methane concentrations in the three reactors were also monitored during the 4-hour tests. The methane profiles (Figure 8-1(B)) suggested that methanogenic activities in the three reactors were very different. The highest methanogenic activity was observed in R3, with a maximum MPR of 6.4 mg/(L·h), followed by R2 with a maximum MPR of 2.0 mg/(L·h). In contrast, the activities in R1 were the lowest, with a maximum MPR of 0.4 mg/(L·h). The comparison of MT profiles and methane profiles in R1–R3 revealed an inverse relationship between methanogenic activities and MT concentrations. This also held true for R4–R7 as shown in Figure 8-1(F). These reactors had relatively high methanogenic activities (Figure 8-S2(A)) with MT degrading during the entire 2-hour test, and the concentration remaining at a low level. The relationship between MT and methane profiles indicated that methanogens could play an important role in MT degradation in anaerobic sewers.
Figure 8-1. Concentration profiles in the anaerobic sewer reactors: (A) MT, (B) methane, (C) sulfide, (D) DMS and (E) acetate in R1 (○), R2 (△) and R3(●). (F) MT profiles in R4–R7.

The sulfide profiles in R1–R3 are shown in Figure 8-1(C). The maximum SPR in the three reactors, as calculated from the sulfide profiles were 4.6 mg S/(L·h) for R1, 8.0 mgS/(L·h) for R2 and 6.5 mgS/(L·h) for R3. There was no clear correlation between sulfidogenic activities and MT
concentration in the reactors as indicated by the sulfide and MT profiles. Although the reactor with the lowest sulfidogenic activities had the highest MT concentration, the converse was not true i.e. MT concentration was not the lowest in the reactor with the highest sulfidogenic activities. In addition, sulfidogenic activities in R4–R7 (Figure 8-S2(B)) were comparable with that in R2, but the MT profiles were significantly different from that in R2.

In R4–R7, the profiles of DMS, another typical VOSC, follows the same trends as that of MT, but the concentrations were more than one magnitude lower than the MT concentration (Figure 8-1(D)). The DMS concentration in R1 increased in the 4-hour test while the DMS concentration in R2 decreased in the last half hour. In R3, the DMS concentration increased slowly in the first 2 h, then decreased. The highest DMS concentrations in the three reactors were 10.8 μg S/L (R1, at 4 h), 7.7 μg S/L (R2, at 3.5 h), and 4.4 μg S/L (R3, at 2 h). A similar trend was observed for acetate. As shown in Figure 8-1(E), the acetate concentration increased continuously in R1 for 4 h but decreased in R2 at end of the test. Slightly different trends were shown in R3, i.e. the acetate concentration decreased at a very slow rate, and unlike MT and DMS, there was no increase observed.

8.4.2 MT degradation in inhibition tests

The results of inhibition tests for identification of MT degradation pathways are shown in Figure 8-2. The degradation of MT stopped in the reactors with BES (a methanogen inhibitor) added, but not in the reactor with molybdate (an SRB inhibitor) added. This indicated that methanogens were responsible for the MT degradation, while SRB were not. No degradation of MT was observed in the reactor with BES plus molybdate. The abiotic loss of MT was negligible as the MT concentration remained constant in the reactor without biofilms (Figure 8-2). Thus, it is likely that MT was primarily degraded by methanogens in the studied anaerobic sewer reactors.
Figure 8-2. The MT profiles in the anaerobic sewer reactors in the presence of different inhibitors.

8.4.3 Stoichiometry of MT degradation
To further confirm the degradation of MT by methanogens, the stoichiometry of MT degradation was also investigated in the control reactor (without any inhibitors). As shown in Table 8-2, a calculated ratio of 0.98 H₂S and 0.74 CH₄ was formed by the degradation of MT. These values agreed well with the theoretical stoichiometric values for the MT dissimilation by methanogens, as shown in Equation (8-1), which further supported that MT is primarily degraded by methanogens.

Table 8-2. Stoichiometry of MT degradation.

<table>
<thead>
<tr>
<th>Test</th>
<th>MT consumed</th>
<th>Molar ratio of H₂S formed to MT dissimilated</th>
<th>Molar ratio of methane formed to MT dissimilated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>1</td>
<td>0.95</td>
<td>0.69</td>
</tr>
<tr>
<td>Test 2</td>
<td>1</td>
<td>1.00</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean</td>
<td>1</td>
<td>0.98</td>
<td>0.74</td>
</tr>
<tr>
<td>Standard error</td>
<td>0</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>

8.4.4 MT degradation kinetics and correlation with methanogenic activities
The kinetics of MT degradation by methanogens was tested in four reactors with four different methane production rates, i.e. 1.7 mg/(L·h), 3 mg/(L·h), 5.5 mg/(L·h) and 8.2 mg/(L·h), respectively. As shown in Figure 8-3, the reactor with a higher methanogenic rate had a higher MT degradation rate. In each reactor, the MT degradation rate declined gradually with the decrease of...
MT concentrations. The different MT degradation rates amongst the four reactors at the same initial MT concentration indicated that the maximum MT degradation rates (k in Equation (8-3)) varied. As MT degradation rate was positively correlated with the methanogenic rate, a modified Monod kinetic model was proposed, as shown in Equation (8-4), to describe the MT degradation in anaerobic sewers. In the modified kinetic model, the maximum MT degradation rate (k) was described as a power function of maximum MPR.

\[
\frac{dS_{MT}}{dt} = -k' \times r_{CH4} \times \frac{S_{MT}}{S_{MT} + K_{MT} + S_{MT}}
\]

Equation (8-4)

Where:

\(k'\) is the reaction rate constant (\(\mu g / (L \cdot h)\));

\(r_{CH4}\) is the maximum methane production rate (\(mg CH_4/(L \cdot h)\));

\(\alpha\) is order with respect to the maximum methane production rate (-).

Figure 8-3 shows that MT concentrations predicted by the modified kinetic model fit well with the experimentally measured data. Parameter values \((k', \alpha, K_{MT})\) giving the optimum model fit with the experimental data (Figure 8-3) are listed in Table 8-3, together with standard errors. The obtained parameter correlation matrix (SI Table 8-S2) shows the correlations among parameters are low (< 0.8). In the uncertainty analysis, 95% confidence regions for the different parameter combinations were investigated to evaluate their identifiability. SI Figure 8-S3 shows the three joint 95% confidence regions for different parameter combinations, together with the confidence intervals for all the parameters. Overall, the 95% confidence regions for all of the three pairs are small, with mean values lying at the center. The 95% confidence intervals for all the single parameters are also small, and generally within 7% of the estimated values (SI Figure 8-S3). Combined, this indicates that all the parameters have a high level of identifiability, and that the estimated values are reliable.

In a real sewer network, pipes of different diameters may be constructed. Therefore, from a practical point of view, the volumetric MT degradation rate illustrated by Equation (8-4) can be easily converted to the areal rate by dividing the area to volume (A/V) ratio. In that case, the \(k'\) value in equation (8-4) equals 815.9 (with the unit of MT degradation rate and methane production rate being \(\mu g / (m^2 \cdot d)\) and \(mg CH_4/(m^2 \cdot d)\), respectively).
Figure 8-3. The degradation of MT in four reactors with different methanogenic activities. Symbols represent experimental measurements and lines represent model fits.

Table 8-3. Estimated parameter values with standard errors.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k'$</td>
<td>81.0±2.9</td>
<td>(μg S/L) (mg CH$_4$/L)$^{-0.65}$$h^{0.38}$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.62±0.02</td>
<td>-</td>
</tr>
<tr>
<td>$K_{MT}$</td>
<td>168.0±12.7</td>
<td>μg S/L</td>
</tr>
</tbody>
</table>

8.5 Discussion

8.5.1 MT degradation pathways in anaerobic sewers

Our results show that under anaerobic sewer conditions, MT is primarily degraded by methanogens. The MT degradation rate increased concomitantly with the increase in methanogenic activity. It follows that with low methanogenic activity, MT can accumulate in sewer systems.

The degradation of MT by methanogens was firstly illustrated by Zinder and Brock (1978). They found in anaerobic freshwater sediment that MT was rapidly metabolized to methane with a ratio close to 4:3, a figure similar to this study. Methanogenic MT degradation was also identified in other environments, such as marine sediment (Kiene et al. 1986, Finster et al. 1990, Visscher et al. 1995), salt marsh sediments (Kiene et al. 1986, Kiene and Capone 1988), and anaerobically digested biosolids (Zitomer and Speece 1995, Chen et al. 2005, Higgins et al. 2006, van Leerdam et
SRB were also found responsible for MT degradation in some anaerobic systems (Kiene 1988, Taylor and Kiene 1989, Tanimoto and Bak 1994, Lomans et al. 2002a, Lyimo et al. 2009). However, the inhibition tests conducted in this study (Figure 8-2) revealed that SRB could not degrade MT in the tested sewer biofilm systems.

MT-utilizing methanogens isolated anaerobic environment such as marine sediment, salt marsh sediments, freshwater sediments and anaerobically digested biosolids are primarily belong to the genera of *Methanolobus, Methanosarcina, Methanosalsus* and *Methanomethylovorans* (Lomans et al. 2002b, Jiang et al. 2005, Cha et al. 2013). However, only MT-utilizing methanogens of the genus, *Methanomethylovorans* were isolated from freshwater environments while others were all from saline-water environments. The presence of *Methanomethylovorans* in anaerobic sewer reactor biofilms was revealed by 16S rRNA gene sequencing (unpublished data) suggesting that this genus may play a role in MT degradation in the reactor. To date, the mechanisms of methanogenic MT degradation have not been well understood (Lomans et al. 2002b). Zinder and Brock (1978) proposed it may be similar to methanol degradation, as there was some reassembly of MT and 2-mercaptoethanesulfonic acid (HS-CoM), a methyl carrier group used by methanogens for methanol degradation. However, a later study (Ni and Boone 1993) suggested that MT and methanol were likely transformed by distinct inducible enzymes.

### 8.5.2 MT degradation kinetics in anaerobic sewers

The kinetics of MT degradation in anaerobic sewers can be described by Monod-type kinetics with the maximum degradation rate correlated to the maximum methane production rate through a power function (Equation (8-4)). The half-saturation concentration ($K_{MT}$) of MT determined in this study i.e. 168 μg S/L (or 5.3 μM) was comparable to that reported by Lomans et al. (Lomans et al. 1999a) in freshwater sediments i.e. 2.2 μM.

The kinetics of MT degradation indicated that low methanogenic activities would lead to a slow MT degradation rate, and consequently, MT could accumulate, as observed in Figure 8-1. This suggests that in sewers with low methanogenic activity, odor problems caused by MT could become more severe. Lomans et al. (2001) believed that methanogens played an important role in the balance between VOSC production and degradation, resulting in little emission of these compounds in freshwater sediments. A similar balance was also reported for anaerobically digested biosolids in that the release of MT and odors normally occurred associated with the inhibition of methanogens (Higgins et al. 2006). The current sewer odor abatement strategies for H$_2$S control in sewers, such as the addition of oxygen, nitrate, iron salts, magnesium hydroxide and caustic, all suppress the
methanogenic activities (Mohanakrishnan et al. 2008, Gutierrez et al. 2009, Zhang et al. 2009, Jiang et al. 2013, Ganigué and Yuan 2014, Gutierrez et al. 2014). Under such conditions, the MT degradation in sewer systems could be inhibited. However, the effects of these strategies on MT balance in sewers would also depend on their effects on the MT production processes. The latter effects are currently not completely understood.

8.5.3 Preliminary assessment of MT presence in anaerobic sewers using acetate concentration as an indicator

Figure 8-2 shows that profiles of MT, DMS and acetate follow the same trend in the sewer reactors. The linear regression of MT and acetate concentrations as well as MT and DMS concentrations in three reactors show high correlations (Figure 8-4(A) and (B)). The $R^2$ value for MT versus acetate was 0.93 and for MT versus DMS was 0.95. Since acetate has not been proven to be a precursor of MT, the high correlation between MT and acetate concentration could be due to similar transformation pathways under anaerobic conditions. Under anaerobic conditions, MT has been reported to originate from sulfur-containing amino acids and derivatives, such as methionine and S-methyl-cysteine (Kadota and Ishida 1972, Kiene and Visscher 1987, Lomans et al. 2002b). Also, it was observed that MT could be generated anaerobically by the methylation of sulfide during the degradation of methoxylated aromatic compounds (Finster et al. 1990, Bak et al. 1992, Lomans et al. 2001). The studies on the microorganisms involved in these processes indicated that fermentative bacteria and some homoacetogenic bacteria play an important role in MT production (Kadota and Ishida 1972, Taylor and Kiene 1989, Bak et al. 1992). These bacteria were also capable of producing acetate during their metabolism (Batstone et al. 2002). In terms of degradation, both MT and acetate can serve as a substrate for methanogenesis. As a result, the production and degradation of MT and acetate could happen simultaneously and the linear correlation between MT and acetate concentrations could establish. Though acetate could also be consumed by SRB (Fedorovich et al. 2003, Sharma et al. 2008b), our results show that the consumption of acetate by SRB seems not significantly affect the correlation. The correlation between MT and acetate profiles revealed that acetate concentration could be used for preliminary assessment of the MT presence in anaerobic sewers. This preliminary estimation could be useful, especially given that the measurement of acetate is much easier that the measurement of MT (Wardencki 1998). However, further investigation, especially the study on MT, is needed to validate this assessment method.
Figure 8-4. Linear regression of (A) MT vs acetate concentrations and (B) MT vs. DMS concentrations

8.6 Supporting Information

**Lab-scale anaerobic sewer systems operation.** Seven 1 L gas-tight cylindrical reactors (R1- R7), made of Perspex™, were operated in parallel to mimic 7 anaerobic sewer pipes (Figure 8-S1A). R1-R3 were at the recovery stage after being previously exposed to free nitrous acid (FNA) for sulfide and methane control (Jiang et al. 2011b). R4-R7 were not treated by any chemicals previously and had reached a pseudo-steady state at the time of the tests. The inner diameter of each reactor was 80 mm and the area to volume ratio (A/V) was calculated to be 55 m⁻¹, with biofilms growing on the wall and top of the reactor considered. Mixing was continuously provided by a magnetic stirrer (Heidolph MR3000) under the reactor, so there was no biofilms growing on the bottom. Domestic sewage, collected on a weekly basis from a local wet well (Brisbane, Queensland), was used as the feed of the reactor. The sewage typically contained sulfate at concentrations of 10-25 mg-S/L, sulfide at < 3mg-S/L, soluble COD at 200-300 mg/L, 50-120 mg-COD/L of VFAs and approximately 50 mg-N/L of ammonium. Negligible amounts of sulfite, thiosulfate (<1 mg-S/L), nitrate and nitrite (<1 mg-N/L) were present. The sewage was stored in a cold room (4°C) to minimize biological transformation, and was heated up to 20±1°C prior to being pumped into the reactors. The sewage was fed to the reactor intermittently by a peristaltic pump to simulate the typical flow patterns of rising main sewers (Figure 8-S1B). Every feeding lasted for 2 min, delivering one reactor volume (1L) of wastewater into the reactor. The hydraulic retention time (HRT) of the wastewater ranged between 15 minutes to 3 hours, which are in the range of HRT observed in real sewer pipes.
Figure 8-S1 (A) A schematic representation of seven laboratory-scale anaerobic sewer reactors. (B) The pumping pattern and HRT of the systems in an 8-hour period. The operation in each day repeated this 8-hour cycle. The vertical solid lines refer to the pumping events and the dashed lines represent the HRT of the wastewater slug in the reactors.
Figure 8-S2. Maximum methane production rate (A) and maximum sulfide production rate (B) in R1-R7.

Figure 8-S3. The 95% confidence regions for the parameter combinations with the best fits in the center as well as the 95% confidence intervals: (A) $K_{MT}$ vs. $k'$, (B) $K_{MT}$ vs. $\alpha$ and (C) $\alpha$ vs. $k'$. 
Table 8-S1. Correlation matrix resulting from parameter estimation.

<table>
<thead>
<tr>
<th></th>
<th>$K_M$</th>
<th>$k'$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_M$</td>
<td>1.000</td>
<td>0.604</td>
<td>-0.072</td>
</tr>
<tr>
<td>$k'$</td>
<td>0.604</td>
<td>1.000</td>
<td>-0.798</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>-0.072</td>
<td>-0.798</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Chapter 9 Research outcomes, conclusions and future work

9.1 Summary of research outcomes

The potential effects of RWC on sulfide and methane production in rising mains were investigated through laboratory study and mathematical modelling. The key findings are:

- RWC increases sulfide concentrations in sewers. The higher sulfide concentration is mainly due to the longer hydraulic retention time of sewage in sewers, as the sulfide-producing activity of sewer biofilms is not significantly affected. The reduced water consumption also results in lower sewage pH. The increased H₂S concentration and lower pH are predicted to enhance odor and corrosion problems in sewers. The volumetric chemical dosing rate for sulfide mitigation would increase; however, due to the lower flow rate, the total sulfide discharge and the daily chemical dosing cost would decrease.

- RWC results in higher methane concentrations in sewers, caused by both enhanced methanogenic activity and longer hydraulic retention time. This could lead to substantial increases in methane emission under reduced flow conditions, thereby increasing greenhouse gas emissions from wastewater collection systems.

The microbial structure of sewer biofilms under RWC conditions was investigated by multiple approaches including microelectrode measurements, molecular techniques and mathematical modelling. The main findings are as follows:

- Sulfide was mainly produced in the outer layer of the biofilm, between 0 - 300 μm, which is in good agreement with the distribution of SRB population. SRB have a higher relative abundance of 20% in the surface layer, which decreased gradually to below 3% at the depth of 400 μm.

- MA mainly inhabited in the inner layer of the biofilm, with the relative abundance increased from 10% at the depth of 200 μm to 75% at the depth of 700 μm.

- SRB in biofilm were mainly affiliated with five genera: Desulfobulbus, Desulfomicrobium, Desulfovibrio, Desulfatiferula and Desulforegula, while about 90% of the MA population belonged to the genus of Methanosaeta.

- Mathematical modelling of the biofilm structure indicated that the coexistence and spatial structure of SRB and MA in the biofilm resulted from the microbial types, their proposed metabolic transformations and substrate interactions.
The impact of iron-rich coagulation sludge discharge on dissolved sulfide concentration in rising main sewers was investigated through a laboratory study. The key findings are:

- The discharge of iron-rich coagulation sludge in rising main sewers can effectively control dissolved sulfide concentration.
- A molar ratio of 0.5-1:1 between the iron contained in the iron-rich coagulation sludge and the expected sulfide formation is required for satisfactory control of sulfide in sewers.
- More research is needed to develop a full understanding of the unintended effects of sludge dosing on sewer sediments and wastewater treatment performance. In this study, we did not observe an increase in in-sewer methane production despite a 12% increase in the total chemical oxygen demand concentration.

An efficient method for VOSC measurement in wastewater was developed based on GC-SCD with static headspace technique. The following conclusions are drawn regarding the suitability of the method:

- VOSCs in the wastewater can be measured by GC-SCD with the static headspace technique. The method is simple and rapid as pre-concentration of samples is not required.
- The calibration curves obtained by this method present good linearity (>0.999). The detection limit is lower than 1.0 ppb.
- The recovery ratio tests and real wastewater sample analysis demonstrate that this method is suitable for routine VOSCs measurement in wastewater.
- VOSCs in wastewater samples can be preserved for at least 24 hours by acidification of wastewater samples (pH ~1.1).

The degradation of MT in anaerobic sewers under different biofilm development stages was investigated through laboratory study. The main findings are:

- MT was mainly degraded by methanogens in anaerobic sewers. The MT degradation rate accelerated with the increase of methanogenic activity of sewer biofilms, resulting in MT accumulation in sewer reactors with relatively low methanogenic activities, and MT removal in reactors with higher methanogenic activities.
- A modified Monod-type kinetic expression was developed to describe MT degradation kinetics in anaerobic sewers, in which the maximum degradation rate was correlated to the maximum methane production rate through a power function.
- MT concentration had a linear relationship with the acetate concentration, which could be useful for preliminary assessment of the MT presence in anaerobic sewers.
9.2 Research outcome synthesis and conclusions

To overcome global water crisis, many countries have started to implement new water management strategies, such as water demand management and decentralized water management. These practices are aimed to optimize the existing urban water cycle and consequently balance the supply and demand of urban water. However, changes in the urban water cycle may have impact on the sewer systems, as an indispensible component of an urban water system. As the cost for odor and corrosion control in sewer systems accounts for a large proportion of the total expenditure of the water industry, it is critically important to identify the potential impacts of the changing urban water management on sewer emissions, which is central aim of this thesis.

Water demand management reduces the water consumption rates of households. The RWC is expected to change the wastewater composition and flow conditions in sewer networks and affect the in-sewer transformation processes. Therefore, in Chapter 4, the impact of RWC on sulfide and methane production in rising main sewers was investigated. The results show that the both sulfide and methane concentrations increases under RWC conditions. The increase of sulfide concentration is mainly due to the longer HRT in sewers, as the sulfide-producing activity of sewer biofilms is not significantly affected. Whereas, the higher methane concentration under RWC condition is caused by both enhanced methanogenic activity and longer HRT. Mathematical modelling reveals the volumetric chemical dosing rate for sulfide mitigation would increase; however, due to the lower flow rate, the daily chemical dosing cost would decrease. These results provide useful information to the water industry to adapt their sewer management strategies in future to reduce sewer emissions.

The different methanogenic activities under normal and RWC conditions imply that RWC may change the microbial community structure of sewer biofilms. Since RWC is expected to be increasingly applied in future for the conservation of global water resources, community structure of sewer biofilms under RWC conditions are investigated in details in Chapter 5. The results show that sulfide is mainly produced in the outer layer of the biofilm, which is in good agreement with the distribution of SRB populations. SRB have a higher relative abundance on the surface layer while MA mainly inhabited in the inner layer of the biofilm. A biofilm models was constructed to simulate the SRB and MA distributions in the anaerobic sewer biofilm. The good fit between model predictions and the experimental data indicates that the coexistence and spatial structure of SRB and MA in the biofilm resulted from the microbial types, their metabolic transformations and interactions with substrates. The finding that interaction between microbial types and substrate determine the biofilm structure and activities can also help to explain why SRB activities under RWC and normal conditions were similar while MA were distinct, as revealed in Chapter 4. The
sulfate concentrations under two conditions were very similar, which lead to the development of similar SRB activities in the biofilms. Although the organic substrates are different under the two conditions, it is apparently not a limiting factor for sulfide production as the concentrations are about 3 times higher than the concentration needed to reduce all the sulfate. However, the difference in organic substrates would affect the methanogenic activities under the two conditions. The higher sCOD concentration under RWC conditions likely favors the growth of methanogens and thus higher methanogenic activity was achieved. The spatial arrangement of SRB and MA in sewer biofilms revealed in this study is of practical importance. Chemicals such as nitrate, oxygen, magnesium hydroxide and sodium hydroxide are frequently added to sewers to control the emission of hydrogen sulfide in sewers (Ganigue et al., 2011). As MA mainly inhabit in the inner layer of the biofilms, they are likely to be protected from being exposed to chemicals added for in-sewer sulfide and methane mitigation. Full penetration of chemicals into biofilms is required to completely control methane production. This is an important consideration for methane abatement strategies in sewers.

Decentralized water management is another emerging strategy to cope with global water shortage. The operation of decentralized systems could generate some waste products such as waste activated sludge or coagulation sludge. Unlike centralized systems, which often include sludge treatment processes, the waste sludge from the decentralized systems is usually dumped into sewers directly due to their relatively small scales. Since the waste sludge could be high in organic matters or metals, the appearance of sludge in sewer might affect the in-sewer processes and sewer emissions. Therefore, in Chapter 6, the effect of iron-rich coagulation sludge on sulfide and methane production in sewer systems was investigated. The results show that the discharge of iron-rich coagulation sludge can significantly reduce total dissolved sulfide concentration in sewers. The decrease of dissolved sulfide concentration is mainly due to the precipitation between iron and sulfide but other reactions might be also involved. The removal of sulfide indicated that iron-rich coagulation sludge could be used to for sulfide control in sewer systems. By adding iron-rich coagulation sludge to control sulfide, the high cost for traditional chemical dosing will be reduced since the sludge is a waste product. The methane formation after sludge dosing was also slightly decreased. In addition, the addition of sludge would not significantly change COD concentration of the wastewater. Phosphate removal was also observed depending on the sludge dosing rate, which can be beneficial to nutrient removal from the wastewater. However, more research is needed to develop a full understand of the unintended effects of sludge dosing on sewer sediments and wastewater treatment performance.
Apart from sulfide and methane, VOSCs are also important sewer emissions causing sewer odor but little attention was paid to this aspect in the past. With the changed urban water management practices, the transformation of VOSCs in sewer systems may also be affected. However, the studies of VOSC are always hindered by its complicated detection methods and reactive nature. Therefore, in Chapter 7, a static headspace method utilising GC-SCD was developed to quantitatively analyze VOSCs in wastewater matrices. The method is simple and rapid as it requires no pre-concentration treatment of samples. It has a low detection limit below 1.0 ppb and a good linearity of above 0.999. The recovery ratio tests and real wastewater sample analysis demonstrate that this method is suitable for routine VOSCs measurement in wastewater. In addition, sample preservation tests showed that VOSCs in wastewater samples could be preserved for at least 24 hours by acidification of wastewater samples (pH ~1.1). Thus, this method can be used for both laboratory studies and field measurements.

The VOSC measurement in both laboratory sewer systems and real sewers reveals that MT presented as the predominant VOSC in sewer systems and it can be produced and degraded simultaneously in rising main sewers. Since the MT degradation could alleviate odor emission from sewers, an understanding of the process is essential for identification of the MT emission hot spots along the sewer pipes, which could then provide guidance for odor abatement strategies development. Therefore, in Chapter 8, the degradation of MT under different sewer biofilm development conditions was investigated. The results show that MT degradation is strongly dependent on the methanogenic activity of sewer biofilms. The MT degradation rate accelerates with the increase of methanogenic activity of sewer biofilms, resulting in MT accumulation (i.e. net production) in sewer reactors with relatively low methanogenic activities, and MT removal in reactors with higher methanogenic activities. A modified Monod-type kinetic expression was developed to describe MT degradation kinetics in anaerobic sewers, in which the maximum degradation rate was correlated to the maximum methane production rate through a power function. It was also found that MT concentration had a linear relationship with acetate concentration, which may be used for preliminary assessment of MT presence in anaerobic sewers. As revealed by Chapter 5, under the RWC conditions, the methanogenic activity of the sewer biofilm increases, which indicates that MT degradation rate in rising main sewers would be accelerated under RWC conditions. However, the higher acetate concentration under RWC conditions and correlation between MT and acetate concentration suggests that the MT concentration is not necessarily decreased, likely due to a higher MT production rate.
9.3 Recommendation of future research

During the whole period of my PhD, many research challenges have been identified that entail further research. Some of the recommendations for the future research are summarized below:

- In this thesis, the impact of RWC on sulfide and methane production in rising main sewers was evaluated. However, the wastewater compositions and hydraulic conditions in gravity sewers would also be changed by the RWC. Therefore, the impact of RWC on sulfide and methane production and emission in gravity sewers should also be assessed.

- The microbial structure of sewer biofilms in rising main sewers was investigated in this thesis. However, the biochemical in-sewer processes would also occur in aerobic sewer biofilms and sewer sediments. Understanding the microbial structures in aerobic sewer biofilms and sewer sediments could provide further fundamental knowledge of ecosystems in sewers, which would help for better understanding the in-sewer processes which is important for sewer management.

- In this thesis, the feasibility of using iron-rich coagulation sludge was demonstrated by laboratory study. However, this method might have some side effects on sewer sedimentation due to the increase of solids and on the WWTPs due to the presence of NOM. These potential issues need to be investigated in future. Also, testing on real sewers is essential to fully demonstrate the effect of this method.

- Gutierrez et al. (2010) recently found that the addition of ferric chloride to sewers for sulfide control enhances phosphorus removal at the downstream wastewater treatment plant. In addition, Ge et al. (2013) found that iron used for phosphate removal in WWTPs could achieve sulfide control in sludge digesters. Therefore, the potential of iron coagulation sludge used for sulfide control in sewer networks for phosphate removal and sulfide control during sludge digestion at down-stream WWTPs is also worthwhile to study for building up an integrated coagulant management strategy.

- Apart from coagulation sludge, waste activated sludge (WAS) could also discharge from the decentralized systems into sewers. Since WAS are high in organic matters and contain different kinds of microorganisms. The impact of WAS discharge on in-sewer processes should also be assessed.
• This thesis explored MT degradation in rising main sewers through laboratory study. The results should be further evaluated in real sewer systems in future. In addition, as MT concentration is determined by both production and degradation processes, the production of MT in rising main sewers should be investigated as well to understand the overall MT transformation in rising main sewers. Moreover, it is also important to understand the transformation of other VOSCs in sewers systems.

Overall, this thesis indicates that changing urban water management practices will pose both challenges and opportunities on sewer management in future. These challenges should be fully assessed and these opportunities should be well exploited.
Reference


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