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FIELD AND LABORATORY TESTING OF CHEMICAL ADDITIVES AND BIOMATERIALS FOR H₂S AND ODOROUS COMPOUNDS CONTROL (ARC LP0882016)

Draft Final Report

This is the draft version of the final report summarizing the key findings of the Sub-project 6 (SP6) studies of the Sewer Corrosion and Odour Research (SCoRe) Project. The project was funded by the Australian Research Council (ARC) and the Australian water industry partners.

**Advanced Water Management Centre
The University of Queensland**

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EXECUTIVE SUMMARY

This research report was prepared by the Sub-project 6 (SP6) of the Sewer Corrosion and Odour Research (SCORe) project, Advanced Water Management Centre at The University of Queensland (AWMC-UQ). The research study on “Field and laboratory testing of chemical additives and biomaterials for H₂S and odorous compounds control” was undertaken by the SCORe SP6 team of AWMC-UQ in conjunction with Sydney Water Corporation, Allconnex Water, South East Water Limited, Melbourne Water, and South Australia Water during June 2008 and October 2011.

The cash budget was \$748,490 including \$428,660 cash contribution from the ARC. Additionally, all partners involved including the University also made substantial in-kind contributions to the sub-project.

The sub-project aimed to develop monitoring protocols based on scientific principles that involve biofilm structure and activities to assess both the short- and long-term impacts of chemicals including biomaterials which are being marketed to the wastewater industry on the control of sulfide as well as other odorous compounds in sewer systems. The establishment of strategies for the use of chemical additives that shown to be effective was also part of the aim.

The sub-project delivered strong outcomes with most milestones achieved. The knowledge generated is being applied by the Australian Industry partners for the management of their sewer systems.

The main outcomes of the project are summarised below.

- A comprehensive compilation of commercial products for sulfide control in sewers has been documented and distributed to the industry partners. It is the first time that full details of these products including results from previous trials are put together in one document. In addition to provide support to SCORe-SP6 for the selection of chemicals to be tested, it is also highly valuable for industry partners in their future endeavour to deal with sulfide problems in sewers.
- Three different commercial biomaterials have been selected and tested in the laboratory. All of the tested biomaterials were found to be ineffective in controlling sulfide and methane formations, contrary to manufacturers' claims. All laboratory tests were carried out under control conditions with the use of a reference reactor. All measurements performed in the tests (sulfide, methane and volatile fatty acid or VFA concentrations, solids analysis inside and discharged from the system), indicated that there were no differences in the performance between biofilms exposed and not exposed to the commercial biomaterials. The results were conclusive.
- The ferric laboratory studies have provided general guidelines for the use of ferric salts for sulfide control in sewer systems.
 - The research findings indicate that ferric salts should preferably be added to upstream locations. In addition to oxidizing and precipitating sulfide, ferric ions (or its precipitation products) also reduce the sulfate reducing bacteria (SRB) and methanogenic Archae (MA) activities, resulting in reduced sulfide and methane production, and hence reduced demand for chemicals.



- A molar ratio of 0.6:1 of Fe^{3+} to sulfide is adequate to control sulfide at low levels.
 - When added to an upstream, ferric salts can be added in excess to the demand for ferric salts at that location, in anticipation of more sulfide production at downstream locations. The excess Fe^{3+} added will bind with phosphate and hydroxide initially. However, it will become available for sulfide precipitation when more sulfide is generated by sewer biofilms, due to the high affinity of sulfide with ferric ions in comparison to phosphate and hydroxide.
 - As found in a related study, ferric salt addition to sewers for sulfide removal is highly beneficial for P removal in the downstream wastewater treatment plant. Almost all ferric added will be regenerated for chemical P removal.
 - Obtained results along with the previous experiments with different iron dosing (5, 10, 15, 21, 27, and 35 mg Fe^{3+}/L), suggested that as the concentration of the iron increased, faster decrease in sulfate reducing activity was observed. Interestingly, regardless of the initial iron concentration, steady state sulfate reducing activity was similar in all cultures, which ranged between 51-62%. Total Solids (TS) and Volatile Solids (VS) measurements have shown that iron dosing caused a slight increase in the TS content (FeS accumulation on biofilm) and significant decrease in the VS content of the biofilm (biomass decay). Suspected mechanism of inhibition is the FeS precipitation in the vicinity of bacterial cell.
- The laboratory study on effects of FeCl_3 dosage into sewers for sulfide control on H_2S removal in the anaerobic digester has been performed. H_2S emissions in biogas produced from anaerobic digesters was effectively controlled by the third use of typical additions of iron salt (FeCl_3) into sewers for sulfide control (e.g. 5-20 mg Fe L-1). The presence of iron precipitates (FeS) had no significant impacts on key sludge digestion processes, including methane production, organic acids consumption, and $\text{NH}_4^+\text{-N}$ and PO_4^{3-} release. The potential of integrated management of sulfide and phosphate-related problems in sewers, wastewater treatment processes and anaerobic digesters has been highlighted, which can substantially reduce chemical consumption costs and operating expenses.
- Lab testings demonstrated the effectiveness of free nitrous acid (HNO_2 or FNA) in controlling anaerobic biofilm activities in sewer. The results show that long-term inhibition on sulfide and methane formations were obtained with a relatively short exposure time. Intermittent dosing is then suitable for this application, resulting in a key advantage of the FNA dosing over many other chemical dosing commonly used by water industry. The inhibition level was found to be dependent on nitrite concentration and exposure time, with stronger inhibition observed at higher nitrite concentration and/or longer exposure time. However, the time required for achieving 50% recovery of both sulfate-reducing and methanogenic activities after the cessation of nitrite dosage only marginally depended on nitrite concentration. The study also shows that level of cell viability had a much stronger dependence upon the FNA concentration, indicating that FNA may directly cause the inactivation of biofilm cells. A preliminary economic analysis revealed that considerable cost savings can also be achieved as a result.
- The biocidal effects of the combination of FNA with hydrogen peroxide (H_2O_2) on anaerobic biofilm activity were investigated. There is a clear synergism between FNA and H_2O_2 in inactivating microorganisms residing in biofilms. The combination increased the biocidal effect of the novel biocide, i.e. FNA, by at least one-log killing. About 2-log of microbial killing (1-log higher than FNA alone) was achieved when biofilms were exposed to $\text{FNA} > 0.2 \text{ mgN/L}$, $\text{H}_2\text{O}_2 > 30 \text{ mg/L}$ for a duration of 6 hours. It



was found that exposure time is critical while FNA and H₂O₂ ranged 0.2 – 0.4 mgN/L, and 30 – 90 mg/L respectively. An exposure time of 6 hours or longer is required to reach 99% microbial inactivation in biofilms. The combination of FNA and H₂O₂ could be used in biofilm control due to its high killing efficiency. The reaction between FNA and H₂O₂ leads to the production of various reactive nitrogen intermediates (RNI). Among different RNIs, the synergistic killing was attributed to elevated generation of biocidal peroxynitrite, and extra microbial killing by hydrogen peroxide in addition to FNA itself.

- Three different strategies of FNA dose trials were conducted in the UC09 rising main sewer, Gold Coast, to implement the results obtained in the lab-scale study. The strategy includes different exposure times and hydraulic retention times (low and high flow periods). The first trial to test FNA exposition during long hydraulic retention time (HRT) has revealed that FNA dosing had a toxic effect on sewer biofilm. MA activity was highly reduced by FNA and the inhibition lasted in time. In contrast, the recovery of the SRB was much quicker. This is possibly due to the insufficient mixing regime inside the pipe, provided by the short pump events, that leads to the limitation of FNA diffusion into the biofilm. Therefore, mixing conditions inside the pipe is identified as a crucial parameter to obtain higher degree of inhibition. The second dosing trial to test FNA exposition during short HRT times (high flow periods) also confirmed the sensibility of MA to FNA. On day 36 after the 3 days addition, the MA activity was still reduced by 96%. FNA dosing is confirmed as a very effective strategy to control methane emissions from rising main pipes. In the second trial, more pump events and periods of turbulence regime that would increase the mixing conditions inside the pipe were expected. Consequently, FNA would penetrate deeper into the sewer biofilms, producing a higher toxic effect. The third dosing trial was performed in two different applications aiming to initially hit hard the biofilm and further shock the biofilms while they were still on the weak state. From the study, FNA addition has generally shown as a competitive option in controlling anaerobic sewer biofilms. The intermittent FNA addition has the potential to achieve long-term sulfide and methane control. The trials also allowed the identification of crucial parameters for the improvement of the nitrite effectiveness (hydraulic regime, dosing rates, etc.).
- The inhibitory effects of high pH shock dosing to anaerobic sewer biofilm activities have been investigated through the laboratory study. Caustic shock laboratory dosing at high pH (10.5-12.5) significantly reduces SRB and MA activities of anaerobic sewer biofilm. Sulfide production rates of the experimental biofilms were reduced to approximately 90% in comparison to the control biofilms operated at pH 7.5. Methane production rates in the experimental reactors were also reduced to >95% in all cases. The inhibitory effect depends on both pH level (alkali concentration) and exposure time with contact time as a more dominant factor. The full recovery of sulfate-reducing activities was reached at day 7, while the recovery of methanogenic activity remained insignificant even until day 17 after dosing. This shows that MA is more susceptible to high pH than SRB.
- The strong toxic effect due to the high pH exposure on SRB and MA activities observed in the lab-scale studies was confirmed in the field trials. It is proven that high pH dosage has a long-term inhibitory effect on both sulfide and methane formations by anaerobic sewer biofilms. Therefore, it is concluded that the dosage of high pH is likely to be applied intermittently in sewer system to control sulfide and methane productions. In the field trial, alkali dosage to increase pH of the sewage up to 11.5 in the wet well (upstream) for 6 hours exposure time was able to reduce 71% and 97% on sulfide and methane productions, respectively. These significant outcomes can be optimized by



improving the dosing strategy particularly for higher level of sulfide control. The main advantage with the intermittent high pH shock dosing includes a short period of alkali addition and a longer period of recovery. The main difference between the lab-scale and field study was that longer control of sulfide production during laboratory experiment was achieved at pH levels of 10.5 and 11.5 for a short exposure time. This dissimilarity could have been caused by different shear stress in two systems that lead to the variation of biofilm structures. During pump event, a shear stress of about 0.3 Pa occurred in the lab-scale sewer reactor, while the shear stress for the UC09 system was estimated to be about 3.0 Pa. With a thinner structure, it was easier for the caustic to penetrate biofilms on the reactor wall causing stronger toxic effects on biomass residing inside. The reduction of boundary layer surrounding biofilms in real sewer systems may possibly happen due to the higher shear stress conditions leading to the resistance to mass transfer.

- Further, the research outcomes from this project provided strong support to enhance the capability of the Australian water industry in managing the corrosion and odour problems in sewers. It is highly recommended to perform a study on the Life Cycle Assessment (LCA) of the impact of chemical dosing in sewers on wastewater treatment plant (WWTP) and sludge management.



1. INTRODUCTION

1.1. BACKGROUND

Sewer system is a very important component of the urban wastewater management system. Although many people consider sewer system as a simple collection and transport system of wastewater from residential areas, commercial areas and industries to wastewater treatment plant (WWTP), it is in fact the most problematic and expensive part of the urban wastewater system. Chemical, biological, and physical transformations happen simultaneously in a sewer network prior to WWTP. In particular, anaerobic environment occurs in a pressure main sewer system during the transportation of sewage caused by the high oxygen consumption of the organic compounds and lack of oxygenation.

Desulfovibrio and *Desulfotamaculum* (collectively called sulfate reducing bacteria-SRB), which mainly present in the anaerobic biofilms on sewer walls reduces sulfate and generate hydrogen sulfide (Thistlethwayte, 1972; Hvítved-Jacobsen, 2002). The formation and its subsequent release of hydrogen sulfide (H_2S) to the gas phase is a major problem in sewer systems, causing serious concern for wastewater authorities (Pomeroy, 1959; USEPA, 1974; Hvítved-Jacobsen, 2002).

Sulfide gas emission causes biogenic sulfuric acid corrosion of concrete and metal that leads to damage of sewer infrastructures, obnoxious odour problems and also health problems to sewer workers (USEPA, 1974). The anaerobic-dominated condition of the sewer may also lead to the formation of methane, which is a potent greenhouse gas (GHG) and explosive at low concentrations (Guisasola et al., 2008; Foley et al., 2010). A great deal of the methane produced in sewer systems will be released to the atmosphere in gravity sections of sewers or at a wastewater treatment plant (Guisasola et al., 2008). Methane formation also consumes valuable COD (Chemical Oxygen Demand) required for biological nutrient removal at the downstream wastewater treatment plant.

There are various operational methods that have been developed to control biogenic corrosion, such as: mechanical cleaning, design optimization of sewer hydraulic, source (sulfate) control by separating urine or pre-treatment, improving sewer pipes resistance, and chemical and/or biomaterial addition (US EPA, 1991; Scrivener et al., 1999; Yamanaka et al., 2002; De Belie et al., 2004; Nielsen et al., 2006; Zhang et al., 2008). This project focuses on the last strategy, which is the reduction of sulfide formation and its release from sewer systems by dosing chemical additives and commercial biomaterials.

A great number of techniques have been used by the wastewater industry to minimise the formation and/or release of H_2S (Thistlethwayte, 1972; Boon, 1995). The most common methods included oxygen injection to pressure mains (e.g. employed by the Allconnex Water), nitrate addition (employed by Sydney Water Corp - SWC), Fe^{2+}/Fe^{3+} (employed by both Allconnex Water and SWC), and $Mg(OH)_2$ dosages (being considered by both Allconnex Water and SWC) to wastewater. There are also numerous products that are being commercialized by various companies for odour and corrosion control in sewers. However, the choice of product, prediction of the optimal dosing rate and the most effective dosing strategy is far from straightforward. Many sulfide control products (chemicals including biomaterials - compounds specifically engineered to target sulfide producing biofilms in sewers) are being marketed to the wastewater industry with little independent proof of the claimed effectiveness. Many utilities are already carrying out tests possibly in parallel, leading to duplicated efforts. Also, the lack of a standard protocol makes it difficult to compare the results obtained with different chemicals and even the same chemicals tested



at different locations. Finally there is no review process for industry to assess whether products are suitable for their needs.

The SP6 was designed to use the above well-established field systems (as well as other laboratory facilities) to test products that are currently on the market for sulfide or 'odour' control. These products may be applicable in a wide range of applications, and would include those that do not rely on the established methods of sulfide removal. The lab-system was used for pre-screening tests for some of the lesser known products. Field-testing was carried out on those that were considered by industry to be appropriate for full scale dosing. A subsequent outcome of this process was the development of a protocol by which industry partners could assess the suitability of products for their specific need and application.

1.2. OBJECTIVES OF THE SUB-PROJECT

The objectives corresponding to this subproject are the following.

1. Compile information about potential products and collect results from previous trials for sulfide control based on information provided by manufacturers and the industry partners.
2. Generate scientific insight and produce conclusive laboratory and field experimental evidence demonstrating the effectiveness of the chemicals tested
3. Provide optimal dosing rates for any successful products, and conduct a cost/benefit analysis for all effective products tested
4. Develop a proven protocol as well as experimental procedures for assessing the suitability of future market products.

The production and control of hydrogen sulfide was chosen to be the primary focus of the sub-project as H₂S is the cause of corrosion and is potentially also a surrogate for other odorous compounds.

1.3. ABOUT THIS REPORT

The key findings of the research project is summarised in this report. Furthermore, to support the conclusions, key experimental evidence is also presented. The summary of the project outcomes is preceded with sections documenting the project methodology/research plan and the key materials and methods, and followed by key issues to be addressed by future research.

During the course of the project (November 2008 and October 2011), 12 Quarterly Project Reports, 5 Detailed Technical Sub-project Research Reports, and 1 Final Report were delivered which contained all the technical details of the sub-project. Readers are referred to these reports for detailed scientific and technical information arising from the project.



2. PROJECT METHODOLOGY AND PLAN

Four different plans of activities were defined to deliver knowledge to the wastewater industry on the dosing and its protocols of chemical additives and commercial biomaterials for H₂S and odorous compounds control. These activities are outlined below.

Activity 1: Identify the Products to Test.

This activity aimed to select the preferred products to be tested in SP6 upon agreement with industrial partners. The final list was selected based on the demands/needs of the industrial partners and also based on the information collected from manufacturers and previous trials carried out by the industry partners. A reviewing process was carried out during the first 4 months of SP6 compiling information available. Once extracted relevant information, this was provided to industrial partners and further discussions was performed in order to reach an agreement with regards the list of 5 top chemical products to be studied.

Activity 2: Setting up field and lab-testing facilities.

This activity aimed to set up testing facilities for chemicals and biomaterials dosing in both lab and field scale. Initially, three main sites were selected for this study. The three main sites were the AWMC laboratory, the UC09 rising and gravity main (Gold Coast). Nevertheless, we canceled the gravity main study after monitoring the activities on one of the gravity main sewer system on the Gold Coast area. The sampling and measurement activities demonstrate that there were no significant differences between the upstream and downstream sulfide concentration of the gravity mains. To anticipate this, more chemical dosing experiment was proposed to replace the activity. The AWMC laboratory rising main facilities were made available to start the chemical testing. Field site UC09 rising main was made ready for chemical testing from March of 2009. The testing sites were equipped with necessary sampling points and monitoring equipment and chemical dosing systems. The industrial partners took responsibility of setting up of these field facilities as in-kind contributions. However, s::can UV/VIS spectrophotometers for sulfide measurement were provided by the project. Negotiation was made with chemical suppliers concerning the use of chemicals and chemical dosing facilities.

Activity 3: Laboratory studies to screen the products.

The laboratory facilities were used to test the ability of the selected products before its field-testing application. Laboratory testing is a low-cost, reliable and quick way to assess the potential of a product, in terms of its ability to remove sulfide in a controlled laboratory environment. It also allows more easily gathering scientific evidence revealing the reasons why a chemical works (or otherwise), increasing the creditability of the experimental findings.

A standard protocol was proposed to study each product, which is described below:

1. Baseline monitoring: establish the normal performance of the reactors in terms of the production of sulfide and several other odorous compounds (OC) prior to the dose of the chemical.
2. Dosing the product: batch/activity test would provide information on the immediate effects of the chemical on the biofilm. The product was applied following the proposed method of the manufacturer. The main stage was the assessment of the effectiveness in reducing sulfide and OC production at short, middle and long term basis. The final step was to evaluate the dosing method and propose an optimal dosing strategy.



3. End of the product application: monitoring of the recovery of the sulfide and OC production by sewer biofilm after the stoppage of the application.

A standard period of 6 months was allocated to test each chemical, corresponding to 1-3 months for each stage. However, some chemicals required less than 6 months, particularly those that do not have an impact on biofilm activities. The application of a new chemical was started only when a full recovery of the biofilm activity was observed. Guidelines and recommendations for field application were provided after each chemical trial.

The first tested product in the laboratory rising main system was Ferric (Fe^{3+}). Based on previous results using ferrous ions (Fe^{2+}) in the same lab system it was observed that, on top of precipitating the sulfides, the iron dose significantly altered the SRB activity. However, it was further revealed that Fe^{2+} did not precipitate with phosphate, and therefore the typically recommended $\text{Fe}^{2+} : \text{S}^{2-}$ dosing ratio of 2:1 (mol/mol) does not apply to rising main sewers. The aforementioned findings dramatically reduced the amount of Fe^{2+} required for sulfide control. It was hypothesized that similar effects could take place when using Ferric ions. However, the use of Fe^{3+} was expected to be substantially more complicated as it is an oxidant as well as a precipitant. Fe^{3+} was expected to oxidize sulfide, forming Fe^{2+} and elemental sulfur, respectively. On the other hand, it was also expected to precipitate with phosphate. The precipitation function of Fe^{3+} is well-known, however the impact of Fe^{3+} addition on SRB activities has not been reported. The project was planned to reveal the optimal use of Fe^{3+} (dosage locations, rates, and frequency). A mechanistic of Fe^{3+} inhibition on SRB was also part of the investigation.

Following the testing of Fe^{3+} , five other chemicals determined in Activity 1 were tested in the laboratory systems. Each of these tests was planned to be conducted for six months period. Some ineffective chemicals were carried out less time to test. However, others took longer than expected.

Activity 4: Testing of successful products using field sites.

Successful products in the lab trials were tested in the UC09 rising main on the Gold Coast area. The same testing protocol applied to lab studies was used for field tests.

The first product proposed to be dosed in the UC09 rising main was nitrite (NO_2^-). Nitrite was chosen based on preliminary work done in the UQ lab. Nitrite was found to considerably inhibit the SRB activity, in addition to their normal oxidation effects. According to previous laboratory results using NO_2^- , it was observed that, after long term exposition to nitrite, rising main SRB populations were inhibited and didn't recover its full activity until 90 days after stopping the dose. Nitrite has a distinct advantage over currently used oxidants like nitrate and oxygen, where sulfide accumulation resumes instantly upon depletion of the oxidant. It suggests that nitrite can be dosed intermittently rather than continuously, which may make it a less expensive sulfide management strategy in the long run. Nitrite therefore appeared to be a very promising chemical for intermittent use to control sulfide production. However, currently there is no information with regard to the minimum concentration of nitrite required for the inhibition, the duration and frequency of application.



3. KEY MATERIALS AND METHODS

The key materials and methods used by the project are summarised in this section to provide readers with background information support. These materials and methods were extensively used in this project to collect the data/information to support the research project.

3.1. NOVEL LABORATORY SEWER SYSTEMS

Figure shows a flexible setup of the reactor-based laboratory-scale system designed in this sewer research project. To simulate rising mains, two completely-sealed reactors (each with a volume of 0.75 L) were connected, representing upstream and downstream part of the sewer system.

Domestic wastewater was collected weekly from the Robertson Park Pumping Station (Indooroopilly, Brisbane) and transported to the laboratory immediately. The sewage typically contains sulfide concentrations of < 3 mg S/L, sulfate concentrations between 10 - 25 mg S/L, and VFA levels of 50 - 100 mg COD/L. Sulfite and thiosulfate were present in negligible amounts (< 1 mg S/L). To minimize biological transformations, the sewage was stored in a cold room at 4°C. The sewage was also heated to 20°C before it was pumped into the reactors. The feeding pump was operated intermittently mimicking the pattern observed for the UC09 pumping station. HRT's between 1 and 9 hours were obtained.

Plastic carriers (Anox Kaldnes) were inserted into all reactors to support biofilm growth. No wastewater recirculation was employed so that different reactors were exposed to wastewaters with different compositions (due to biotransformation in upstream reactors) potentially leading to growth of biofilms with different characteristics.

Two such systems were established at the beginning of the project; one was used as the experimental and the other as a reference system. The design allows the spatial and temporal variations of sewer biofilms to be examined at both macro- and micro-scales. Each reactor can be isolated for batch tests and the carriers in all reactors can be removed for micro-scale measurement.



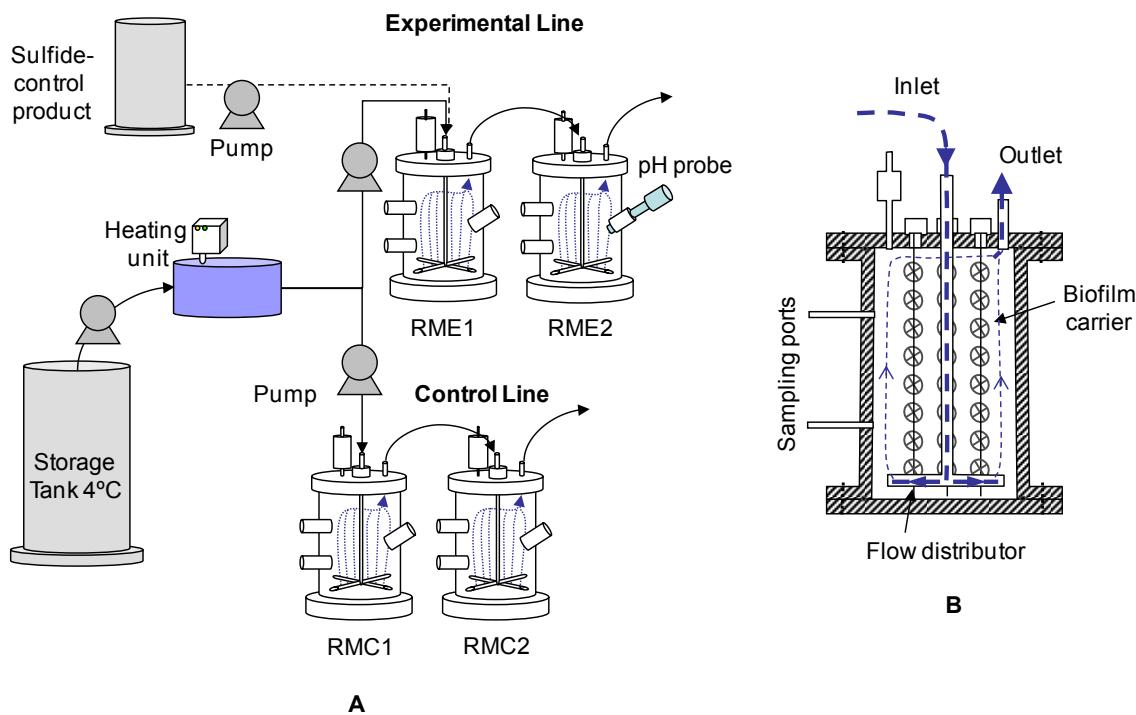


Figure 1: Reactor-based lab-scale sewer systems. A: Rising main reactors; B: Sectional view of a lab reactor. Biofilms carriers (appearing in white strings inside reactors) are placed in the reactors for biofilm growth. The carriers can be removed for micro-scale studies on biofilm structure and function.

The laboratory system was used mainly to test the effectiveness of various H₂S control strategies and to optimise these strategies.

3.2. REAL SEWER SYSTEMS

The UC09 rising main sewer was used extensively in the project. The UC09 is 1080 m long and has diameter of 150 mm, located on the Gold Coast, Australia. The pipe mainly receives domestic wastewater with an average flow of 206 m³/day. The hydraulic retention time (HRT) of sewage in the UC09 pipe varied from 1.7 to 5.7 h. UV-spectrolyser units that are able to continuously monitor online sulfide concentration in the sewage were installed during the entire course of the study at the UC09 wet well (Fig. 2A) and 828 m (Fig. 2B) of the downstream part of UC09.





A

B

Figure 2: The UC 09 rising main sewer system, Gold Coast. A: Wet well, B: 828m downstream location.

3.3. THE S::CAN UV/VIS-BASED SPECTROMETER

The S::CAN Spectro::lyzer (Messtechnik GmbH, Austria) as can be seen in Figure 3, measures the attenuation of light in the UV-VIS spectrum between 200 and 730nm across a measurement gap of between 0.5 and 2mm. Different components of wastewater absorb light in different regions of the UV-VIS spectrum, and the spectrometer can detect the total absorption from all the compounds present. Each measurement takes about 15 seconds. The probe is kept clean automatically with a compressed air system and data is logged on a control computer and hence can operate independently for long periods of time. The methods for these measurements can be found in Sutherland-Stacey et al. (2008)



Figure 3: The s::can spectro::lyzer used in the project.

Previous project on the “Understanding the Biotransformation Processes in a Sewer System to Achieve Optimal Management” (LP0454182) conducted by AWMC-UQ has used the sensor for the on-line measurement of dissolved sulfide in wastewaters. The sensor has since been applied extensively in both laboratory and field studies with highly satisfactory results in most cases.

3.4. ANALYTICAL METHODS

Filtered liquid samples for the analysis of soluble inorganic sulfur species (SO_4^{2-} , HS^- , $\text{S}_2\text{O}_3^{2-}$ and SO_3^{2-}) were measured using ion chromatography (IC) with a UV and conductivity detector (Dionex ICS-2000) using the protocol described in Keller-Lehman et al. (2006).

Methane was sampled into freshly vacuumed BD vacutainer tubes using a hypodermic needle attached to a filter and a 10 mL plastic syringe that contained the sewage sample. The tubes were mixed overnight in a shaker to allow equilibration of gas and liquid phases. Most of the methane (~ 97 % at 25°C) was transferred to the gas phase in this process. Then, methane concentration in the gas phase of the tubes was measured using a Gas



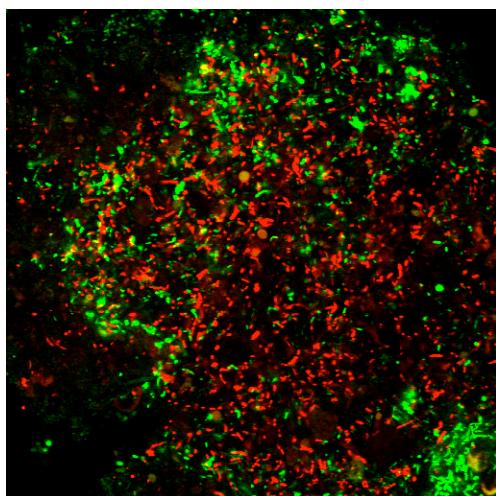
Chromatograph (Shimadzu GC-9A) equipped with a flame ionization detector (FID) using the protocol described in Guisasola et al. (2008). The concentration of methane in the initial liquid phase was calculated using a mass balance and Henry's law. Effluents from each reactor were collected over 24 hr (composite sample technique) before and after dosing NaOH to investigate the amount of solids detached due to the dosing process. Volatile fatty acid (VFA) concentrations were measured by gas chromatography (PerkinElmer, Inc.).

Total suspended solids (TSS) and volatile suspended solids (VSS) were analysed as per Standard Methods 2540D (APHA, 1998). Sulfide and methane production rates were calculated from the slopes of the data points using linear regression.

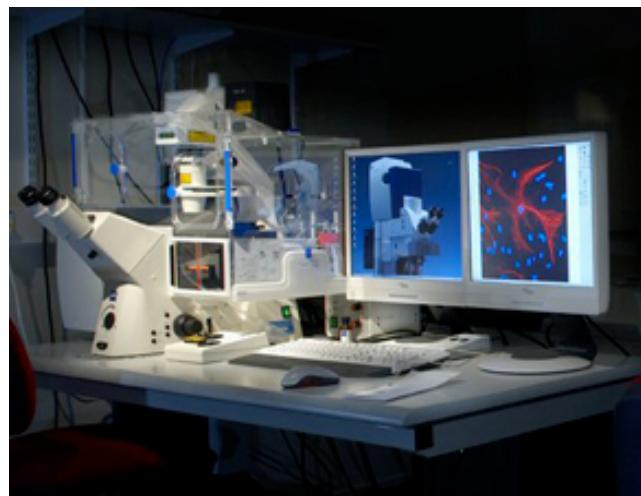
3.5. LIVE/DEAD BACTERIAL VIABILITY TEST

A staining procedure using the LIVE/DEAD® BacLight™ bacterial viability kits (Molecular Probes, L-7012) was used to determine the cell viability in biofilms before and after being exposed to chemical additives. The LIVE/DEAD® BacLight™ bacterial viability kits use two different nucleic acid stains that are red-fluorescent Propidium Iodide (PI) and green-fluorescent SYTO-9. The green PI stain only characterizes bacteria with damaged membranes (dead cells), while the red SYTO-9 stain penetrates all bacteria that have both damaged and intact membranes (viable cells) as shown in Figure 4A.

Microscope slides with stained biofilm samples were subsequently prepared prior to observation under a confocal laser scanning microscope (Zeiss LSM 510 META), equipped with a Krypton-Argon laser (488 nm) and two He-Ne lasers (453 and 633) as illustrated in Figure 4B. For each sample, at least twenty photos were captured for different areas under the microscope. Cell viability was quantified by counting the relative abundance of green and red pixel using the Image J software. The percentage of viable cells to the total cells (viable + dead) in the biofilm is indicated by the percentage of green fluorescence to the total fluorescence (red + green fluorescence).



A



B

Figure 4: The LIVE/DEAD bacterial viability test. A: an image of cell viability in biofilms B: a confocal laser scanning microscope.

4. SUMMARY OF OUTCOMES

4.1. UPGRADING RISING MAIN LABORATORY REACTOR SYSTEM

The laboratory facilities were used to test the ability of the selected products before its field-testing application. Laboratory testing is a cheap, reliable and quick way to assess the potential of a product, in terms of its ability to remove sulfide in a controlled laboratory environment.

Previously, through the ARC Linkage Project LP454182 “Understanding the Biotransformation Processes in a Sewer System to Achieve Optimal Management”, the AWMC developed a laboratory pilot plant, built to mimic a real sewer, complete with a rising main and gravity system. This was used to test the effectiveness of injection of oxygen, nitrate, alkali and metal ion addition, and provided a deeper understanding of the in-sewer biotransformation processes at a fundamental level (sulfide saturation/inhibition, impact on carbon compounds consumption, biofilm structure and functioning).

To cope with the needs of the actual SCORe SP6 project, where more additive products were trialed, rising main AWMC Lab facilities were upgraded. It aimed to ensure that the systems were equipped with the online monitoring equipment such as S::CAN UV/VIS spectrophotometers for sulfide, nitrate and other relevant measurements, variable dosing pumps, installation of multi probe units to measure and log pH, dissolved oxygen, temperature, and sampling points. Rising main AWMC Lab facilities was ready for the chemical testing from July 08. A mature biofilm has to be developed prior to the chemical dose on it.

During the course of the project, a standard protocol was applied to study each product. Same standard protocol was used for all the chemicals in the lab tests. The general sections of the screening test protocol are the following:

1. **Baseline behavior lab system determination:** establish the normal performance of the reactors in terms of sulfide production capacity and carbon sources consumption prior to the dose of the chemical.
2. **Start dosing the product:** Preliminary batch test will help to know the immediate effects on the biofilm. Secondly, the product will be applied following the proposed method of the manufacturer. Assess the effectiveness in reducing sulfide production at short, middle and long term time basis. Evaluate the dosing method and propose an optimal dosing strategy.
3. **End of the product application:** monitoring of the recovery capacity of the sulfide producing biofilm once stopped the application. Re-growth period. New and fully biofilm will have to be developed in the system prior to the application of the next product.



A period from 6 months was allocated to test each chemical, corresponding to 2 months for each stage. However, the length of each product testing period was quite flexible depending on variable like time to reach the maximum effectiveness after start dosing, evaluation and application of the optimal dosing and capacity of recovery of the biofilm after stopping product addition.

As shown in Figure 1, the laboratory setup consists of two separate systems, each consisting of 2 parallel lines. Each line is composed of 2 reactors simulating up- and down-stream sections of a sewer pipe. For each system, one line is used a reference and the other line the experimental line, where the chemical will be dosed. The impact of chemicals to be tested on the production of sulfide and other odorous compounds will be assessed through comparing the performance and biofilm activity of the experimental system with those of the reference.

The system is intermittently fed with sewage through a peristaltic pump and was exposed to twelve sewage pump cycles on a daily basis. Variation of sewage hydraulic retention time (HRT) is maintained, with minimum and maximum HRTs of 2 to 6 hours respectively.

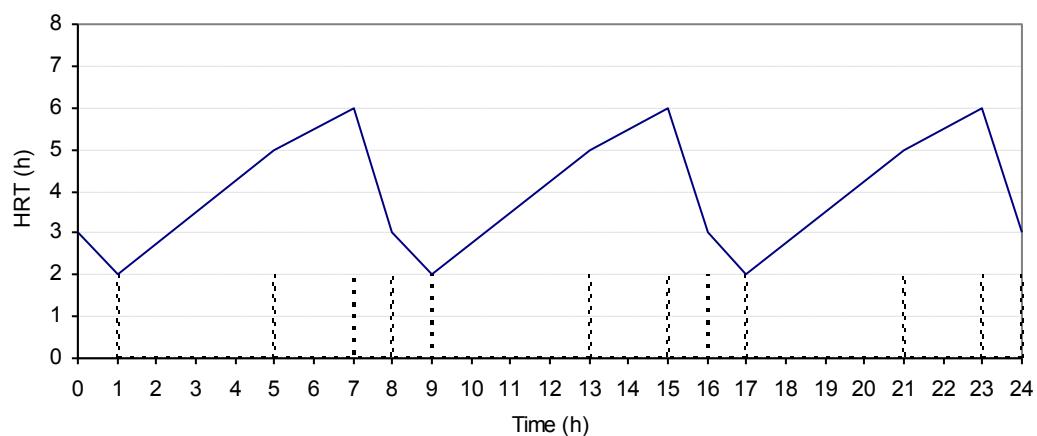


Figure 5: Pump events (vertical dashed line) and HRT of the sewage leaving the reactors (solid line) corresponding to the upgraded laboratory rising main system.

4.2. SELECTION OF THE CHEMICALS TO BE TESTED

A total of 45 products were identified to have odour control properties. A document summarizing the information about products available in the market for sulfide control was completed and distributed to industry partner (Appendix 1).

Three different sources of information were used in compiling the data:

- Industry partners' previous experience in trials and information.
- Literature review: an examination involving scientific journals, reports and web research.
- Manufacturer's information: commercial reports, brochures, flyers and other materials.



In general, the products can be separated in two main categories:

- 1) Liquid phase chemical treatments, such as: injection of air or oxygen, nitrate, nitrite, metal salts, and pH adjustments.
- 2) Liquid phase biological treatments. The uses of biomaterials to modify the microbial metabolism, change microbial populations, or otherwise, affect the microbes responsible for generating odours in wastewater collection systems.

Based on the information compiled, five products were planned to be tested in the laboratory facilities:

1. Biosol
2. Probac
3. Bio-kat
4. Actizyme
5. ByoGon

In the study, only the first three products were tested as the outcomes of the study showed all the three biomaterials were ineffective in controlling sulfide and methane productions in lab-scale reactors. The last two aforementioned biomaterials study was replaced with chemical dosing tests. Details on the results of biomaterials testing will be provided in the next sections.

4.3. EFFECTIVENESS OF BIOMATERIALS IN CONTROLLING ANAEROBIC BIOFILM ACTIVITIES IN LAB-SCALE SEWER SYSTEM

As mentioned in the previous section, only three (3) commercial biomaterials were tested in the lab-scale sewer system. The three biomaterials were Biosol, Probac, and Bio-kat. In this section, the key results of the investigation are revealed.

1) Biosol

The manufacturer claims that Biosol is able to down regulate both the metabolic and reproduction rates of the bacteria found in sewers, using trace levels of chemical cell signals. Biosol mechanism is based on “cell signalling chemicals”. The chemical cell signals seem to consist of the communication signals between cells via trace levels of chemicals that can slow down the reproduction and metabolic rates of the bacteria. If occurring, the down-regulation of the bacteria would weaken the biofilm, which will then be removed from the pipe by the shear stress of the flowing sewage.

The laboratory reactor system to test Biosol (and all other tested biomaterials) and the pump event applied in this study are described in Fig. 1A and Fig. 5, respectively. The length of the study was 180 days. During this time, operations of the reactor system were divided into four periods, depending on batch of Biosol dosed (Table 1) and shear conditions applied:

- **Baseline Period:** Baseline stage (Days -60 to 0). The system was operated for two months to establish pseudo steady-state conditions and to develop mature anaerobic biofilm on the walls of the reactor and the carriers, both in experimental and control lines. Low mixing regime was applied, 200 rpm of magnetic stirrer, equivalent of a shear stress of 0.29 Pa.



- **Period Test 1:** Biosol (batch 1) was dosed to the upstream section of the experimental line (Days 0 to 40) at a concentration indicated by the manufacturer which was 10 ppm (10 parts per million, in volume). Low mixing regime was applied, 200 rpm of magnetic stirrer, equivalent of a shear stress of 0.29 Pa.
- **Period Test 2:** Biosol (batch 1) was dosed at 10 ppm and mixing was increased to 750 rpm, equivalent of a shear stress of 2.97 Pa (Days 41 to 90).
- **Period Test 3:** Biosol (batch 2) was dosed at 10 ppm to the upstream section of the experimental line and shear stress was maintained at 750 rpm regime, equivalent of a shear stress of 2.97 Pa (Days 91 to 120).

Detailed tests were done in all periods to determine the impacts of Biosol dosing on biofilm activities. All tests were carried out in a temperature-controlled lab ($20^{\circ}\text{C} \pm 1^{\circ}\text{C}$).

Table 1. Characterisation of Biosol by usual lab parameters

Parameter	Unit	Batch 1	Batch 2
		Average value	Average value
pH	-	3.2	3.5
Volatile Fatty Acids	mgCOD/L	886	975
Soluble COD	mgCOD/L	89,700	85,819
Total COD	mgCOD/L	152,552	86,726
Ammonia, NH ₄ -N	mg/L	17	23
Phosphorus, PO ₄ -P	mg/L	31	33
Total Organic Carbon, TOC	mg/L	803	2,776
Chloride, Cl	mg/L	85.4	1510

Figure 6 show that Biosol did not reduce the sulfidogenic and methanogenic capacity of the system. After 110 days of Biosol exposition, the performance off-line where Biosol was added was the same as the line fed only with domestic sewage. No significant reduction of sulfide and methane was observed for the whole length of the study.

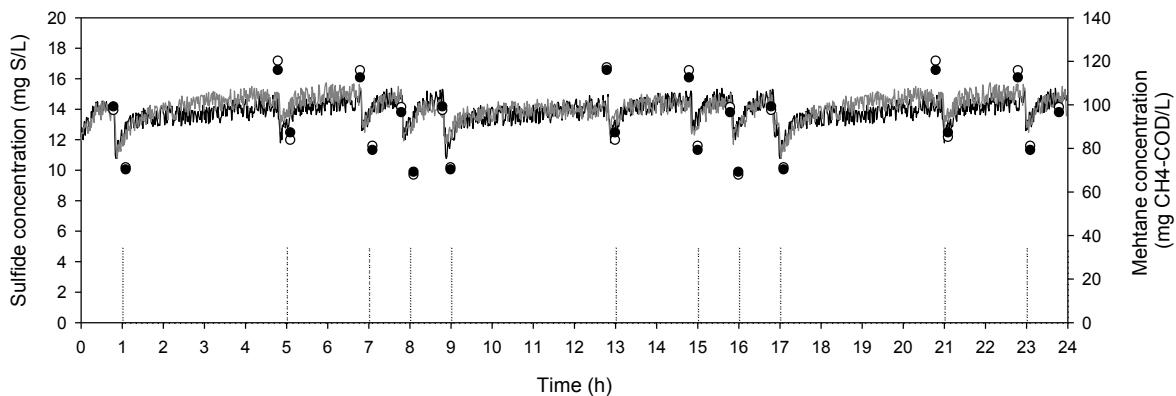


Figure 6: 24 hour methane and online sulfide data from RME2 and RMC2 corresponding to Days 109-110 after Biosol was initiated. (—): Sulfide online in RME2; (—): Sulfide online in RMC2; (o): Methane in RMC2; (•): Methane in RME2; (...): pump events.



The compilation of sulfide production rates in all 4 reactors of the system is presented in Figure 7 below. Sulfide production rates in upstream reactors, RMC1 and RME1, presented steady values around 6 mgS/L.h during the whole study, except for a period of time around Day 30 of continuous dose of Biosol when the values climbed up to 8-9 mgS/L.h (Fig. 7A). This change occurred in both experimental and control lines; therefore it was related to higher content of sulfate and organic matter in the fresh sewage used during this period. The same pattern was observed in the downstream reactors (Fig. 7B) with slightly lower sulfide production rates but also comparable performance between control and experimental reactors. In all, the activity tests confirmed that the dose of Biosol did not reduce the capacity of producing sulfide in the reactors. For the whole period of exposition to Biosol (120 days), the experimental reactors performed at the same level as the ones only fed with sewage.

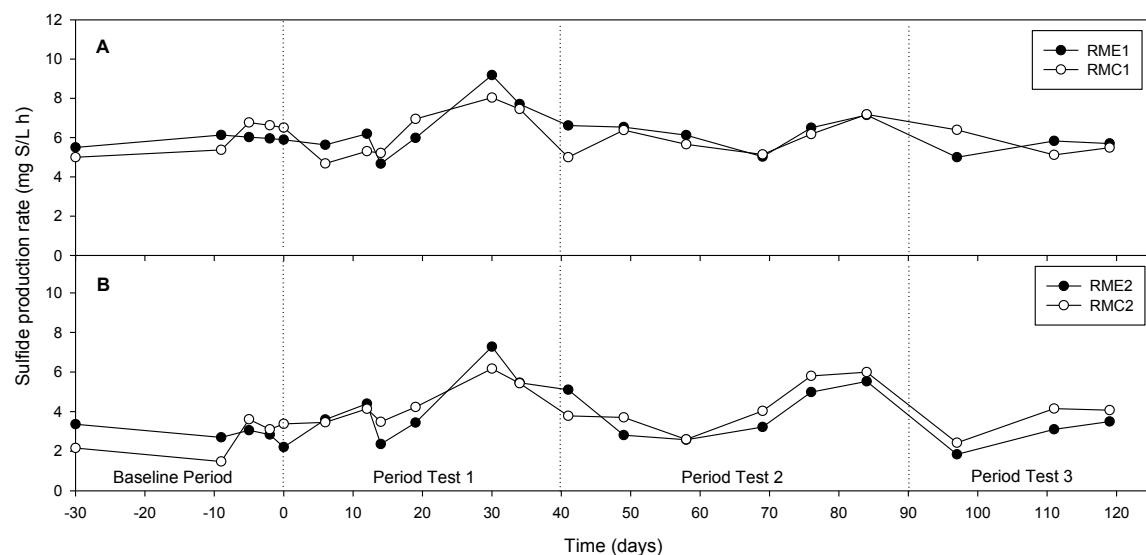


Figure 7: Sulfide production rates obtained in the activity tests from Biosol. (A): Upstream reactors rates. (B): Downstream reactors rates.

With regards to the methane production activities, the batch tests again showed no significant differences between reactors exposed to Biosol and reactors feed only with domestic sewage (Fig. 8). Methane production rates were generally higher in the upstream reactors of the system because were directly receiving fresh sewage with high VFA content.

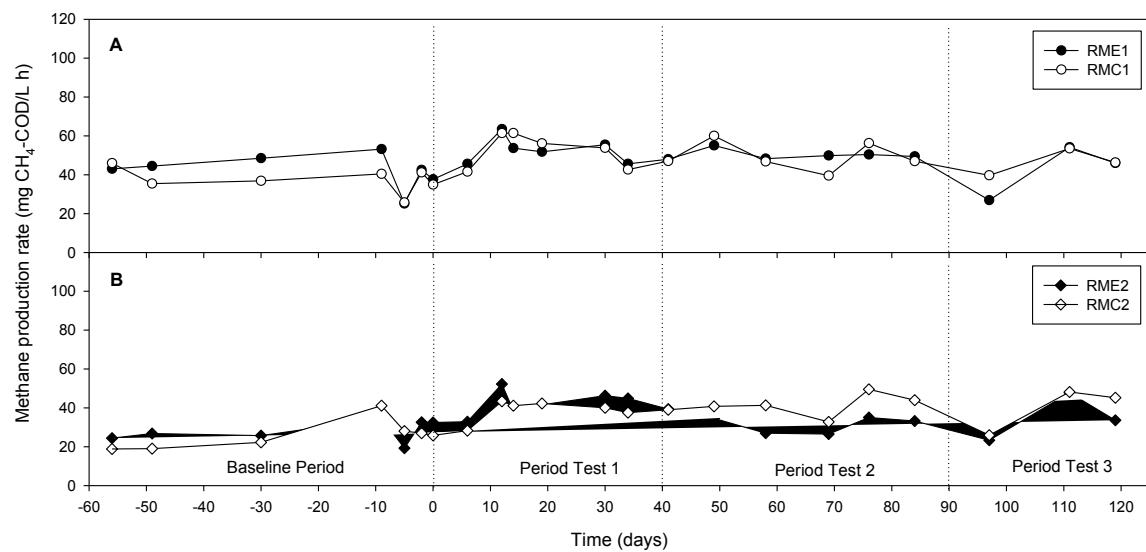


Figure 8: Methane production rates obtained in the activity tests from Biosol. (A): Upstream reactors rates. (B): Downstream reactors rates.

The results of TSS measurements are presented in Figure 9 below. The measurements of the TSS and VSS attached in the plastic carriers inside the reactors showed stable concentrations all over the study for both lines. Solids in the effluent of both lines were similar through the whole study. Most of the time concentrations varied between 40 and 80 mg TSS/L, which was in the usual variation range of systems feed with varying real sewage. In case Biosol worked as described by manufacturer, a substantial decrease in the solids content would be expected in the experimental line, especially in Period Test 2 and 3 when the biofilms were submitted to a higher shear. As can be seen in Fig. 9, this did not occur and biomass content in the carriers was very similar both in exposed and not exposed to Biosol reactors.

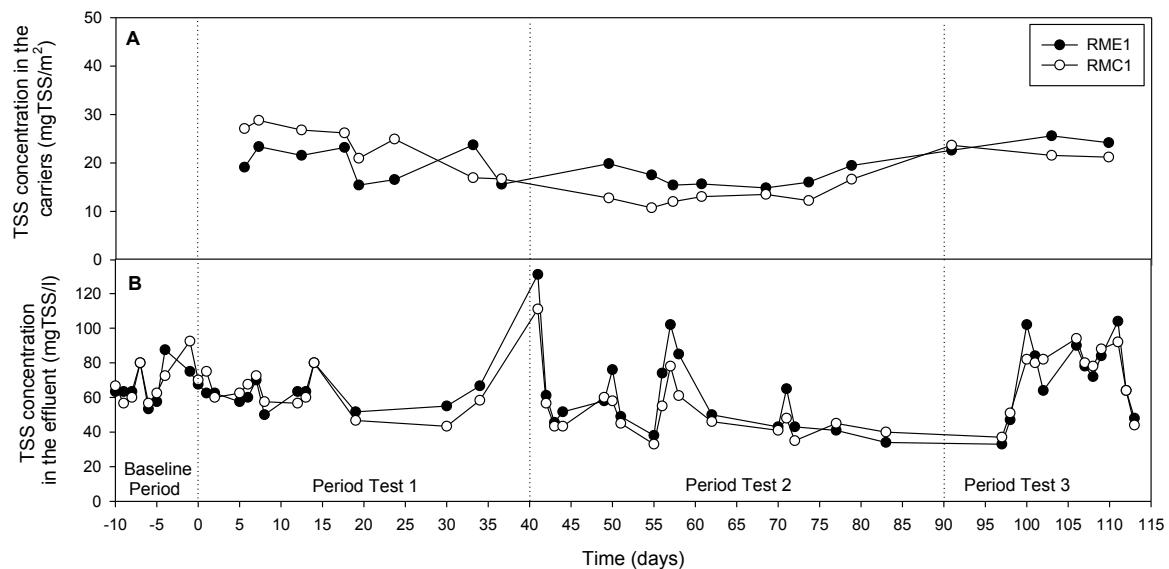


Figure 9: Solids concentration (A): Total suspended solids content measured in the sewage effluent (24 hour composite samples). (B): TSS content in plastic carriers.



The results obtained in the laboratory studies clearly demonstrate Biosol was not effective in reducing sewer biofilm activities. All measurements performed in the present study (sulfide, methane and VFA concentrations, solids analysis inside and discharged from the system), indicates that there was no difference in the performance between biofilms exposed and not exposed to Biosol. Therefore, Biosol is deemed not suitable to be tested in field scale.

2) Biokat

Based on the information acquired from the manufacturer, Biokat is claimed as an innovative cellular bio-activation liquid (100% natural and non-toxic) that stimulates indigenous bacteria to accelerate biological activity. It functions by providing many of the missing or deficient intracellular micro enzymes that are lacking in the nutrient constituent of the wastewater. This may further increase the metabolism of microorganisms to the highest rate. By accelerating the metabolic rate of the indigenous organisms, the manufacturer believes that the following results can be achieved:

- The reduction of fats, oil and grease
- Atmospheric hydrogen sulphide reduction
- Significant corrosion reduction

The application of Biokat is also believed to reduce total suspended solids, promotes odour reduction, substantially accelerates bio solids reduction and in general improves effluent quality. According to the supplier, in the real application, one thousand times dilution of a concentrated Biokat solution is directly dosed into the wet well. The manufacturer claims Biokat can stimulate the aerobic bacteria, which may be present in the wet well, to reduce the organic electron donor molecules. In this case, the SRB in sewer systems will possibly have not enough sulfate to consume, and as a result, less H₂S are produced. However, there has been no scientific evidence to support the effectiveness of Biokat so far. Biokat was dosed into the laboratory sewer systems to assess its effectiveness. Analysis was performed to characterize this biomaterial. Biokat (diluted a thousand times) composition was analysed and results are presented in Table 2.

Table 2. Characterisation of Biokat

Parameter	Unit	Diluted Biokat (1000x)
pH	-	7.63 ± 0.04
Volatile Fatty Acids	mgCOD/L	1.50
Total COD	mgCOD/L	5.30
Ammonia, NH ₄ -N	mg/L	0.00
Phosphorus, PO ₄ -P	mg/L	0.01
Total Organic Carbon, TOC	mg/L	2.46
Chloride, Cl	mg/L	2.00
TSS	mg/L	0.00

The length of the study was 136 days. During this time, operation of the reactor system was divided into four periods, depending on batch of Biokat dosed applied:

- **Baseline Period:** Days -28 to 0. The system was operated for two months to establish pseudo steady-state conditions and to develop mature anaerobic biofilm on the walls of the reactor and the carriers, both in experimental and control lines. Low mixing regime



was applied for control and experimental reactors at 200 rpm, equivalent of a shear stress of 0.29 Pa.

- **Period Test 1:** Dose 1, days 0 to 35. Probac was added into the mixing tank at the initial dose of 1 ppm as indicated by the manufacturer.
- **Period Test 2:** Dose 2, days 35 to 69. A higher concentration of Probac (2 ppm) was added into the mixing tank.
- **Period Test 3:** Dose 3, days 69 to 108. The dose was increased to 3 ppm.

Figure 10 shows that the dose of Biokat did not significantly reduce the capacity of producing sulfide in the reactors. For the whole period of exposition to Biokat (136 days), the experimental reactors perform at a similar level as the ones only fed with sewage.

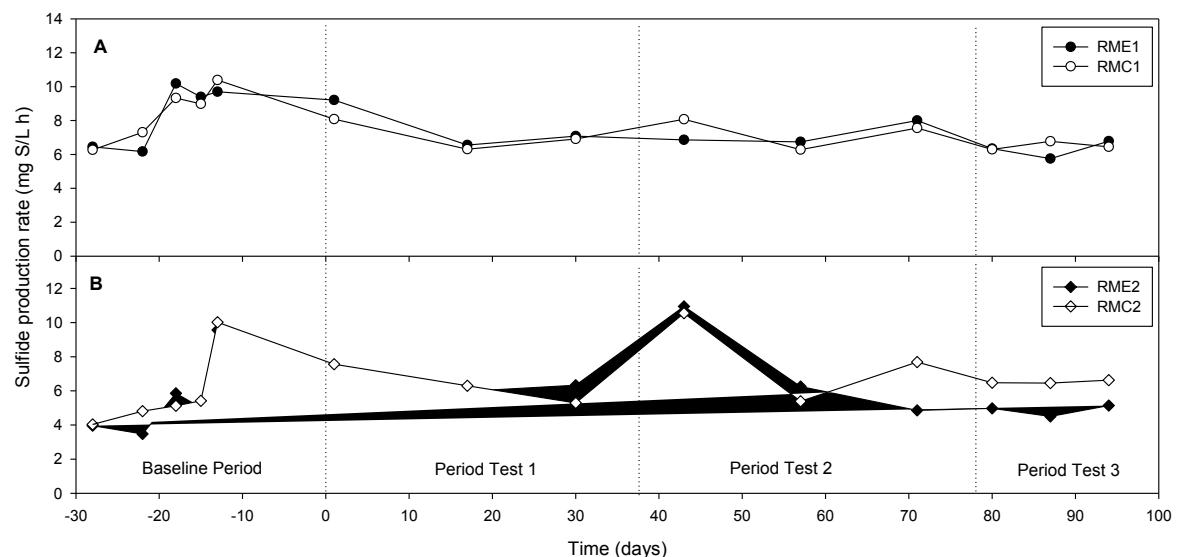


Figure 10. Sulfide production rates obtained in the activity tests from Biokat. (A): Upstream reactors rates. (B): Downstream reactors rates.

With regards to the methane production activities, the batch tests again showed no significant differences between reactors exposed to Biokat and reactors feed only with domestic sewage (Fig. 11).



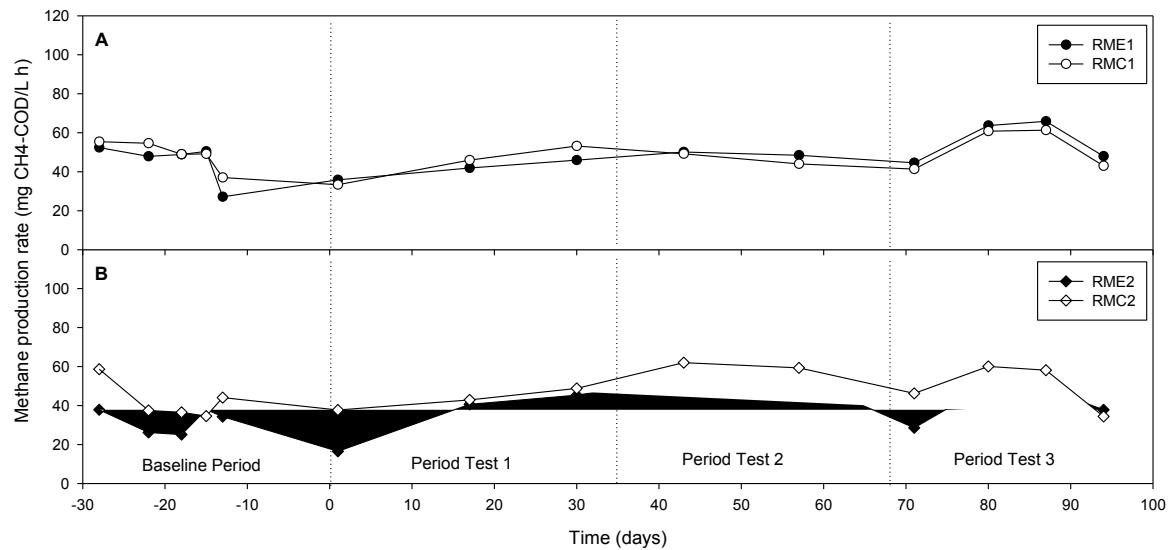


Figure 11. Methane production rates obtained in the activity tests from Biokat. (A): Upstream reactors rates. (B): Downstream reactors rates.

The laboratory studies have revealed that biokat was not effective enough to control both H₂S and methane productions from the sewer system. In general, the obtained results of all measurements in the present study indicate that there was a similar performance between biofilms in both experimental and control lines. Insignificant reduction of sulfide and methane parameters only occurred in the downstream part of the experimental line in one of the three dosing periods. It is not recommended to test the product for practical application in field scale.

3) Probac

According to the supplier, Probac is not a live cell bacterial (bio-culture) or a mixture enzyme and cannot introduce renegade bacteria into sewer systems. It is a pure organic concentrate (extracted from selected plant materials), 100% biodegradable, user and environmentally friendly, non flammable, non corrosive and non toxic. It prevents septicity, strips off any grease, slime and scum that have built up in the sewer pipelines and pumping station wet wells. It works at the cellular level, to stimulate both the metabolic and reproductive rate of the naturally occurring desirable bacteria that may be functioning improperly because they have been inhibited by oxidants, poisons, or extremes of temperature or age. This will cause the desired bacteria to become and remain dominant, thereby out-competing the odorous bacteria for the same food source. The supplier also claims that Probac should be effective within two weeks of dosing; a significant decrease of sulfide concentration can be measured especially in the downstream pipe.

Probac was dosed into the lab-scale sewer systems to assess its effectiveness. Analysis was performed to characterize this biomaterial. The composition of Probac was analysed and results are presented in Table 3. We can see from its physical characteristics that the pH of the solution is low. Its chemical characteristics show high concentrations of soluble and total COD (chemical oxygen demand), TOC (total organic carbon), Chloride and TSS (total suspended solid).



Table 3. Characterisation of Probac

Parameter	Unit	Concentration
pH	-	3.6 ± 0.05
Volatile Fatty Acids	mgCOD/L	43.7
Total COD	mgCOD/L	39712.5
Ammonia, NH ₄ -N	mg/L	5.5
Phosphorus, PO ₄ -P	mg/L	32.3
Total Organic Carbon, TOC	mg/L	7910.1
Chloride, Cl	mg/L	1034.1
TSS	mg/L	8390.0

The length of the study was around 50 days. During this time, operation of the reactor system was divided into four periods:

- **Baseline Period:** Baseline study (Days -21 to 0). The system was operated for two months to establish pseudo steady-state conditions and to develop mature anaerobic biofilm on the walls of the reactor and the carriers, both in experimental and control lines. In this period, both control and experimental reactors were fed only with fresh sewage and a similar performance between the control and experimental lines on sulfide and methane production rates is expected. A mixing regime was applied for control and experimental reactors at 500 rpm, equivalent of a shear stress of around 1.58 Pa.
- **Period Test 2:** Dose 1 (Days 0 to 2). Probac was added into RME1 at the initial dose of 15 ppm as indicated by the manufacturer.
- **Period Test 3:** Dose 2 (Days 2 to 4). A lower concentration of Probac (10 ppm) was added into RME1.
- **Period Test 4:** Dose 3 (Days 4 to 32). The dose was decreased to 5 ppm.

Figure 12 shows the comparison of average values of sulfide concentrations during baseline and dosing periods in both experimental and control lines. A comparable performance between experimental and control reactors were observed in the upstream and downstream reactors. In general, the activity tests indicated that the dose of Probac in fact did not reduce the sulfide producing capacity of sewer reactors. For the whole period of exposition to Probac (32 days), the experimental reactors were observed to perform at a similar level as the ones without receiving Probac.



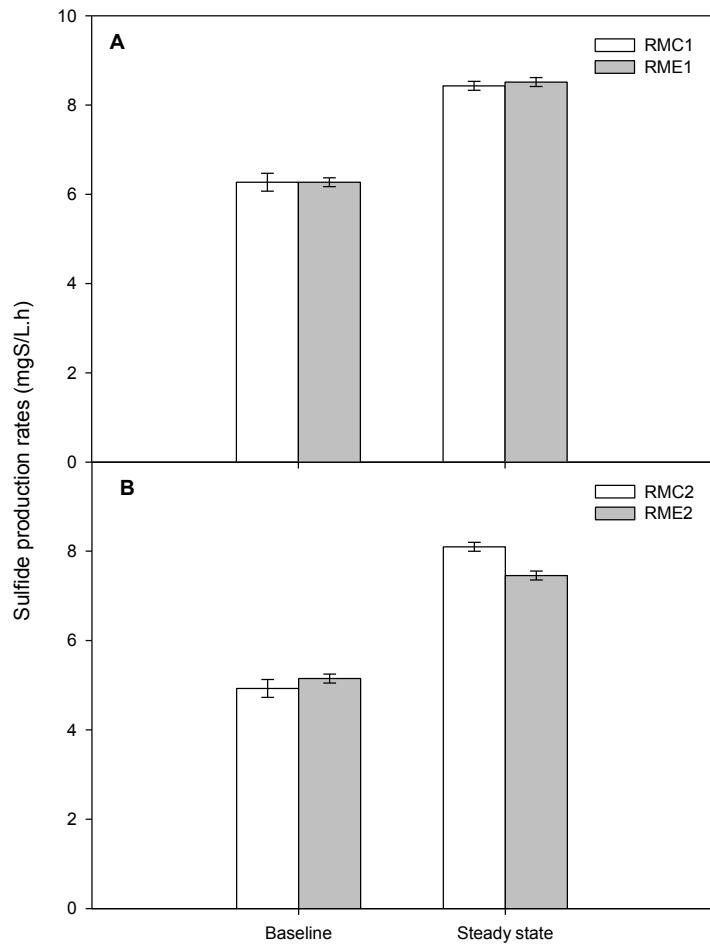


Figure 12. Sulfide production rates obtained in the activity tests from Bioproduct C. (A): Upstream reactors rates. (B): Downstream reactors rates.

With regards to the methane production activities, the batch tests again showed no significant differences between reactors exposed to Probac and reactors feed only with domestic sewage (Fig. 13).



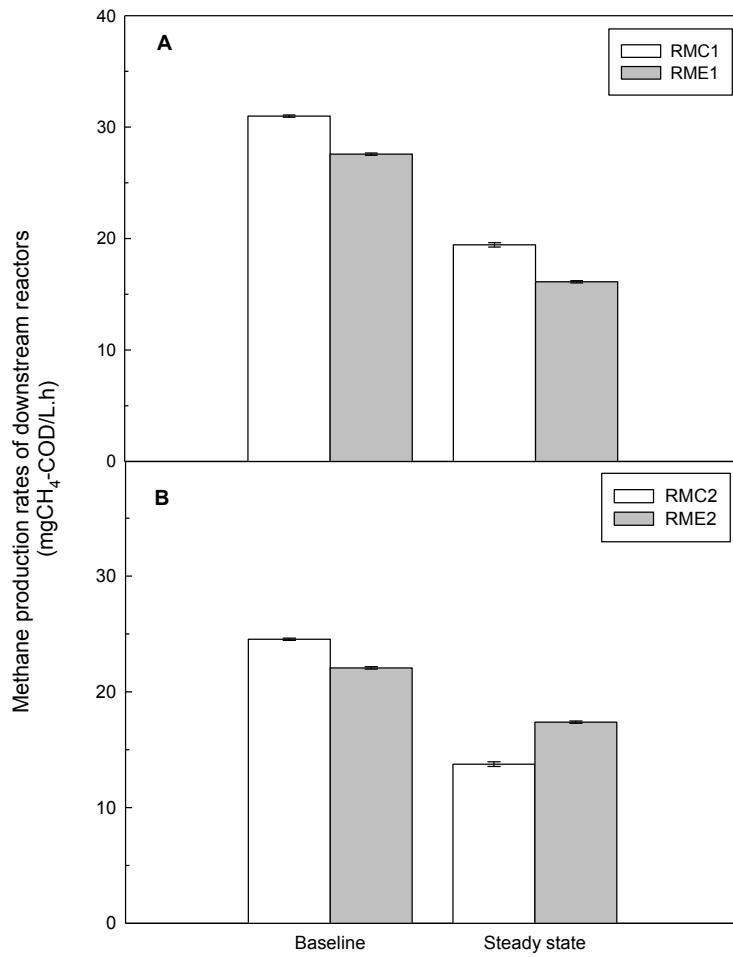


Figure 13. Average methane production rates obtained in the activity tests from Probac. (A): Upstream reactors rates. (B): Downstream reactors rates.

The outcomes of this study shows that Probac had no, or minor, ability to control the formation of sulfide and methane in the sewer system. A similar performance was obtained between biofilms in both experimental and control lines. Some minor reduction of sulfide and methane parameters only occurred in the downstream part of the experimental line in one of the three dosing periods, which may or may not be related to the dosage of the product. The field scale trial of Probac was not recommended.

To summarize, none of the selected bioproducts (Biosol, Biokat, Probac) showed their effectiveness in reducing sewer biofilm activities when dosed to rising main sewers. The lab system has been used for testing a number of “traditional” products with obtained results mimicked quite well its field’s site application. Therefore, the selected bioproducts are categorized as not suitable to be applied in field scale.

4.4. TESTING OF FERRIC ION AS A MEANS FOR SULFIDE CONTROL IN LABORATORY SYSTEM

The investigation on ferric salt addition to control sulfide formation in sewers started as part of the previous ARC Linkage Project (LP454182) between The University of Queensland, Allconnex Water and Sydney Water Corporation. When the SCORE project was started, the study was included in SP6, according to the research plan endorsed by the TAC.



The key findings of the work can be summarized below:

1. A molar ratio of 0.6:1 between Fe^{3+} and sulfide is adequate to control sulfide to below 0.1 mg/L in rising main sewers. This ratio is substantially lower than the industry practice.
2. The addition of ferric salts to sewers induces inhibition to both sulfate reducing bacteria and methanogenic archaea. It was observed that sulfate reduction and methane production rates of the sewer biofilms were reduced by 50% and 80% respectively. This implies that ferric addition achieves sulfide control through not only the oxidation and precipitation of sulfide, but also through reducing the production of sulfide. This is a benefit that was not realised before. It implied that ferric addition should be added at upstream locations in order to achieve both effects.
3. Sulfide is more competitive than phosphate and hydroxide in precipitating with ferric ions. When ferric salts are added to sewers in excess of demand for sulfide precipitation, it will bind with phosphate and hydroxide. However, the bound ferric ions will become available for sulfide precipitation when sulfide is generated again.

The focuses of the ferric study under the SCORe SP6 project were:

1. Confirming that better sulfide control can be achieved by adding ferric salts under upstream locations, as implied by the previous work;
2. To elucidate how quickly the inhibitory effects will be induced;
3. To elucidate how long the inhibitory effect will last after the addition of ferric salt is stopped;
4. To gain some information with regard to the mechanisms of inhibition.

In this study, a laboratory system as described in Fig.1 was used. Ferric chloride was only dosed in one of the lines, the experimental line, while the other was used as reference.

4.4.1. IMPACT OF DOSING LOCATION ON SULFIDE CONTROL

The study confirmed the findings reported in Zhang et al. (2009) that addition of ferric chloride can effectively control dissolved sulfide in rising main sewers (Fig. 14). For the typical domestic sewage composition, sulfide can be reduced to below 0.1 mg S/L with ferric chloride addition dosing a Fe^{3+} to S^{2-} molar ratio of 0.6:1.



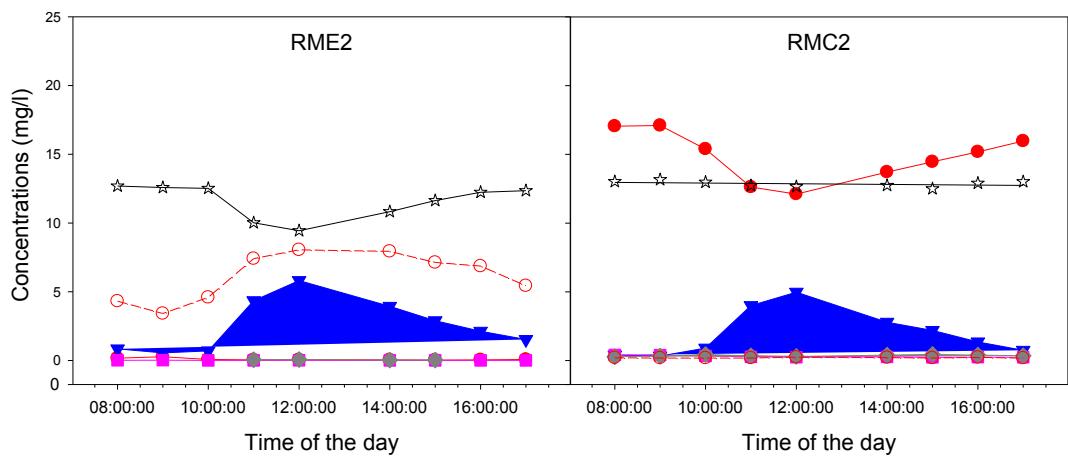


Figure 14. Profiles of dissolved sulfur species in the downstream sewer reactors after long-term addition of Fe^{3+} to RME2. RME2, experimental line; RMC2, control line; ● dissolved sulfide, ○ soluble iron, ▼ sulfate, ★ orthophosphate.

The study also revealed that:

1. Overall, ferric addition significantly inhibited sulfate reduction capacity of SRB's.
2. Addition of ferric in upstream sections of rising mains was more effective in reducing sulfide production than the injection in downstream parts. When ferric was dosed in downstream reactor RME2, similar concentrations of sulfate were detected in RME2 and RMC2, only 0.7 mgS-SO₄/L of difference (Fig. 14). In this case, sulfate reduction occurred mainly in the ferric free upstream reactor and inhibition occurred only in the downstream reactor. On the other hand, when ferric was dosed in the upstream RME1, the inhibitory effect of iron was maximized along the pipe and higher concentrations of sulfate (around 3.9 mg S-SO₄/L) were usually detected at RME2 reactor when compared to RMC2.
3. The inhibition of iron salts on biofilms activities is induced in 3-7 days after the initiation of ferric salt dosage. The inhibitory effect of Ferric would also persist for a time period even the chemical dosage is stopped.

4.4.2. EFFECT OF INITIAL FERRIC ION CONCENTRATION ON SULFATE REDUCTION ACTIVITY OF ANAEROBIC SEWER BIOFILM

Previous study has revealed that the addition of iron resulted in decrease of sulfate reducing and methane producing activities of sewer biofilms by 60 and 80%, respectively (Zhang et al., 2009). The effect of initial iron concentration on the sulfate reducing activity of anaerobic sewer biofilms has been examined in this study. Reactors simulating anaerobic sewer biofilms were amended with 5, 10, 15, 21, 27, and 35 mg Fe^{3+} /L iron and the dynamic as well as the steady state response of the biofilm was investigated.

In general, the key outcomes of the dynamic ferric dosing study demonstrated that:

1. Decrease in sulfate reducing activity of the anaerobic sewer biofilms was observed for all the applied iron dosages (Fig. 15A). However, as the concentration of the iron



increased, faster decrease in sulfate reducing activity was observed. Interestingly, regardless of the initial iron concentration, steady state sulfate reducing activity was similar in all cultures, which ranged between 51 to 62 %.

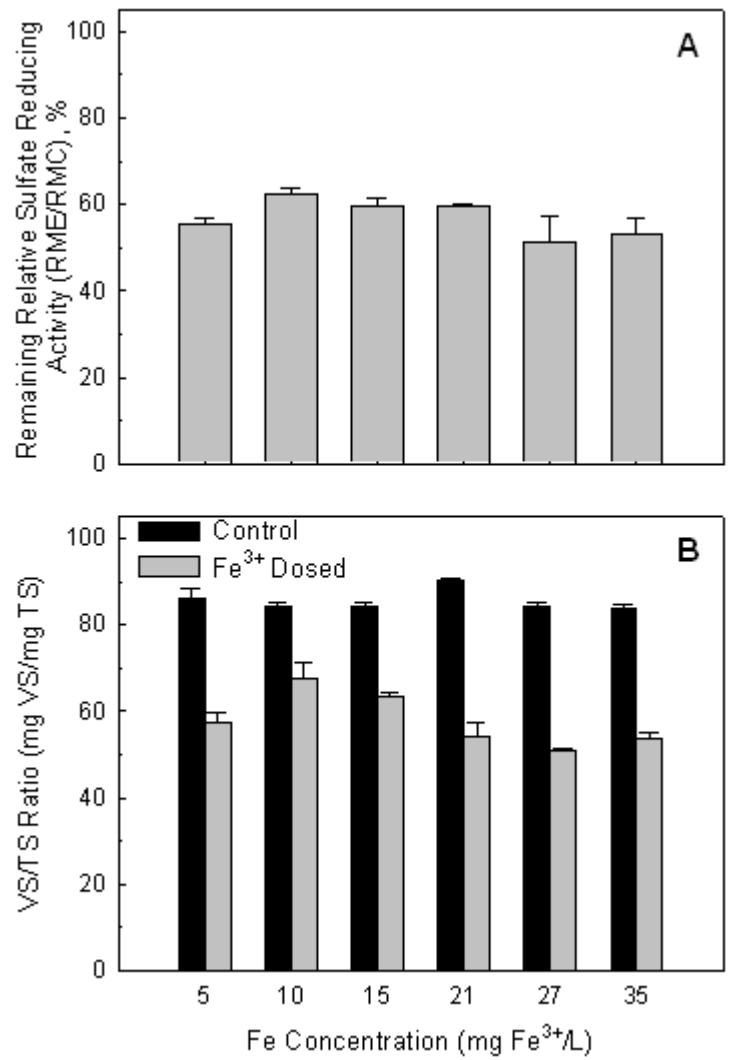


Figure 15. Steady state response of anaerobic sewer biofilm. (A): Remaining relative sulfate reducing activity of cultures exposed to different iron concentrations over long periods of time. (B): Steady state VS/TS ratio of control and ferric dosed biofilm reactors.

2. A decrease in the VS/TS ratio was observed in the iron dosed cultures (Fig. 15B). Steady state VS/TS ratio of the iron dosed cultures ranged between 51 to 67% and the observed decrease in the VS/TS was consistent with the observed remaining sulfate reducing activity of cultures dosed with 5, 10, 15, 21, 27, or 35 mg Fe³⁺/L (Fig. 15B). TS and VS measurements have shown that iron dosing caused a slight increase in the TS content and significant decrease in the VS content of the biofilm. VS is generally considered to be the biomass content of a sample, therefore, decrease in VS content suggest decay of biomass in biofilm. Slight increase of the TS content may be the result of accumulation of inorganic FeS precipitates on biofilm, blocking the diffusion of substrates, which may have caused a decrease in VS content.
3. Termination of iron dosing resulted in 94% recovery of sulfate reducing activity in the 15 mg Fe³⁺/L dosed anaerobic sewer biofilms within 3 days (Fig. 16A). However, very slight increase in the VS/TS ratio of the iron dosed culture was observed (Fig. 16B).



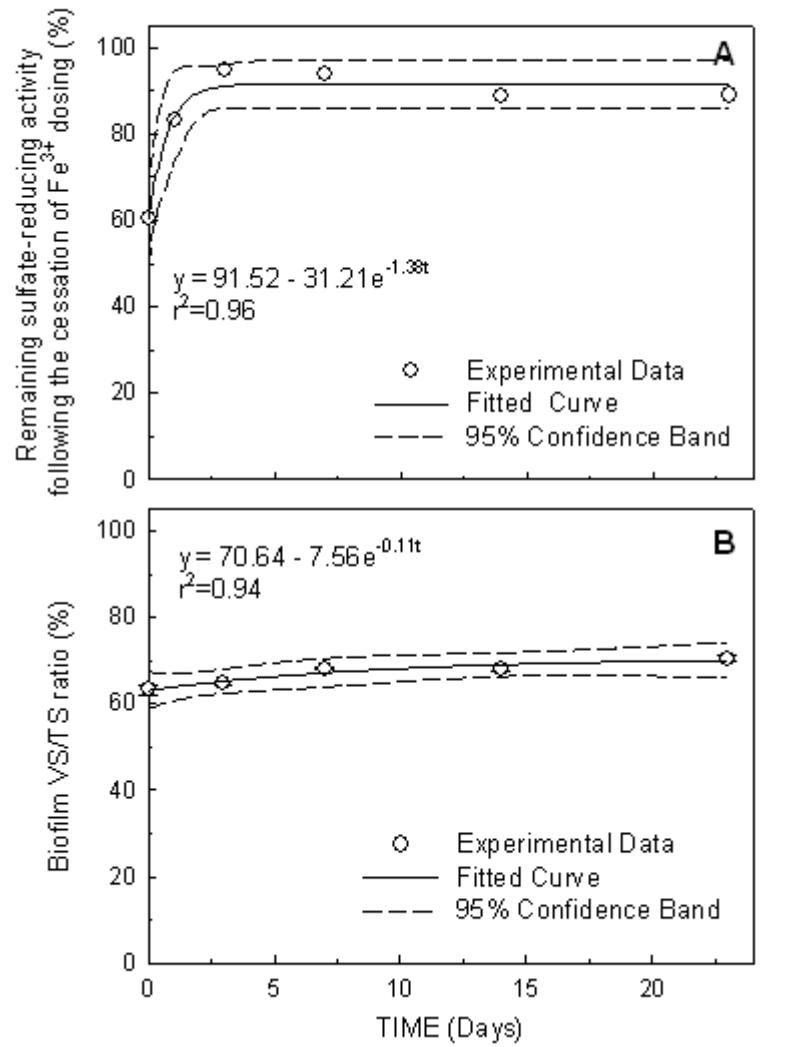


Figure 16. Response of the 15 mg Fe³⁺/L dosed culture subsequent to cessation of iron dosing. (A): Remaining relative sulfate reducing activity. (B): VS/TS ratio of the culture previously exposed to iron.

4. VS/TS ratio was increased to 71% in 23 days. As mentioned before, one of the reasons for decreased VS/TS ratio was the decrease in VS content of the biomass. Slow increase in the VS/TS ratio may suggest slow growth of methanogens and sulfate reducers that were washed out due to continuous iron dosing.

4.4.3. POSSIBLE MECHANISM OF IRON INHIBITION ON SULFIDE REDUCING ACTIVITY IN SEWER SYSTEMS

Although the inhibitory effect of ferric to sewer biofilms has been confirmed, the exact mechanisms of iron inhibition remain to be discovered. Several possibilities were hypothesized (like enzymes disruption, physical blockage of sulfate diffusion into the biofilm due to metal sulfide precipitates, etc). These investigations can be crucial to further improve the effectiveness of the ferric dose strategy.



To date, the possible hypothesis is that slight increase of the TS content may be the result of accumulation of inorganic FeS precipitates on biofilm, blocking the diffusion of substrates, which may have caused a decrease in VS content.

As an extension (additional work) of the ferric ion testing, laboratory study was performed using mixed suspended culture enrichment as inoculums to determine the possible mechanism of iron inhibition on sulfide reducing activity in sewers. A set of adaptation protocols has been designed to increase the concentration of suspended SRB culture up to 200 mgVSS/L. This concentration is required to achieve SRB activity in producing sulfide around 5 mgS/L.h in the system.

The assay was conducted in 120 mL serum bottles. In the study, a non Fe-treated culture was used as a control and four Fe-treated cultures were prepared with different scenarios:

- 1) FeS treatment was applied to determine the effect of ex-situ formed FeS on the SRB activity.
- 2) FeCl₃ treatment was applied to determine the effect of iron-hydroxide formation on the SRB activity.
- 3) FeCl₃ and SO₄²⁻ treatment will involve the addition of FeCl₃, SO₄²⁻ and glucose at the same time in order to determine the effect of in-situ FeS formation during sulfate reduction, which is assumed to take place around the cells causing diffusion limitation or interactions with enzymes.
- 4) FeCl₃ and S²⁻ treatment was applied to determine the effect of FeS formation in the culture on SRB activity. Different from #3, FeS is formed through externally provided sulfide.

Table 4 shows that the experiment was divided into pre-treatment and after pre-treatment. pH was adjusted to approximately 7 at the beginning of each incubation. Assays were conducted in triplicates. During pre-treatment, sulfide in test 4 and sulfate in test 3 should be in excess. This will ensure that Fe in these two cases should be locked in precipitates with sulfide rather than being present as Fe(OH)₃. All five cultures were amended with 100 mg/L SO₄²⁻ and 400 mg glucose-COD/L after pre-treatment. The sulfate reduction rate was then monitored periodically for 1 hour.



Table 4. Matrix of the experiment

		Pretreated Cultures					
Contents		Control	FeS	FeCl ₃	FeCl ₃ and S ²⁻	FeCl ₃ and SO ₄ ²⁻	Total (mL)
<i>Pre-treatment</i>	Innoculum	+	+	+	+	+	+
	Media	+	+	+	+	+	+
	FeS Solution (100 mg Fe ³⁺ /L)			+			
	FeCl ₃ Solution (100 mg Fe ³⁺ /L)			+	+	+	+
	S ²⁻ Solution (100 mg S/L)					+	
	SO ₄ ²⁻ Solution (100 mg S/L)				+		
	Glucose Solution (400 mg COD/L)				+		
<i>After pre-treatment</i>	SO ₄ ²⁻ solution (100mg S/L)	+	+	+	+	+	+
	Glucose Solution (400 mg COD/L)	+	+	+	+	+	+

It can be seen from Figure 17 that in most cases ferric addition has reduced the activity of sulfate reduction. The effect of in-site FeS formation during sulfate reduction (treatment #3) seems to be the cause of ferric inhibition compared to the impacts by the other treatments. This supports the possible hypothesis of ferric inhibition due to the accumulation of inorganic FeS precipitates on biofilm, which limits the diffusion of substrates.



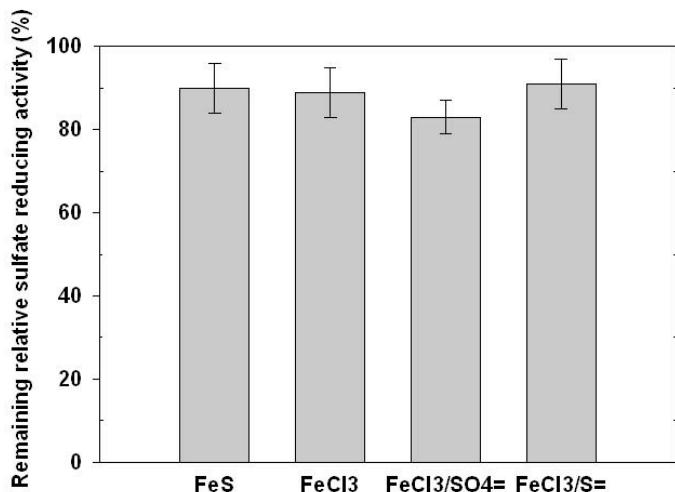


Figure 17. Remaining relative sulfate reducing activity due to ferric addition

4.4.4. IMPACTS OF IRON DOSING INTO SEWER TO ANAEROBIC SLUDGE DIGESTER

A recent study of Gutierrez *et al.*(2010) reported that ferric addition for sulfide control in sewers induces chemical phosphorus removal during wastewater treatment, as ferric ions could be regenerated from ferrous sulfide (FeS) precipitates (previously formed in sewers) in aeration tanks (in wastewater treatment plants), and used to precipitate phosphate. These ferric phosphate precipitates will settle in activated sludge streams and subsequently enter anaerobic sludge digesters, where ferric ions may be regenerated by the third time under anaerobic conditions. Therefore, a new hypothesis has been proposed; whether these ferric ions can be used again in anaerobic digesters to precipitate with dissolved sulfide to control H₂S gas emission from sludge digestion.

As part of the additional task of SP6, the impacts of iron dosing into sewer to anaerobic sludge digester was studied to evaluate the feasibility of H₂S removal in anaerobic digesters by the third use of a typical ferric dosage (5-20 mg Fe L⁻¹) in upstream sewers for sulfide control. Meanwhile, impacts of ferric addition on sludge digestion performances (e.g. methane production) were also assessed.

Some main findings from the study are:

1. In both low-sulfur and high-sulfur tests, H₂S gas produced from digesters with different iron additions was not completely controlled initially, reflected by a transient increase of H₂S concentration, but completely removed by the end of both tests (as shown in Fig. 18). This was likely because the regenerated ferric ions (from ferric phosphate precipitates) was initially in a shortage compared to the sulfide production, but then became in excess, resulting in the ultimate absence of H₂S gas. While H₂S gas from the blank digester (without the iron addition) was consistently higher, reflected by the high concentration of approximately 350 ppm in the low-sulfur test and 530 ppm in the high-sulfur test.



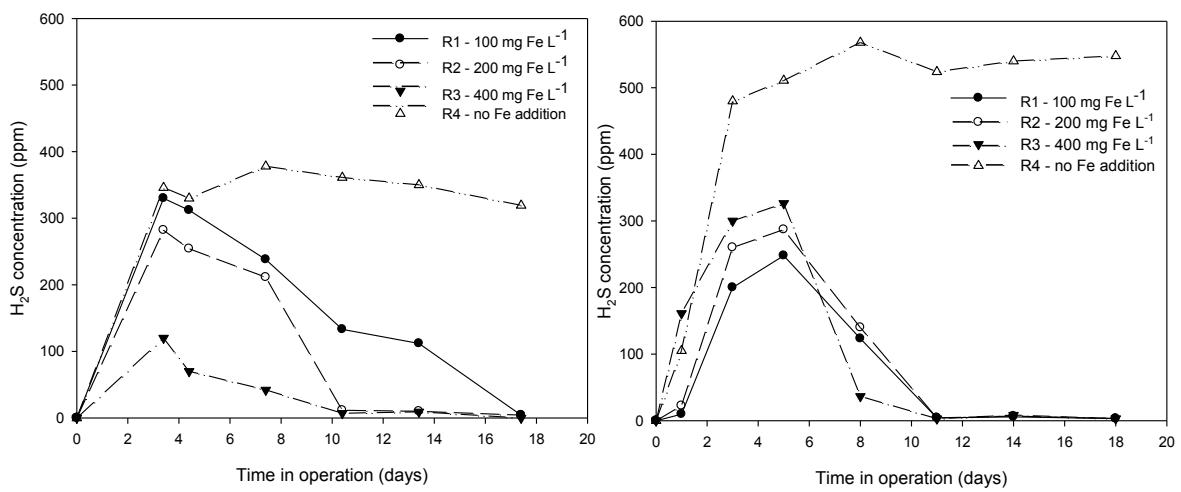


Figure 18. H_2S removal in the low-sulfur (13 mg L^{-1} , left) and high-sulfur (40 mg L^{-1} , right) anaerobic digestion batch tests.

2. Complete sulfate reduction was achieved in all batch tests, as shown in Fig. 19. However, dissolved sulfide remained below 0.3 mg L^{-1} in the digesters with iron additions, while maintained at 0.5 and 1.2 mg L^{-1} in the blank tests in the low-sulfur and high-sulfur tests, respectively. This was consistent with the H_2S gas results, and confirmed that the sulfide production during sludge digestion was removed by precipitating with ferric ions released from previous ferric additions. Accordingly, the ferric reuse efficiency was estimated between 2% and 11% in the low-sulfur test, while increased to $5\text{-}20\%$ in the high-sulfur test. This indicates that the typical ferric dosage ($5\text{-}20 \text{ mg Fe L}^{-1}$) for sulfide control in sewers has been effectively reused for the third time to control H_2S gas emission in anaerobic digesters, in addition to benefiting phosphate removal during wastewater treatment (Gutierrez et al., 2010).

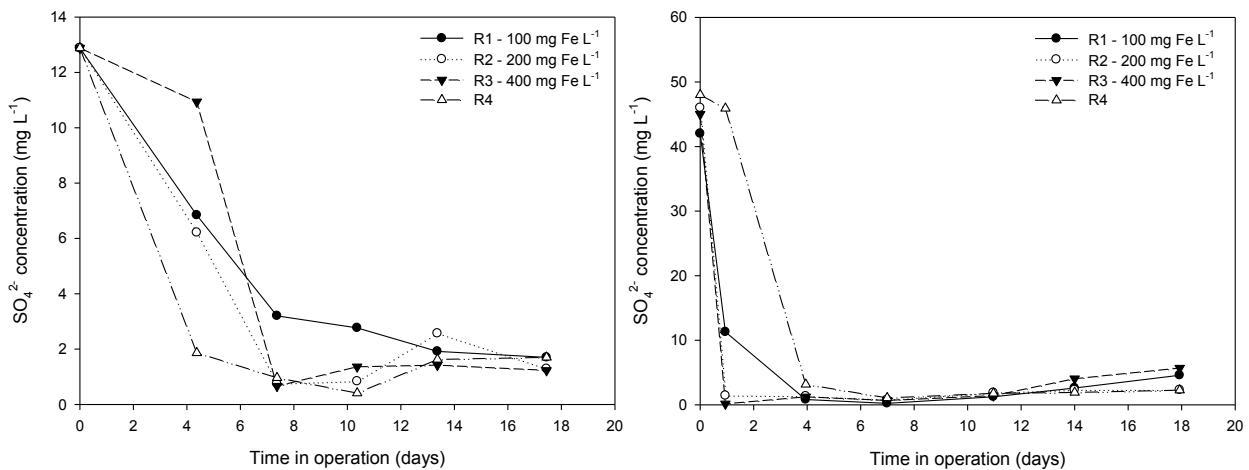


Figure 19. Sulfate reduction rates in the low-sulfur (13 mg L^{-1} , left) and high-sulfur (40 mg L^{-1} , right) anaerobic digestion batch tests.



- Cumulative methane production in the low-sulfur and high-sulfur anaerobic digestion tests is shown in Figure 20. It indicates that the methane production from the digesters with previous iron additions in both tests was not influenced by iron precipitates existed in anaerobic digesters, either in the form of FeS (created by regenerated ferric during sludge digestion) or ferric phosphate precipitates (previously formed in FeS reoxidation tests).

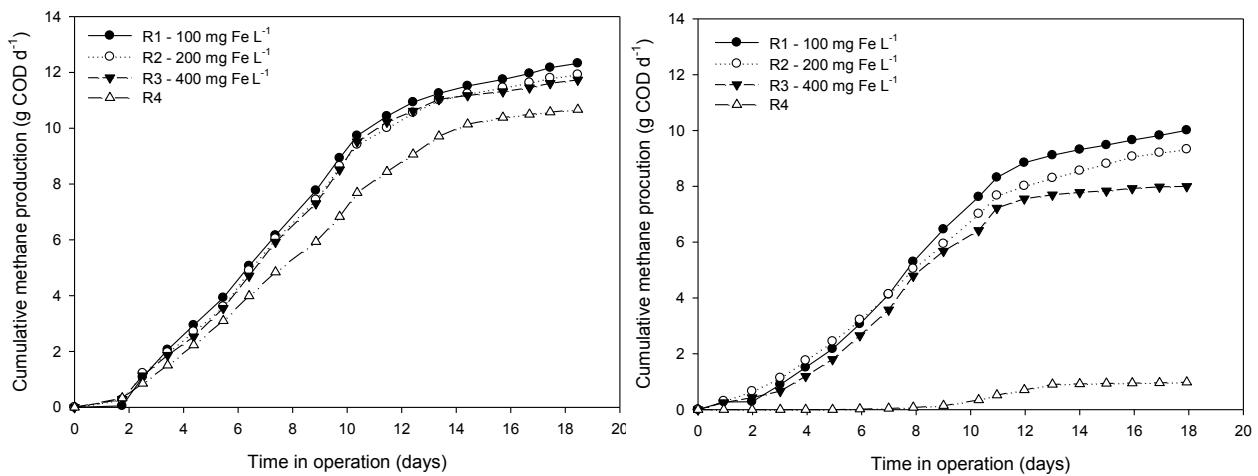


Figure 20. Cumulative methane production in the low-sulfur (13 mg L⁻¹, left) and high-sulfur (40 mg L⁻¹, right) anaerobic digestion batch tests.

- In addition, iron precipitates in anaerobic digesters had no significant impact on other digestion processes according to measurements of organic acids, ammonium-nitrogen and phosphate. The full methodology and all results obtained in this study can be found in Appendix 2 (Ge, H., Zhang, L., Batstone, D., Keller, J., Yuan, Z. 2011. Impact of iron salt dosage to sewers on sulfide control and methane production in anaerobic sludge digesters. To be submitted in the Water Research).

4.5. TESTING OF NITRITE AS A MEANS FOR SULFIDE CONTROL IN LABORATORY SYSTEM

The effectiveness of a specifically prepared nitrite solution (free nitrous acid, FNA) on sulfide control in rising main sewers has been assessed. A patent application is being prepared. The University of Queensland has offered the technology to the project as it platform pre-existing intellectual property. The general key findings of this work are:

- Lab-scale studies revealed the effectiveness of the FNA solution in controlling sulfide and methane production. The results show that even with a very short exposure time, long-term inhibition on sulfate reduction and methane production can be achieved.
- Intermittent addition could be applied to achieve long-term sulfide and methane control, resulting in a key advantage of this chemical over many other chemicals such as oxygen, nitrate, iron salts and alkali, where continuous addition is typically required.
- A preliminary economic analysis revealed that considerable cost savings can be achieved as a result.



4.5.1. BIOCIDAL EFFECT OF FREE NITROUS ACID (FNA) ON ANAEROBIC SEWER BIOFILM

Several recent studies showed that nitrite dosage to wastewater substantially reduces the sulfate-reducing and methanogenic activities of anaerobic biofilms. This study aimed to demonstrate that the reduction in these activities was due to the biocidal effect of free nitrous acid (FNA), the protonated form of nitrite, on bacteria residing in biofilms. The viable cell percentages in intact and disrupted biofilms, after being exposed to various FNA concentrations up to 3 mg-N/L for 6, 12, and 24 hours, were investigated using LIVE/DEAD staining. Nitrite concentrations at 0, 30, 60, 90, and 120 mg-N/L were employed to achieve the specified FNA concentrations at pH 5, 6, 6.5, and 7. Additionally, FNA was dosed directly into rising main sewer reactors and the subsequent recovery was monitored to verify the biocidal effects on sulfide and methane production from sewer biofilms.

Some important findings are revealed through this study:

1. There was a general negative impact of nitrite on the biofilm cell viability As shown in Figure 21A,. Higher nitrite concentration induced lower cell viability. However, the correlation between the extent of inhibition and the concentration of nitrite was not evident. pH also had an impact on the extent of viability decrease. The lower the pH, the lower the cell viability. However, there is no clear correlation between biofilm cell viability and pH shown in Figure 21B. This suggests that pH was not the main toxic factor either.
2. The level of cell viability had a much stronger dependence upon the FNA concentration (Fig. 21C), indicating that FNA may directly cause the inactivation of biofilm cells. Also shown in the figure is the fit between the experimental data and the predictions by an exponential model. It is seen that the toxicity of FNA to anaerobic biofilm cells and FNA concentration could well be described by an exponential function.



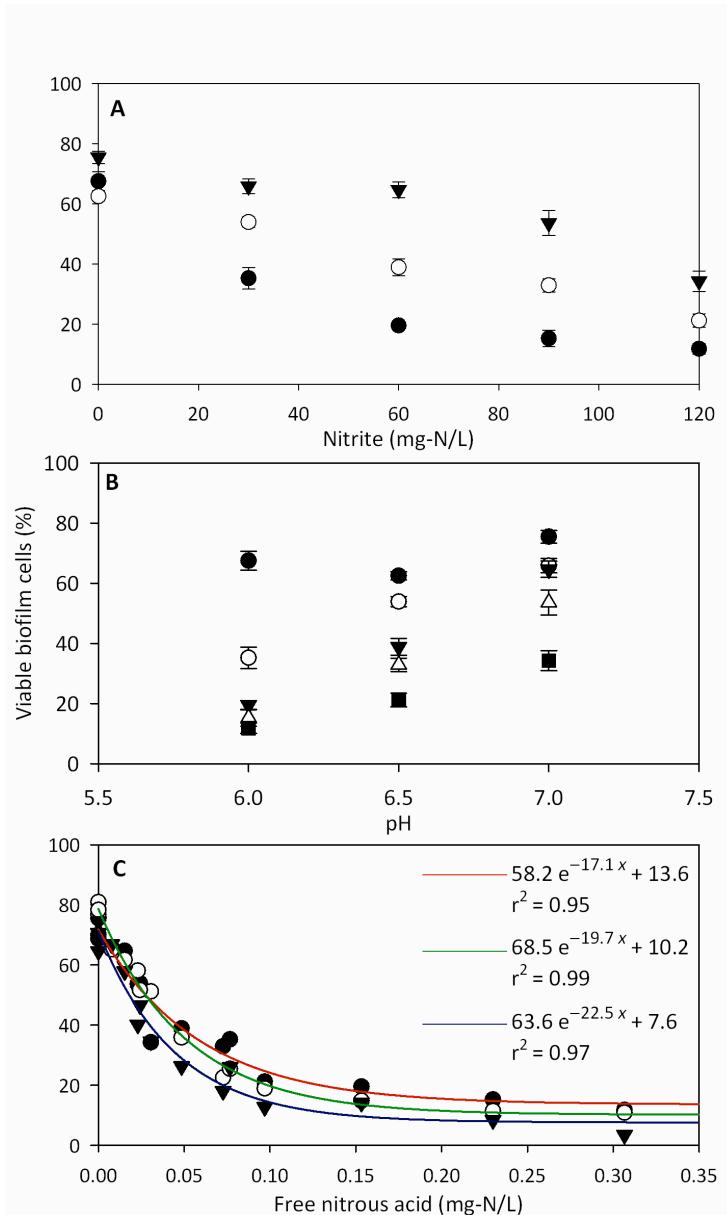


Figure 21. The dependence of viable biofilm cells (%) on nitrite concentrations (A), pH (B) after 12-hour of exposure, on FNA concentration (C) ranging from 0 to 0.3 mg-N/L after exposure for 6 hour, 12 hour, and 24 hour. In subplot A, pH=5 (●), 6 (○), 6.5 (▼), and 7 (△). In subplot B, nitrite concentration = 0 (●), 30 (○), 60 (▼), 90 (△), and 120 (■) mg-N/L. In subplot C, exposure time = 6 (●), 12 (○), and 24 (▼) hour, with regression line in red, green, and blue respectively. The regression is done with a 3-parameter exponential decay model.

3. Disrupted biofilm were more sensitive to FNA toxicity (Fig. 22). The viable cells in disrupted biofilm after exposure to FNA was 10-20% lower than that in intact biofilm. It is clear that FNA toxicity to biofilms can be augmented by physical disruption. It was also found that disruption made the biofilm more susceptible to pH change. In the absence of nitrite, pH change from 7.6 to 6 decreased the viability by 25% in the disrupted biofilms. This decrease was negligible for the intact biofilm samples.



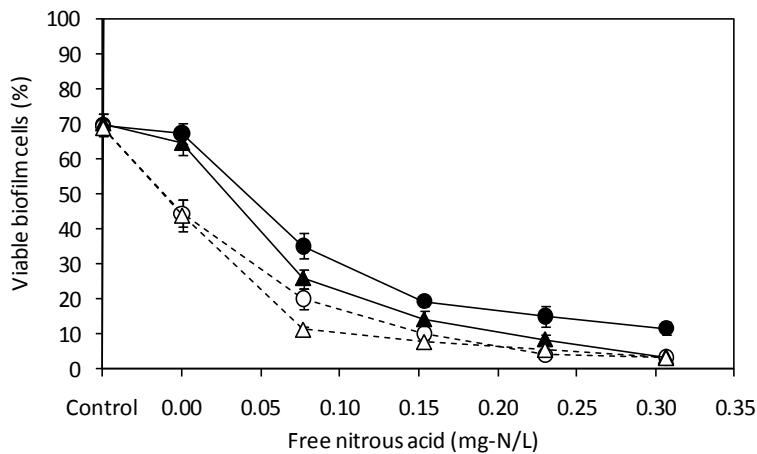


Figure 22. The viable biofilm cells (%) in intact (filled symbols) and disrupted (empty symbols) biofilms after being exposed to FNA at pH 6 for 6 (● and ○) and 24 (▲ and Δ) hours. The first datapoints for each series are the control biofilm samples ($\text{pH} = 7.6$).

4. The recovery of biofilm activities after being exposed to FNA at 0.18 and 0.36 mg-N/L (Fig. 23) resembled the re-growth of residual sulfate-reducing bacteria and methanogens, further confirming the biocidal effects of FNA on bacteria in biofilms.

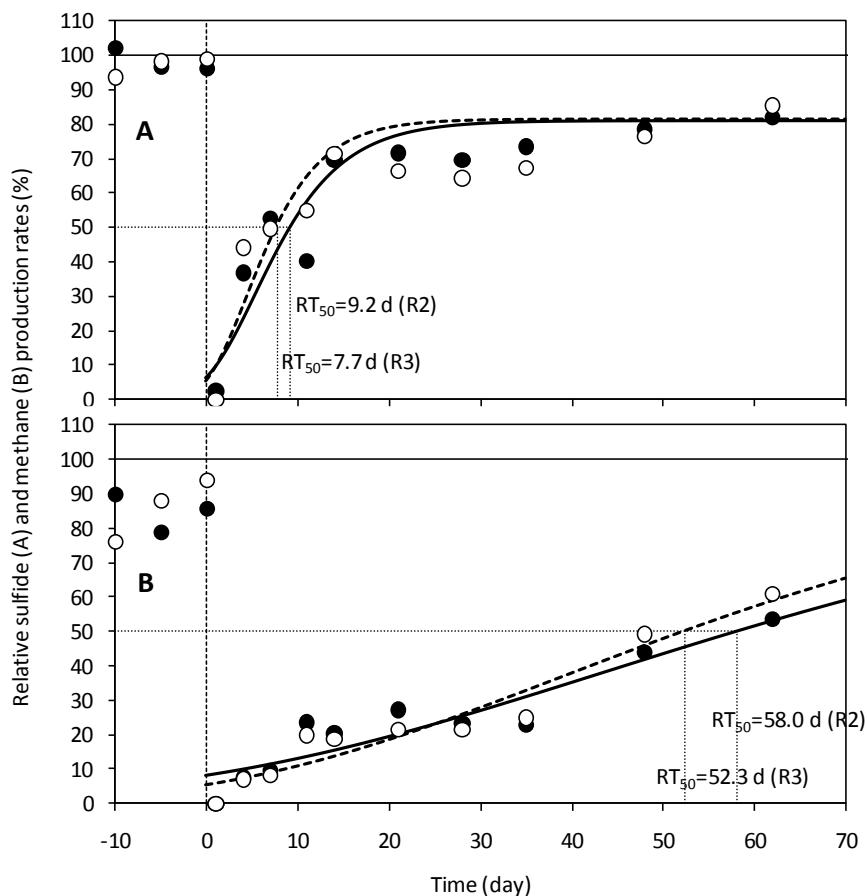


Figure 23. Relative hydrogen sulfide (A) and methane (B) production rates during the stabilization and recovery periods after being exposed to free nitrous acid at 0.18 (□) or 0.36 (□) mg-N/L, in reactor R2 and R3 respectively, on day 0. The solid (—) and dashed (---) lines are regressions with Gompertz growth equation.



5. Cost estimation was also calculated for the FNA dosing (Fig. 24). The estimation is compared to other chemical dosing, such as iron salts, nitrate, air injection, and nitrite. The figure demonstrates that FNA dosing is very competitive as it offers the lowest cost estimations compared to the others.

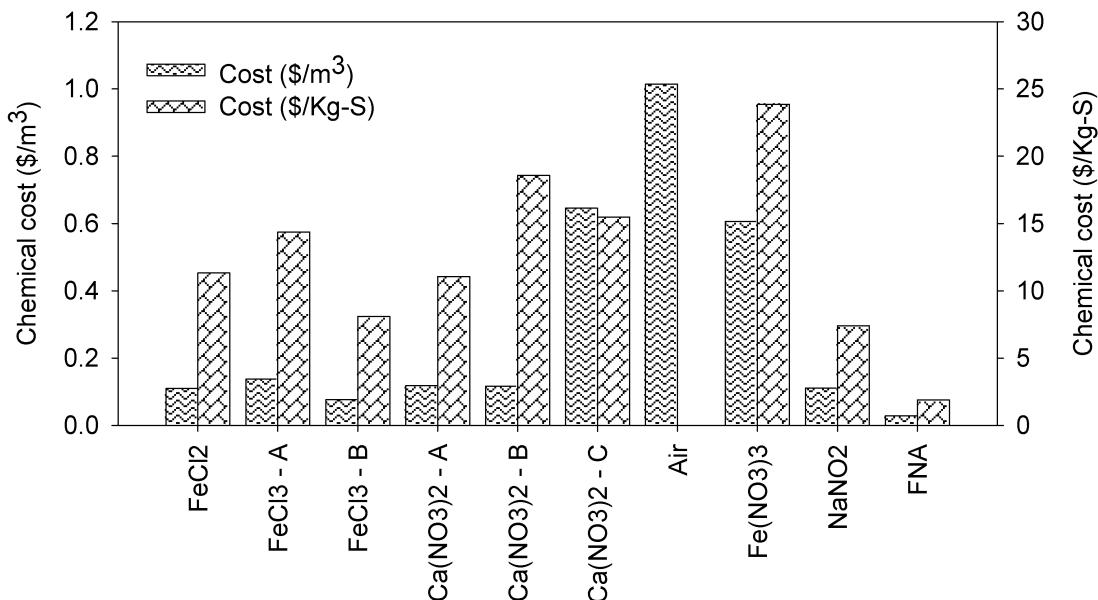


Figure 24. Comparison of chemical addition costs in sewers

4.5.2. SYNERGISTIC INACTIVATION OF ANAEROBIC SEWER BIOFILM MICROBES USING FREE NITROUS ACID WITH HYDROGEN PEROXIDE

Synergistic biocidal effect of free nitrous acid (FNA) combined with hydrogen peroxide (H_2O_2) on anaerobic wastewater biofilms was investigated in this sub project as an additional task (extension). Laboratory-scale rising main sewer systems were used to grow anaerobic wastewater biofilms. Intact biofilm samples attached to plastic carriers taken from the reactors were incubated in wastewater containing FNA, H_2O_2 , or both, for 6 hours. The killing efficiency of microorganisms in biofilms by the biocide treatments was measured using a LIVE/DEAD staining assay, which assesses cell viability by verifying cell membrane integrity. These tests were employed to testify the synergistic killing by the formation of FNA and H_2O_2 . To optimize the dosing parameters (i.e. FNA concentration, H_2O_2 concentration and exposure time) to achieve high killing of anaerobic sewer biofilms, a central composite design was employed to design a series of viability tests. The response surface methodology (RSM) was used to analyze the obtained data and to determine the optimal combination of parameters. The formation of peroxy nitrite and its decay dynamics was analyzed using kinetic simulation of reactions to explore the potential mechanisms for the observed synergism.

Interesting results were obtained from this study. The key outcomes are:

1. The microbial killing efficiency increased with FNA concentration when only FNA was applied at 0 to 0.4 mg-N/L (Fig. 25). Around 90% of microbial inactivation could be achieved with FNA above 0.4 mg-N/L. The biocidal effect of H_2O_2 was not as effective as FNA, only achieving 58 – 75% kill of biofilm microbes at 30 – 90 mg/L.
2. The killing percentage could be increased by 43 – 51% when H_2O_2 was simultaneously added with FNA compared to the addition of FNA alone at 0.05 mg-



N/L. At FNA concentration higher than 0.2 mg-N/L, about 99% killing efficiency was achieved consistently for H₂O₂ at 30 – 90 mg/L. There is a strong elevation of FNA's biocidal effect when it is combined with H₂O₂. About 2-log of microbial inactivation could be achieved for the tested FNA and H₂O₂ concentration ranges.

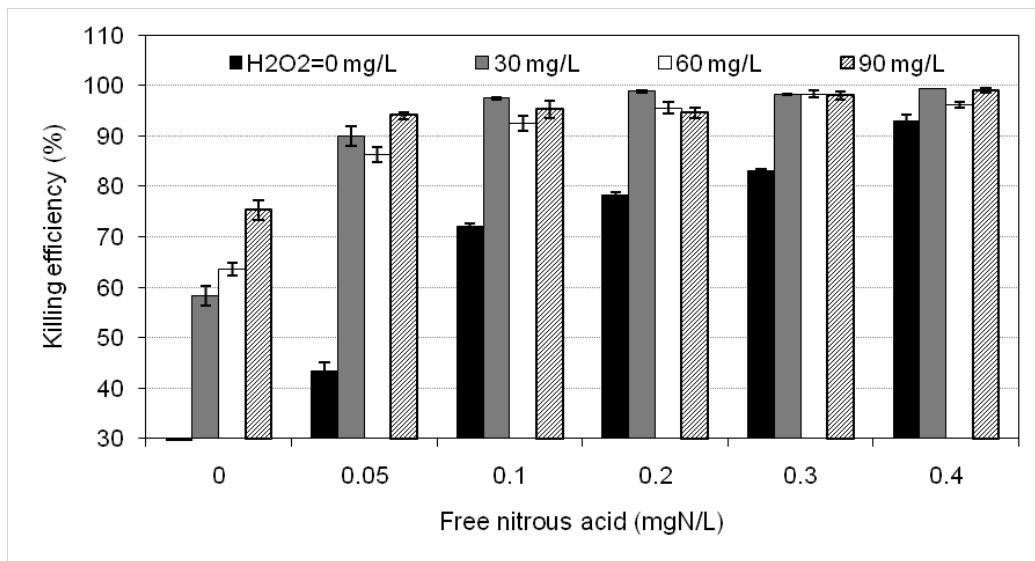


Figure 25. Killing efficiency of free nitrous acid (0, 0.05, 0.1, 0.2, 0.3, 0.4 mgN/L), hydrogen peroxide (0, 30, 60, 90 mg/L) and their combination.

3. FNA + H₂O₂ can achieve faster inactivation for low-resistant biofilm microbes, supported by higher killing rate constant $K_1 = 10.5 \text{ L-h/mgN}$ compared to $K_1 = 5.0 \text{ L-h/mgN}$ for FNA alone (Fig. 26). Also, the fraction of low-resistant biofilm microorganisms was increased from 0.55 for FNA to 0.93 for FNA + H₂O₂. This implies that FNA + H₂O₂ combination was more effective than FNA to inactivate the mixed-culture wastewater biofilms.

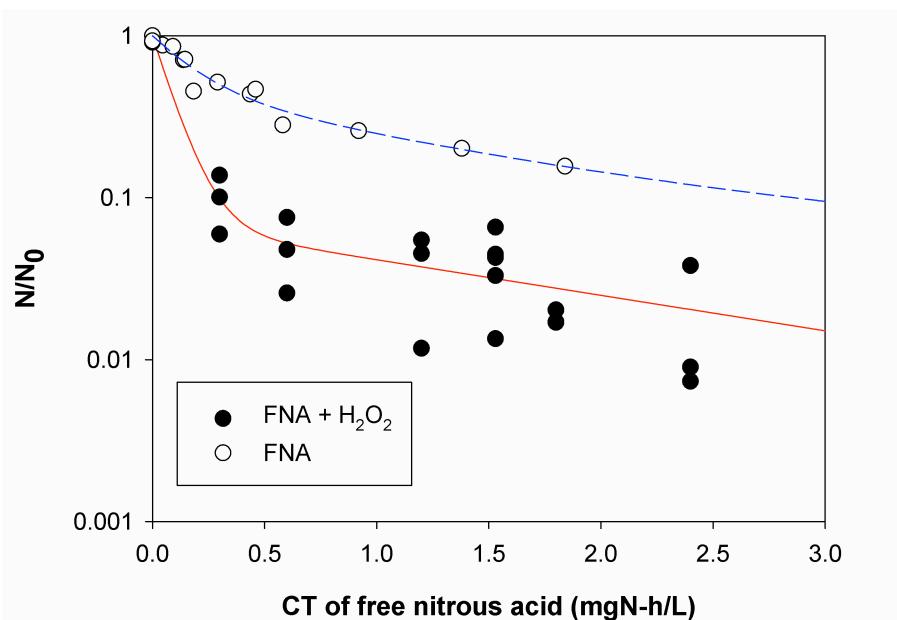


Figure 26. Effects of hydrogen peroxide (10-90 mg/L) on the FNA inactivation kinetics of biofilm microorganisms at pH = 6.



4. FNA + H₂O₂ finished its first stage of inactivation (killing low-resistant microbes) around Ct = 0.5 mg-N·h/L, similar to that of FNA. Interestingly, the killing rate constants for high-resistant microbes using FNA and FNA + H₂O₂ were similar, K₂ = 0.7 and 0.5 respectively. The percentage of persisters (fr) for FNA dosing was reduced from 0.05 to 0 when FNA + H₂O₂ combination was dosed.
5. Exposure time had a major impact on the killing efficiency (Fig. 27). The killing efficiency increased with increasing exposure time from 2 – 6 h and flattened out at longer exposure time. Experiment I has demonstrated that addition of H₂O₂ along with FNA increased the microbial killing efficiency by one-log, the RSM results further showed that sufficient exposure time was needed to achieve the elevating effects.
6. The RSM analysis indicated that the stationary point (the optimal levels of experimental factors) on the fitted response surface was at FNA = 0.35 mgN/L, H₂O₂ = 29.6 mg/L, and exposure = 7.4 hour. The perspective 3D plots in Figure 26 clearly confirmed that 2-log killing of biofilm microbes could be achieved around the stationary point. About 1% of microbes were indicated to be still alive after treatment for about 7.4 h. There was a slight increase of killing efficiency with increasing H₂O₂ concentration from 20 to 40 mg/L (Figure 27B). In contrast, killing efficiency barely increased with higher FNA from 0.2 to 0.4 mgN/L. Nevertheless, ridge analysis indicated that killing efficiency could be increased at higher FNA, or higher H₂O₂ concentrations.

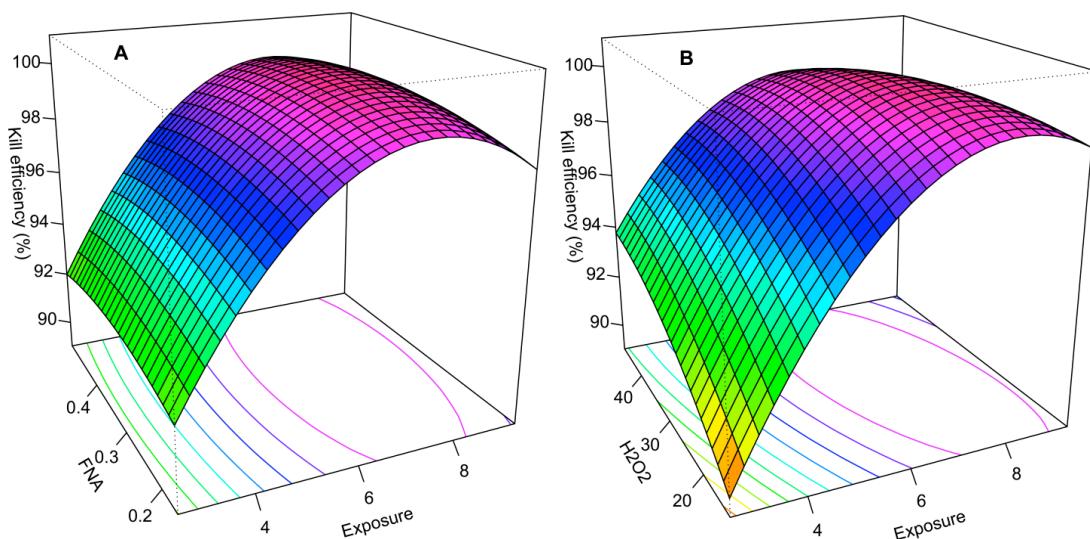


Figure 27. Fitted response-surface perspective plots near the stationary points. A: is the response surface at H₂O₂ = 29.6 mg/L. B: at FNA = 0.35 mgN/L.

7. A potential mechanism of the synergistic killing caused by FNA + H₂O₂ can be postulated based upon the dynamic reaction analysis (Fig. 28). Among the various compounds, NO, NO₂, N₂O₃, N₂O₄, ONOO⁻/ONOOH, and H₂O₂ are all known biocides. The addition of H₂O₂ to FNA led to significant ONOO⁻ production at the cost of negligible consumptions of H₂O₂ and FNA. ONOO⁻ was a stronger biocidal agent than NO and it was not present in FNA solution. Much higher NO₂ concentration plays an augmenting role in the synergism. In addition to these reactive nitrogen compounds, hydrogen peroxide itself is also a strong biocide. Thus, the synergism is likely based



upon massive production of ONOO^- , elevated NO_2 concentration, and extra microbial killing caused by hydrogen peroxide itself.

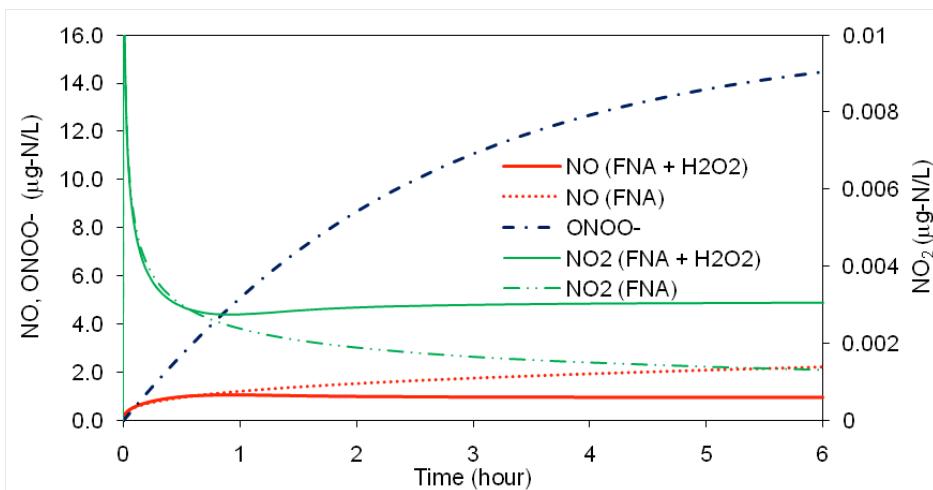


Figure 28. Simulated profiles of important biocidal chemical compounds generated by FNA only, or by the combination of FNA and H_2O_2 when being mixed in a pH = 6 buffer solution.

(Detailed information of this research, including holistic description of methods, results, and full discussion, can be found in Appendix 3. This Appendix has been prepared as a manuscript to be submitted to Environmental Science & Technology.)

FNA+H₂O₂ cost estimation is requested by SWC to be included in this report-> Guangming

4.6. TESTING OF CAUSTIC SHOCK (EXTREME pH ELEVATION) AS A MEANS FOR SULFIDE CONTROL IN LABORATORY SYSTEM

Effects of extreme pH elevation (10.5 – 12.5) and different exposure times (0.5 to 6 hours) in sewer biofilms was studied. An extensive lab test including the LIVE/DEATH cell staining analysis in biofilms were performed to determine the best pH-time combination.

Some major findings are revealed in this study:

1. Decrease in sulfate reducing activity of the anaerobic sewer biofilms was observed for all of the pH shock dosages (Fig. 29). In general, as the pH level increased, higher sulfate reducing activity was observed. Figure 29A shows that sulfate reducing activity in experimental reactors decreased to approximately 10% in comparison to the control reactor operated at pH 7.5 after 6 hours exposure time to three different pH dosages (10.5, 11.5 and 12.5). These results indicate that caustic intermittent dosage to increase pH levels to 10.5 and above could effectively control sulfate reduction.
2. Higher NaOH concentration increased pH level which had a significant impact on the cell viability decrease. Compare to the 6 hours test, a similar initial value of sulfate reducing activity occurred when pH 10.5, 11.0 and 11.5 were dosed in the system with 1 hour exposure time (Figure 29B). However, sulfide production recovery of the system with a shorter exposure time tends to be faster than the longer exposure. A shorter exposure time may merely cause a temporary inactivation of anaerobic biomass in sewer systems as a result of insufficient contact time. With a longer exposure time, inhibitory chemical could possibly penetrate into a deeper layer of biofilms which grow on the pipe wall. This condition may possibly improve the inactivation process resulted in a slow recovery of sulfide and methane formations.



3. The full recovery of biofilm activities after being exposed to pH 10.5 with different exposure times generally obtained for approximately 7 days (Fig. 29C). The pH shock lab study at pH 10.5 demonstrates that the highest reduction of sulfide productions occurred at the longest exposure time of 6 hours. It shows that exposure time holds a significant role in controlling biofilm activities.

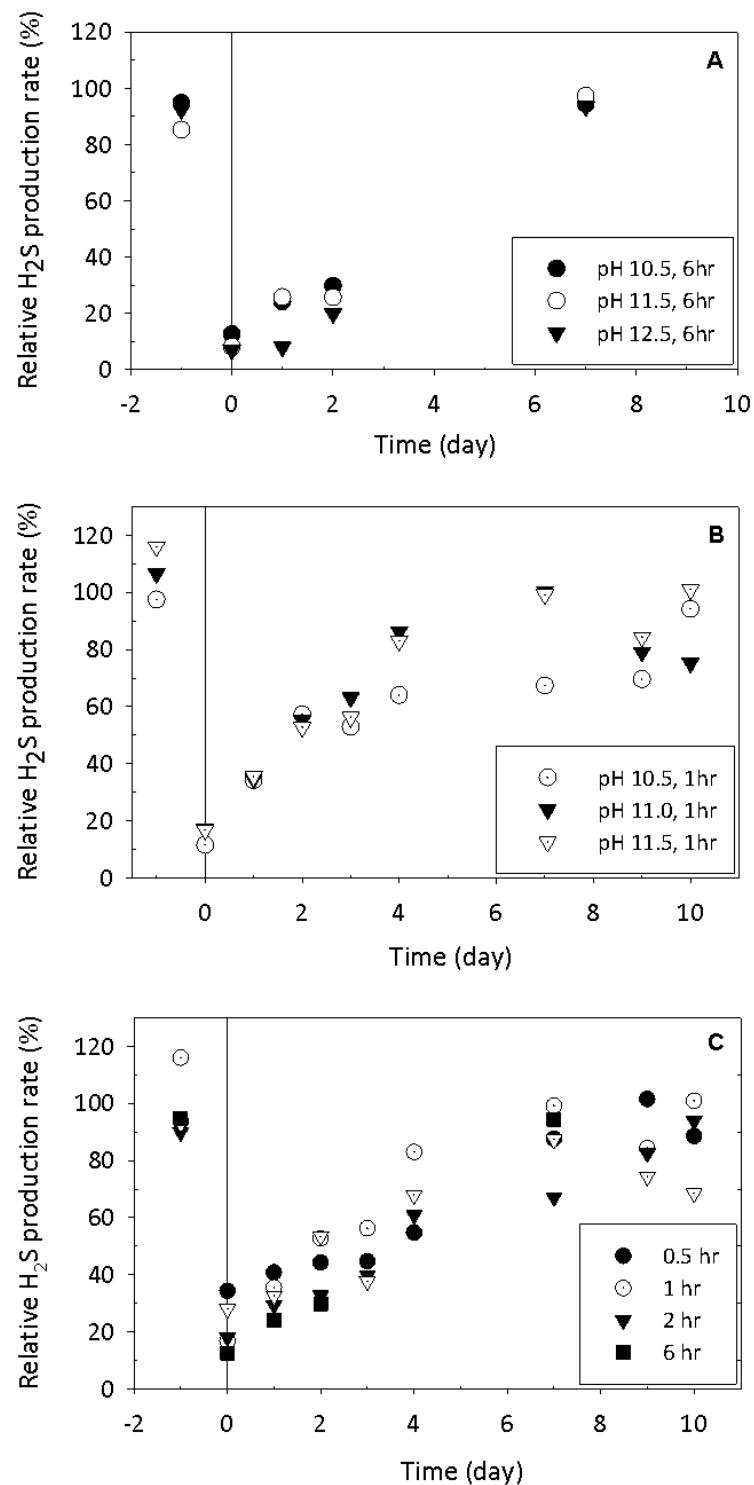


Figure 29. Relative sulfide production rates. (A): 6hr exposure time of different pH. (B): 1hr exposure time of different pH, and (C) different exposure time of pH 10.5.



4. Decrease in methane production was also observed during the pH shock lab test (Fig. 30). Different from sulfide, after pH shock dosing, the recovery of methane production was generally low for a long period of time. Only 5-15% of relative methane production rate was noticed until day 17 from the initial dosing. This lasting inhibitory effect shows that high level of pH has significantly inactivated the MA, which was more susceptible to high pH than SRB.

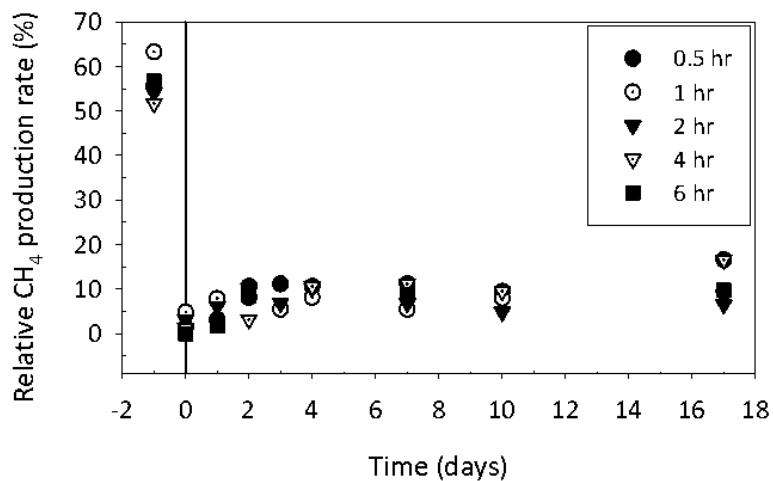


Figure 30. Relative methane production rates at pH 10.5 with different exposure times.

5. It is demonstrated from the study that the higher the pH, the lower the cell viability. Figure 31A shows that the level of cell viability had a strong dependence upon the pH, indicating that high NaOH concentration may directly cause the inactivation of biofilm cells and significantly decreased SRB and MA activities. The study also reveals lower cell viability with higher exposure time (Fig. 31B). Based on the previous work conducted by Baatout et al. (2007), a radically change of cell physiology including significant depolarization and decay of the cell membrane and its permeability due to high pH shock exposition may occurred causing cell death.



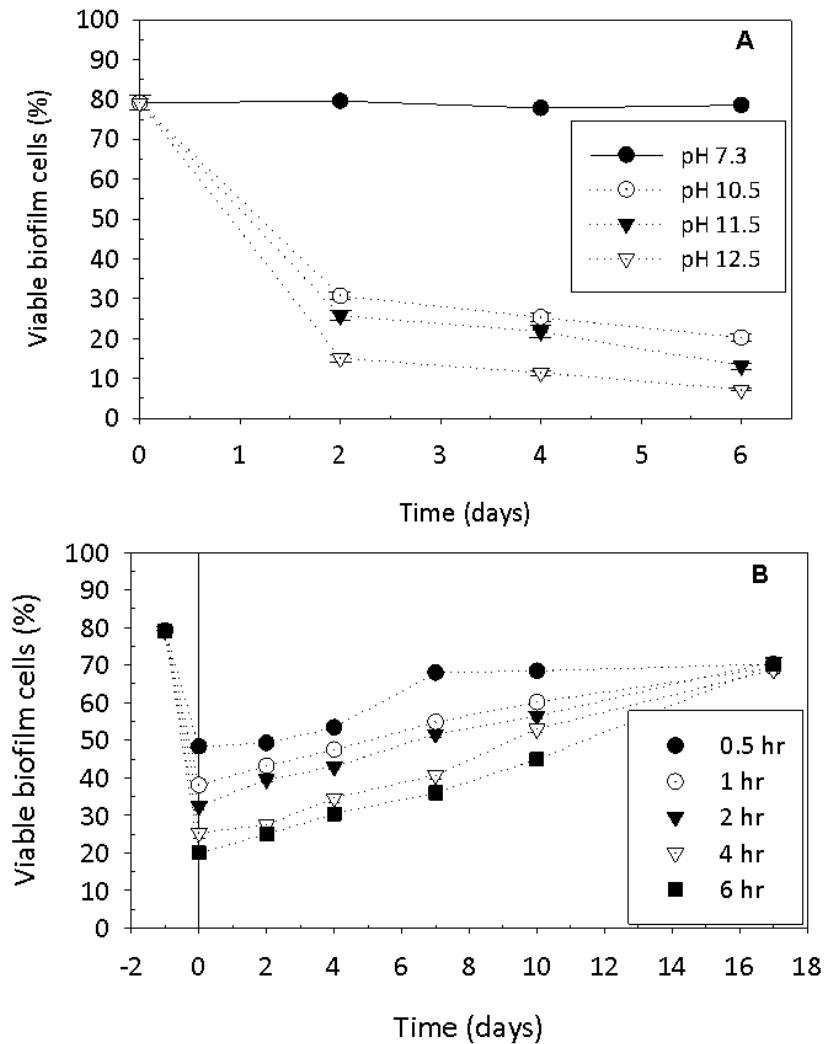


Figure 31. The viable biofilm cells (%) at pH 10.5 (A) different pH level, (B) different exposure times.

6. The dosage of high pH has also resulted in the detachment of biofilm from the wall of the experimental reactor. It can be clearly seen from the graph that higher solid concentration was detached from the reactor with higher exposure time. Figure 32 illustrates solids detachment which was indicated by the increased of solids by up to 76 % at the dosing day (day 0) after high pH was added and up to 75 % at day 1 after the dosage was given.



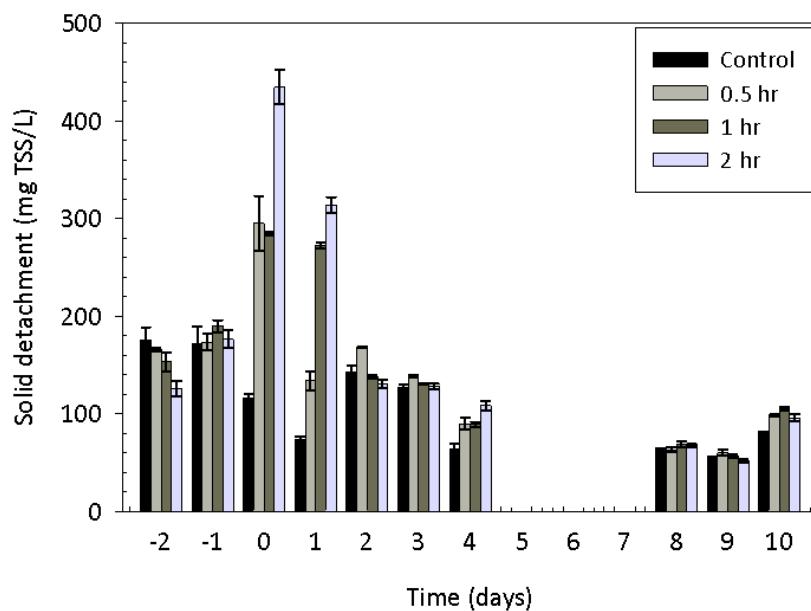


Figure 32. Solids detachment concentration at pH 10.5 with different exposure times.

4.7. TESTING OF FREE NITROUS ACID AS A MEANS FOR SULFIDE CONTROL IN FIELDS SITE

The testing aimed to verify the valuable results obtained from the UQ lab-scale studies in the field site. The key results showed that with a very short exposure time, long-term inhibition on sulfate reduction and methane production can be achieved. This means intermittent addition could be applied to achieve long-term sulfide and methane control, resulting in a key advantage of this chemical over many other chemicals, where continuous addition is typically required. Three free nitrous acid dose trials have been performed in the UC09. In these trials the addition of free nitrous acid was carried out manually due to practical and safety reasons.

4.7.1. UC09 NORMAL FUNCTIONING

As described in Section 3.2, UC09 rising main which is located on the Gold Coast was used for the free nitrous acid field testing. Figure 33 displays the typical pump events and HRT over a 24 hour of the UC09.



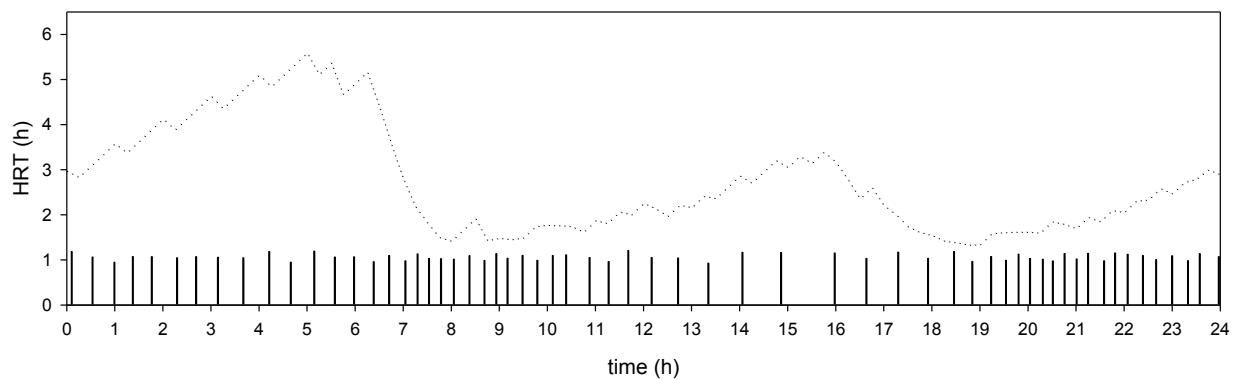


Figure 33. UC09 normal functioning (....) Sewage Hydraulic Retention Time (HRT) and (—) pump events corresponding to UC09 rising main.

Figure 34 displays a typical profile of sulfide concentration 828 m downstream of UC09 wetwell corresponding to the period prior to any dose. In this particular day, the average sulfide concentration was 4.9 mgS/L, with a variation range from 2.2 to 8.4 mgS/L depending of the HRT of the sewage in the pipe. The methane concentrations varied between 15 and 25 mgCH₄-COD/L with average concentrations around 20 mgCH₄-COD/L.

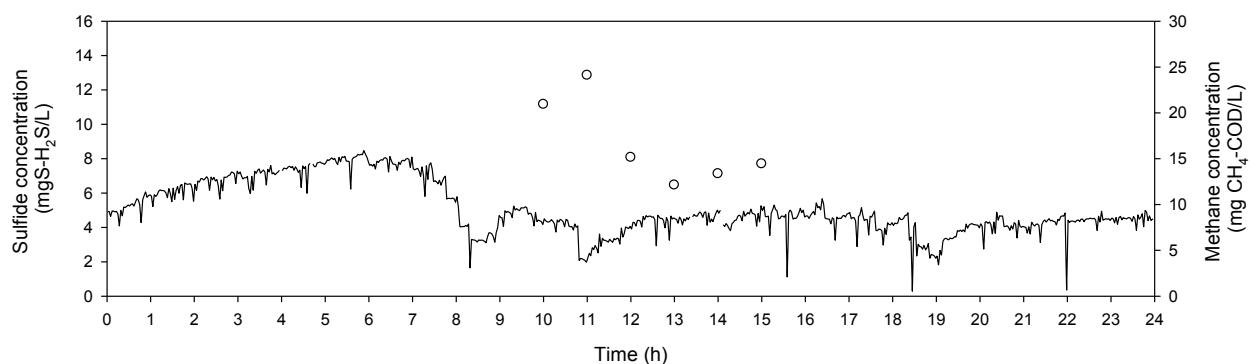


Figure 34. Typical 24h profile in UC09 rising main 828m downstream location for the background period. (—): Sulfide concentration measured by an on-line S::CAN sensor. (○): Methane data from offline sampling is also displayed.

4.7.2. FREE NITROUS ACID DOSE DESIGN

The SCORe-SP6 studies revealed the effectiveness of free nitrous acid in controlling sulfide and methane production. Three FNA dose trials have been performed in the UC09. The FNA solution was dosed to the UC09 pump station following a flow paced pattern. The target concentration of nitrite in sewage was 100 mgN-NO₂/L in the three trials.

- **Nitrite solution dose 1:** On the 17th of November, FNA was dosed overnight for 2 consecutive days, from 8:00 PM to 6:30 AM, to test FNA exposition during long HRT times in the UC09. It can be seen in Figure 35 that HRT was higher during overnight times due to the low flow in the pipe. In addition to usual pump events, extra short pump events (20 seconds) were manually performed to produce mixing conditions inside the pipe.



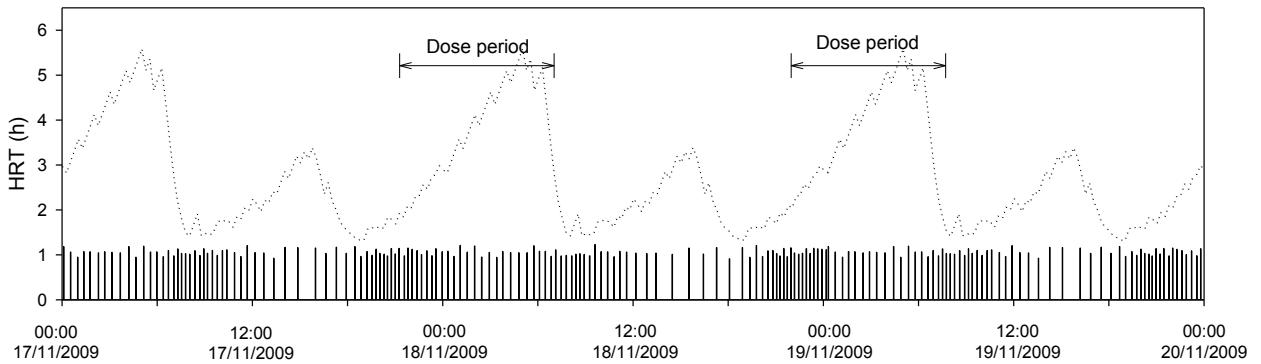


Figure 35. Nitrite solution addition period corresponding to the 1st dose trial. HRT and UC09 pump events are also presented.

- **Nitrite solution dose 2.** On the 16th of December, FNA was added on 3 consecutive days from 8:00 AM to 7:00 PM to test nitrite exposition during high flow periods in the UC09 (Fig. 36). No manual pump events were performed.

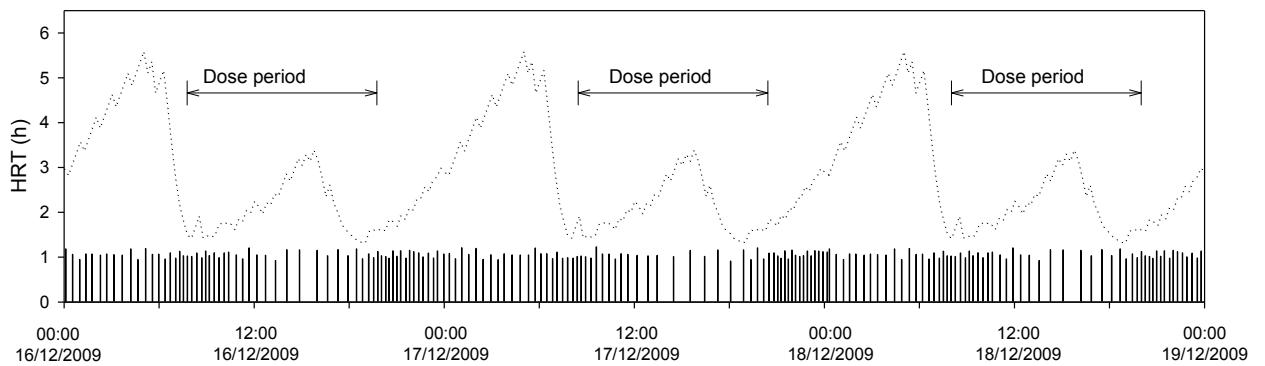


Figure 36. Nitrite solution addition period corresponding to the 2nd dose trial. HRT and UC09 pump events are also presented.

- **Nitrite solution dose 3:** The 3rd dosage trial was designed based on the observations from the previous field trials and lab studies that showed sewer anaerobic biofilms seemed to be more vulnerable to chemicals in the initial days after an exposition to FNA. Therefore, in order to extend the toxic effect of FNA, the trial was divided in two different applications:
 - Initial dosage (100 mgN-NO₂/L): 20 hours (6:00 AM to 2:00 AM) of continuous flow pace addition of FNA solution at UC09 wet well, aiming to hit hard the biofilm. Figure 37 shows the initial dosage plan.
 - Second dosage (100 mgN-NO₂/L): flow pace addition of FNA solution was performed for 4 days after the initial dosage for a period of 8 continuous hours (7:00 AM to 3:00 AM). The objective of the second dosage was to further shock the biofilms while they were still on the weak state. Figure 38 shows the second dosage plan.



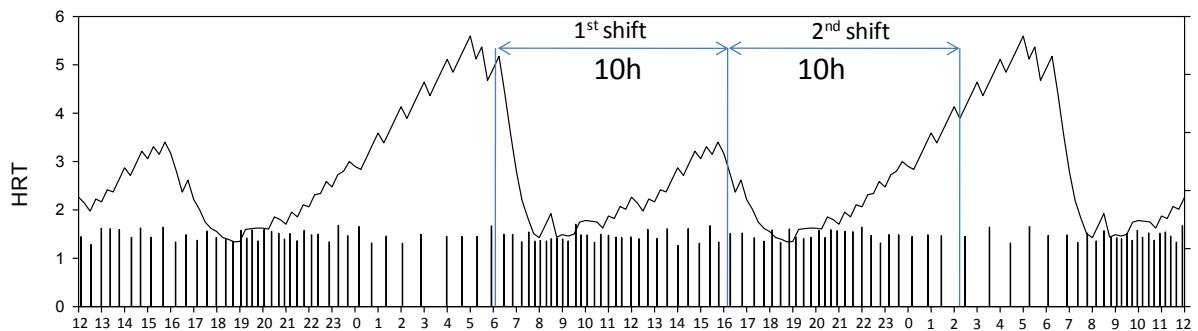


Figure 37. Proposed periods corresponding to Dosage 1. UC09 HRT and Pump events are presented.

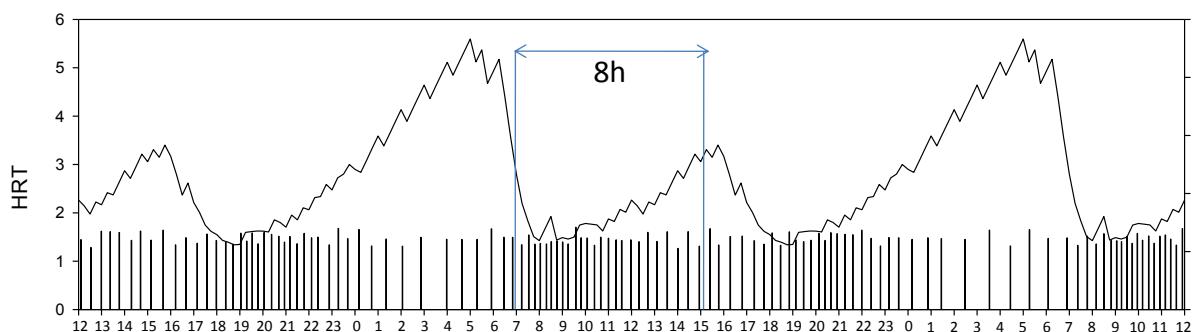


Figure 38. Proposed period corresponding to Dosage 2. UC09 HRT and Pump events are presented.

4.7.3. RESULTS OF FREE NITROUS ACID DOSE TRIAL 1

The free nitrous acid was added during the long HRT periods in the UC09. It was hypothesized that lower HRT in the pipe would significantly reduce the amount of FNA required to ensure sulfide control.

The findings of the first field FNA dosage trial are:

1. The addition of FNA prevented the sulfide production in UC09 (Day +1 profile in Figure 39, below). Unfortunately, the inhibitory effect did not last long and the sulfidogenic activity recovered steadily after the cessation of the FNA dose. Five days after the start of FNA dose the sulfide concentrations reached similar levels to prior to the chemical dose.



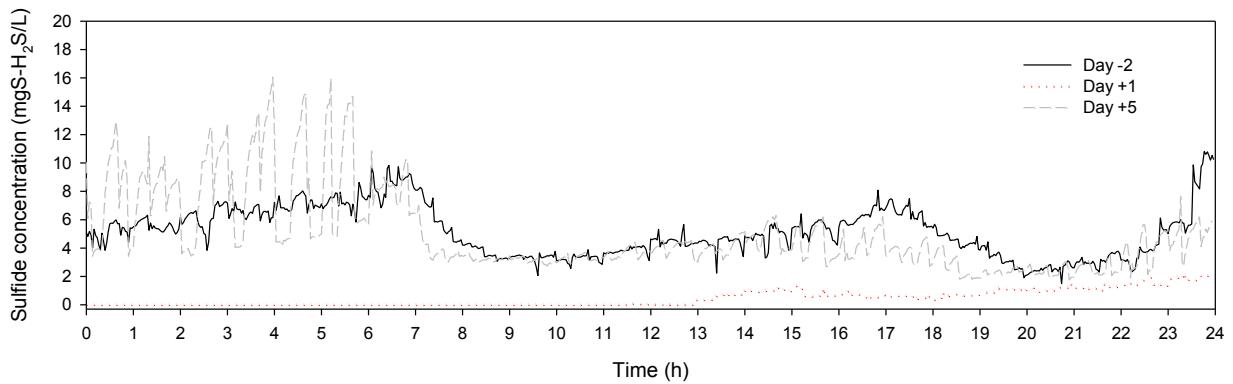


Figure 39. 24 hour online sulfide profiles corresponding to 1st trial of FNA addition in the UC09. Sulfide concentrations corresponding to 2 days before addition and 1 and 5 after special nitrite solution addition in the UC09 wetwell.

2. The daily average values of sulfide detected at 828m downstream location corresponding to the first FNA dose trial is illustrated in Fig. 40. The baseline sulfide average concentration, around 5mgS-H₂S/L, was quickly reduced after the 2 days addition of FNA solution, down to 1mgS-H₂S/L. As described above, the SRB activity recovered steadily once FNA dose was stopped, reaching the initial baseline values 5 days after the first exposition to FNA.

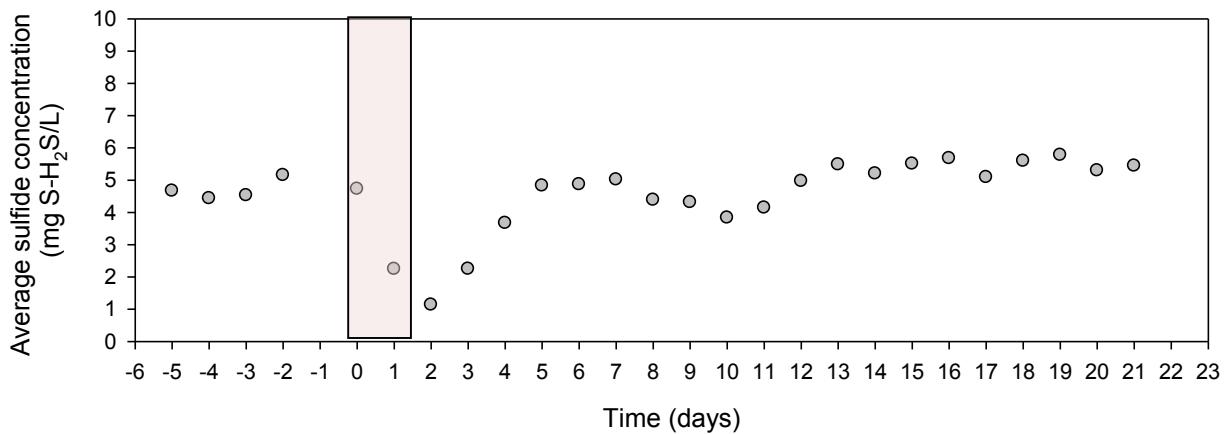


Figure 40. Daily average sulfide concentrations corresponding to the 1st FNA trial. Days -6 to 0: baseline period; Days 0 to 1: FNA solution dosing period; Days 2 to 21: recovery after stoppage of FNA dosing.

3. The injection of FNA solution has drastically reduced the methane production in the pipe (Fig. 41). The net production of methane during the baseline period was 15.7 mgCH₄-COD. After the FNA first dose trial, the methane concentration was only 1.8 mgCH₄-COD, which corresponded to 90% reduction when compared to the baseline period. Opposite to the SRB, the toxic impact to MA lasted for a longer period of time. The MA activity did not show signs of recovery for a period up to 30 days.



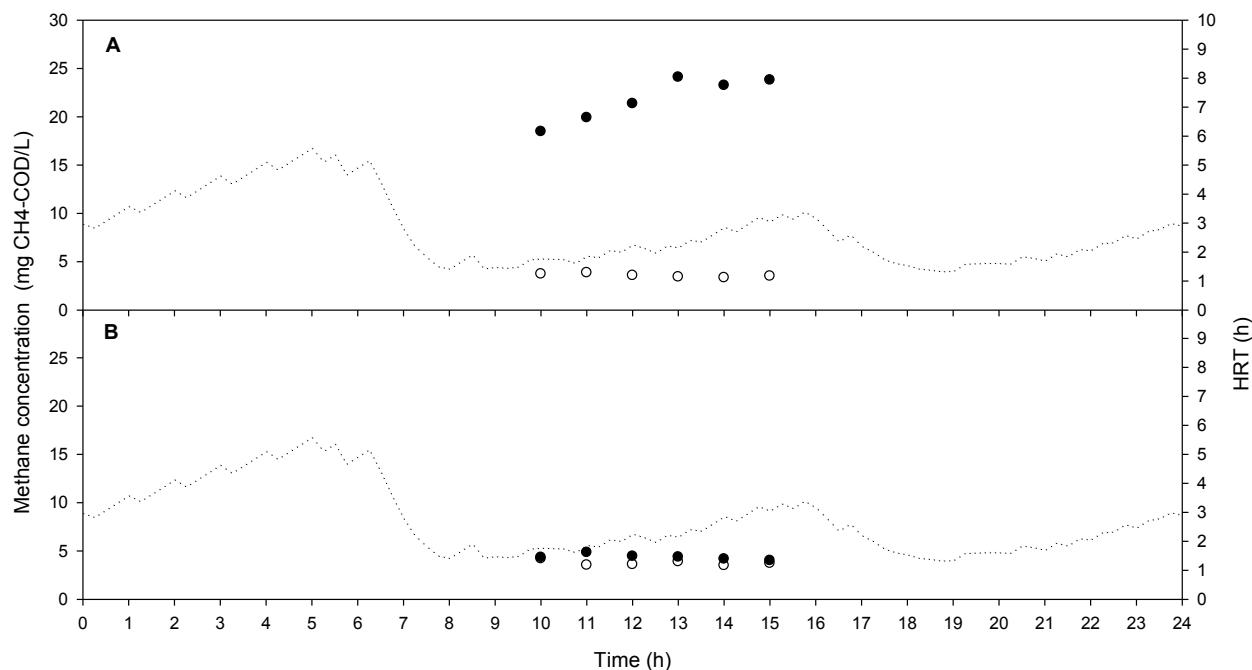


Figure 41. (○) Methane concentrations corresponding to the UC09 wetwell and (●) the UC09 828m downstream sampling point. (A): Baseline, 10 days prior to 1st nitrite solution addition; (B): 15 days after nitrite solution.

4. Methanogens activity was highly reduced by FNA first dose. Moreover the inhibition lasted in time. This supports prior lab observations where methanogens have been more sensitive to nitrite solution toxicity than SRB and need more time to recover. While the recovery of the MA activity was slow, as previously revealed by laboratory studies, in the first trial, SRB recovered much more quickly than that observed in the lab. This could be explained by the insufficient mixing conditions inside the pipe provided by the short manual pump events. The volume of water displaced during the manual pump events most likely mixed the initial sections of the pipe. That means that the FNA was not sufficiently diffused into the biofilms, reducing the expected inhibitory effect. Mixing conditions inside the pipe was then identified as a crucial parameter to obtain higher degree of inhibition.

4.7.4. RESULTS OF FREE NITROUS ACID DOSE TRIAL 2

In the second trial, the FNA dosing time was shifted from night period (8:00PM to 6:30PM) to day period (8:00AM to 7:00AM). Higher flow was expected during daytime, therefore more pump events and more periods of turbulence regime that would increase the mixing conditions inside the pipe. It was expected that the FNA would penetrate deeper in to the sewer biofilms, producing a higher toxic effect.

Some outcomes from the second field FNA dosage test are:

1. The results of 24h sulfide profiles obtained in the FNA second trials are presented in Figure 42. Complete sulfide control was achieved directly after dosing, with no sulfide concentrations detected in the pipe (Day +1). The SRB activity gradually increased afterwards but in much slower than in the first trial. Seven days after the solution dose addition, the sulfide production was around half of that in the baseline. It took up to 22 days to reach similar sulfide concentrations as prior to the FNA addition.



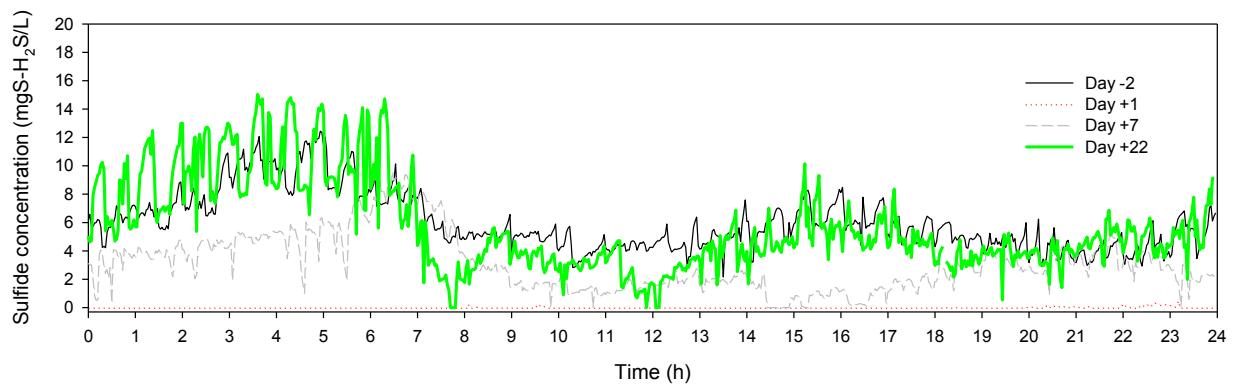


Figure 42. 24 hour online sulfide profiles corresponding to 2nd trial of FNA addition in the UC09. Sulfide concentrations corresponding to 2 days before addition and 1, 7 and 22 days after FNA addition.

2. The recovery of the sulfide production capacity in the UC09 after the 2nd dosing was irregular through time. After the expected initial drop to zero due to the FNA exposition, a period of 10 days of constant SRB recovery occurred (Fig. 43). At this point, sulfide concentrations stopped its increase and stabilized at 3 mgS-H₂S/L day for period of 5 days. Surprisingly the SRB activity experienced then a second drop to zero without any further FNA solution addition. Once SRB touched ground, the sulfidogenic activity steadily increased again until reach the baseline levels of 6 mgS-H₂S/L 20 days after the nitrite solution was halted.

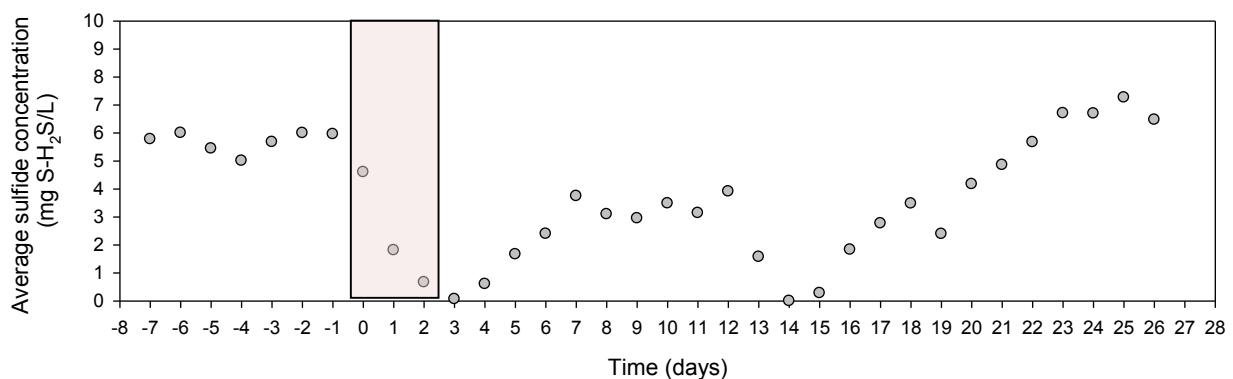


Figure 43. Daily average sulfide concentrations corresponding to 2nd FNA trial. Days -8 to 0: baseline period; Days 0 to 2: FNA dosing period; Days 3 to 26: recovery after stoppage of FNA dosing.

3. With regards to the methane production, the 2nd FNA addition confirmed the sensibility of methanogens to FNA. On day 36 after the 3 days addition, the MA activity was still reduced by 96% (Fig. 44). FNA dosing is confirmed as a very effective strategy to control methane emissions from rising main pipes.



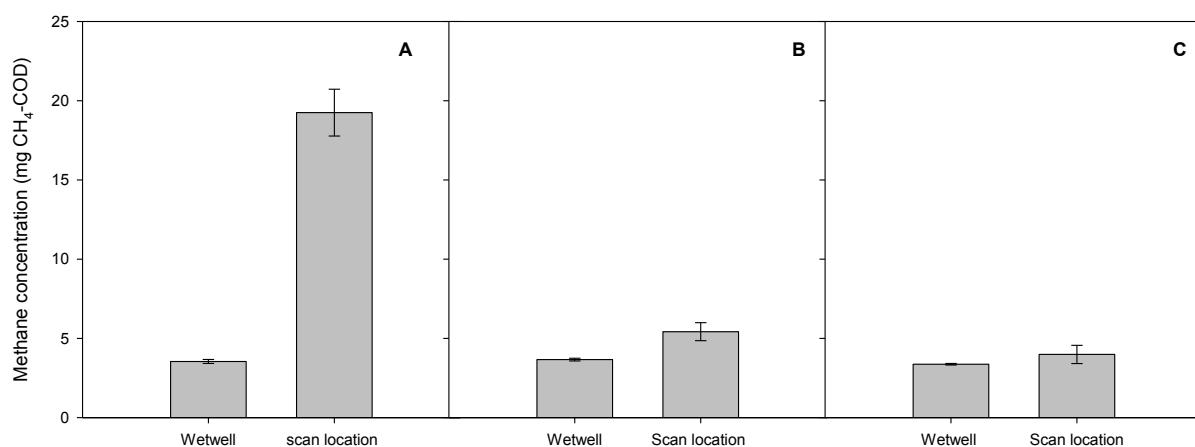


Figure 44. Average methane concentrations in the UC09 wetwell and 828m downstream location. A: Baseline period, before FNA addition; B: 33 days after 1st FNA dose trial; C: 36 days after 2nd FNA trial.

4. The dose of FNA during high flow periods substantially increased the inhibitory effect to SRB. The mixing conditions inside the pipe were crucial to enhance the toxic effect of FNA on sewer biofilms. For a period of 19 consecutive days, the dosing strategy applied was able to keep the sulfide concentrations levels below 50% of those of the baseline. The FNA toxic effect on methane control in field site conditions was very strong. A high degree of inhibition (>95%) was maintained for long periods of time (>35 days).

4.7.5. RESULTS OF FREE NITROUS ACID DOSE TRIAL 3

The third trial resulted in sulfidogenic activity in the UC09 inactivation for few days only. Figure 45 below presents the average sulfide concentration calculated from the online sulfide data recorded at 828m downstream of the rising main. As previously seen, the additions of nitrite solution produced an immediate reduction of the sulfide generated in the pipe. However the extent of the reduction was not lasting and recovery of sulfide concentrations was observed within the days after both additions. On day 8 after the first dosage, the sulfide average concentration was 90% similar to the average values recorded prior to the FNA dosage. In the third dosing trial, methane analysis was not performed. The reason is that the impact of the FNA dosing on methanogens was very conclusive as has been demonstrated in the previous trials.



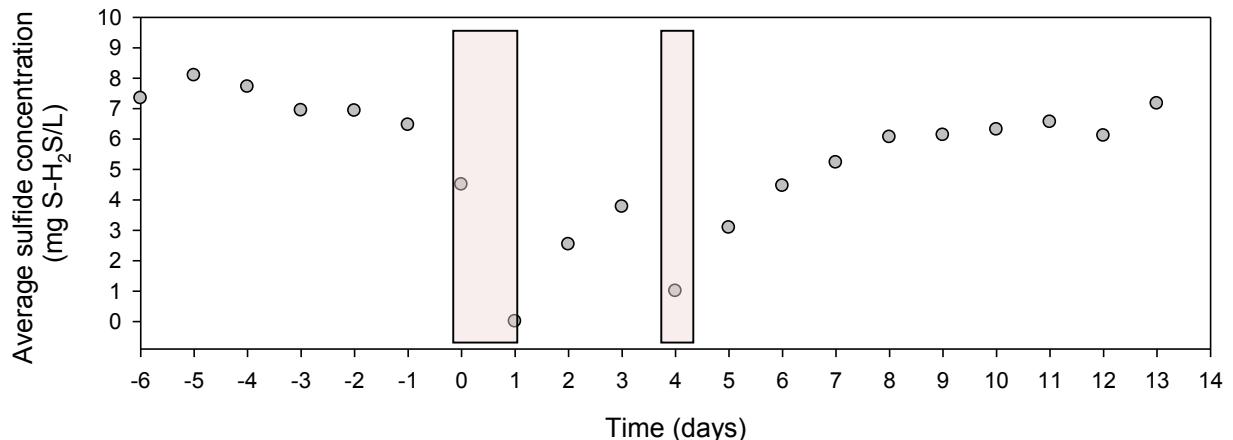


Figure 45. 24h average sulfide concentrations at the downstream section of the UC09 rising main. Dark sections indicate period of FNA dosage.

From the study, FNA addition has shown as a competitive option in controlling anaerobic sewer biofilms. The intermittent FNA addition has the potential to achieve long-term sulfide and methane control. The trials also allowed the identification of crucial parameters for the improvement of the FNA effectiveness (hydraulic regime, dosing rates, etc.). For example, automatic continuous dose for more days, would definitely help to extend inhibitory effect. Unfortunately that has not been possible to date due to practical and safety issues.

4.7.6. CONCLUSIONS

The FNA dosage field study clearly confirmed that FNA addition into a sewer system had a toxic effect on both SRB and MA as also observed in the laboratory studies. The obtained results to date showed that FNA addition can be a competitive option when controlling anaerobic sewer biofilms to field site sewers. The intermittent FNA addition has the potential to achieve long-term sulfide and methane control. However, there is always room for optimisation and make FNA addition more effective, such as by combining it with other chemicals (H_2O_2 , caustic, oxygen, etc.). The trials allowed the identification of crucial parameters for the improvement of the FNA effectiveness (hydraulic regime, dosing rates, etc.).

4.8. TESTING OF CAUSTIC SHOCK (EXTREME pH ELEVATION) AS A MEANS FOR SULFIDE CONTROL IN FIELDS SITE

4.8.1. INTERMITTENT DOSING OF HIGH pH SHOCK

Similar to the FNA field study, the intermittent dosing of high pH shock study aimed to verify the results obtained from the lab-scale studies in the field site. Three field trials have been carried out in the UC09. The addition of caustic was carried out manually due to practical and safety reasons. The main key finding revealed that with a very short exposure time, long-term inhibition on sulfate reduction and methane production can be achieved.

In general, the key results of the caustic shock field study showed that:

- The field trials confirm the significant suppression of anaerobic sewer biofilm activities due to the toxic effect caused by shock dosage of high pH. Inhibition of sulfate reduction in the field tests was somewhat lower than the lab-scale experiments. This



may possibly caused by different shear stress that leads to a diverse biofilm structures between the two systems.

- Intermittent dosage of high pH shock is possible to effectively control odour, corrosion and the release of greenhouse gas emission from the sewer systems. The benefit will include a short period of alkali dosing with a minimum consumption of chemicals and a long period of inhibition.

In the field study, pH of 10.5 with 2 hour exposure time was chosen to be initially tested based on its performance during the lab trials (Figure 28C). According to this figure, relatively similar performance was obtained when pH 10.5 was compared to the other pHs (11.0-12.5). The selection of pH and exposure time was also preferred with the consideration to minimize chemical usage which relates to the costs. The lab results also demonstrate that approximately 7 days were required to completely recover the biofilm activities. This means that in the real application, once to twice of caustic dosing is required to control the anaerobic biofilm activities in sewer systems. Taking into consideration that the lab reactors do not precisely mimic a real sewer, online sulfide measurement was conducted during dosing until recovery period was achieved.

Some other outcomes from the caustic shock field trial are:

1. The trials have suppressed the sulfide production in the UC09. Figure 46 presents the sulfide concentrations at 828m downstream location corresponding to the first trial. In the first test, the baseline sulfide average concentration, around 6 mgS-H₂S/L, was quickly reduced after the 2 hours addition of caustic shock, down to <1 mgS-H₂S/L (>85% inhibition). However, the inhibition only occurred for a short period of time (around 4.5 hours), thus the recovery of the SRB was much quicker than expected.
2. The second field trial was conducted to improve the first test by increasing the pH to 11.0 with the same exposure time of 2 hours. Nevertheless, quite similar results were obtained from the second test. In general, the results obtained from the first and second trials reveal that the 2 hours pH shock dosing was not effective enough to stop the anaerobic biofilm activities of the UC09 system for a long period of time. Lab studies show a more effective control to anaerobic biofilm activities (for the same pH and time expositions) compared to the field study. It is likely that the results were affected to the fact that a relatively more controlled experiment can be performed in the laboratory than in the field site.
3. The third trial was performed by increasing the pH shock to 11.5 with a longer exposure time of 6 hours. Figure 47 demonstrates the average sulfide concentrations corresponding to the third dose trial. The baseline sulfide average concentration, around 12 mgS-H₂S/L, was reduced after the 6 hours addition of caustic shock, down to around 3.5 mgS-H₂S/L. The inhibition took place for about one week. This confirmed a similar recovery process of the SRB to the results obtained in the laboratory test. Furthermore, pH data of all field trials (trial 1 to 3) showed that the pH level in the downstream area never reached the initial pH level as we dosed in the wet well. During trials 1 to 3, pH decreased when sewage was travelling along the 1km pipe (Figure 48). This may be caused by the production of proton in the system, which lower the pH and shorten the duration of pH peak.



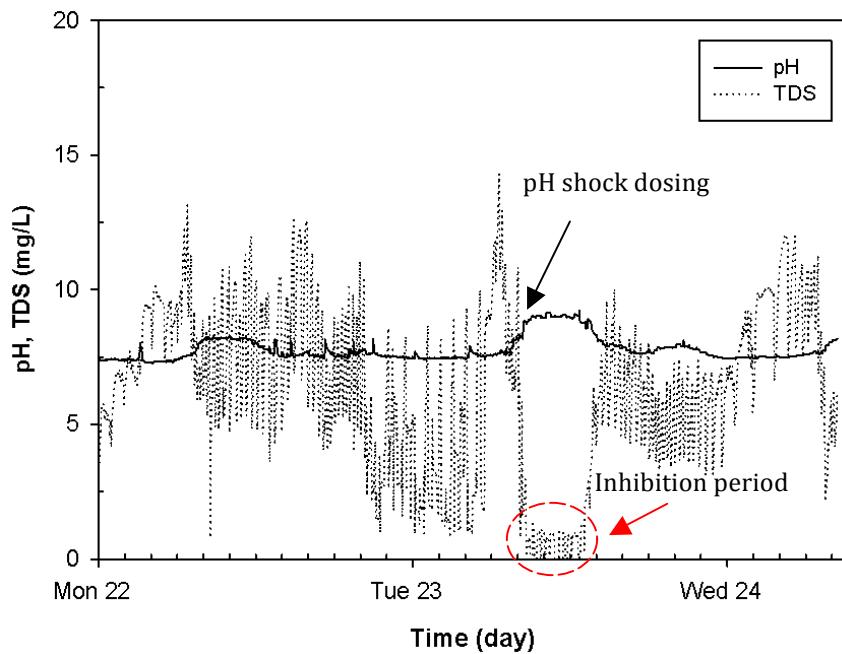


Figure 46. Online sulfide profiles of pH shock dose in the UC09 (828m downstream location) corresponding to the first trial

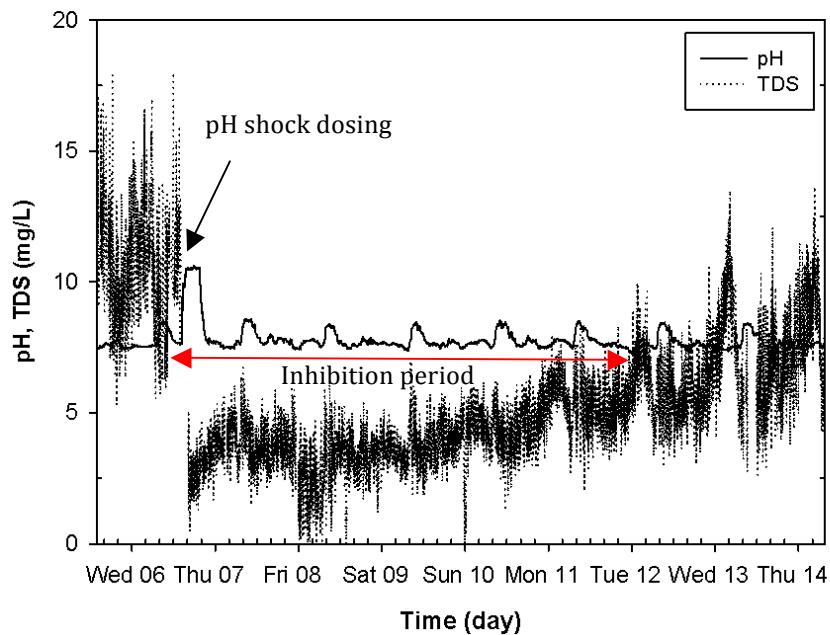


Figure 47. Online sulfide profiles of pH shock dose in the UC09 (828m downstream location) corresponding to the third trial



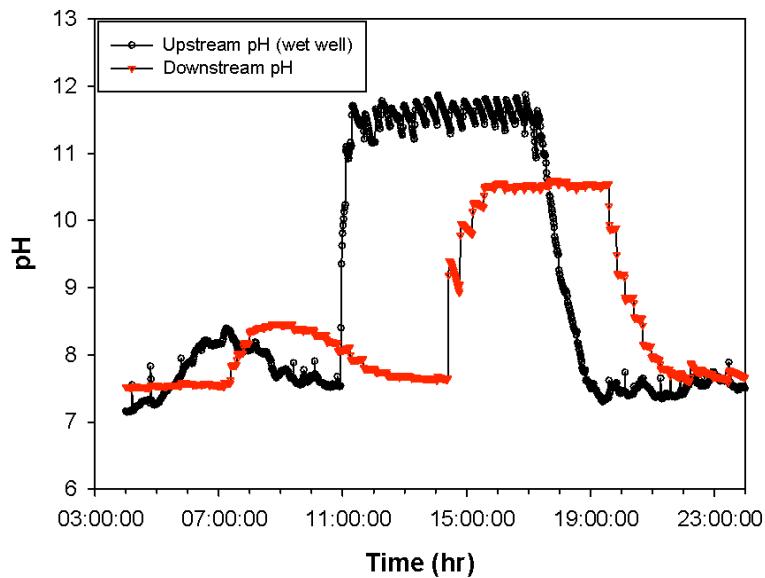


Figure 48. Online pH measurement during the third trial in both the upstream (UC09 Wet-well) and downstream locations (828m).

- Average methane concentration in the UC09 wet well and 828m downstream location before and after the third pH shock field trial is illustrated in Fig. 49A. The methane formation in the downstream location has been reduced by up to 97%. At the same time, solid concentrations in the downstream area have increased to 32% compared to prior dosing Figure 49B. The increase in solid concentration is mainly due to the detachment of solids (biofilms) from the pipe wall.

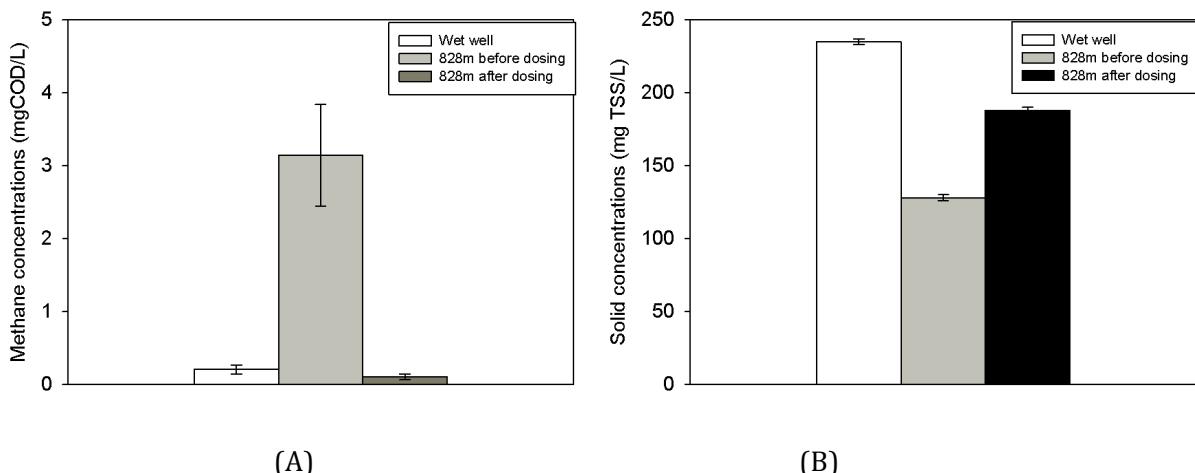


Figure 49. Average methane and solid concentrations in UC09 wet well and 828m downstream location as a result of the third caustic shock trial. (A) methane, (B) solids.

- An estimation of chemical cost for caustic shock dosing application was calculated. The cost estimation presented in this report is based on the third trial of caustic shock field test performed by the SOCRe-SP6. In this field trial, a pH level of 11.5 with the exposure time of 6 hours was applied. A total volume of 26.55 m³ sewage was treated by about 15.95 L of caustic (NaOH 50% w/w, priced at \$69.63/20L) during the third trial. Therefore, based on the data, an approximate price of \$2.09 is required to treat a cubic meter of sewage.



4.8.2. CONCLUSIONS

The strong toxic effect due to the high pH exposure on SRB and MA activities observed in the lab-scale studies was also proven in the caustic shock field dose trials. It is confirmed that high pH dosage has a long-term inhibitory effect on both sulfide and methane formations by anaerobic sewer biofilms. Therefore, it is concluded that the dosage of high pH is likely possible to be applied intermittently in sewer systems to control sulfide and methane productions.

The main difference between the lab-scale and field study was that longer control of sulfide production during laboratory experiment was achieved at pH levels of 10.5 and 11.5 for a short exposure time. This dissimilarity could have been caused by different shear stress in two systems that lead to the variation of biofilm structures. During pump event, a shear stress of about 0.3 Pa occurred in the lab-scale sewer reactor, while the shear stress for the UC09 system was estimated to be about 3.0 Pa. With a thinner structure, it was easier for the caustic to penetrate biofilms on the reactor wall causing stronger toxic effects on biomass residing inside. The reduction of boundary layer surrounding biofilms in real sewer systems may possibly happen due to the higher shear stress conditions leading to the resistance to mass transfer.

4.9. FERRIC DOSING INHIBITION CASE STUDY: BELLAMBI PRESSURE MAIN

Previously, the AWMC-UQ laboratory studies through the SCORe-SP6 Project have demonstrated the effectiveness of ferric dosing to control anaerobic biofilm activities in sewers. However, no systematic study has been done to investigate this in the field conditions. The collaborative research project between SCORe-SP6 and Sydney Water aimed to investigate and monitor the inhibitory effects of FeCl_3 dosing in the Sydney Water's Bellambi sewerage system. The study will involve a mass balance analysis of sulfate and iron concentrations in the system.

Sewage from Bellambi catchment drains to a pumping station (SPS 796). The sewage is then pumped to Wollongong STP through a rising main sewer (10 km long) with the HRT between 2 and 9 hours. At the SPS 796, ferric chloride is dosed to control sulfide.

Pumping events and HRT of Bellambi Pressure Main (a 24-hours period) on the sampling day (Sept 21st, 2011) is presented in Fig. 50. Sampling campaign was conducted on the 21st of September 2011 with the design based on the flow data as shown in Table 5. During the sampling campaign, a total of 16 (Sixteen) grab samples were collected from the Bellambi pressure main at certain locations and times. Sulfur species, volatile fatty acids (VFA), pH and Iron parameters were measured from the collected sample. The correlation between sulfate reduction and iron concentration will be observed.



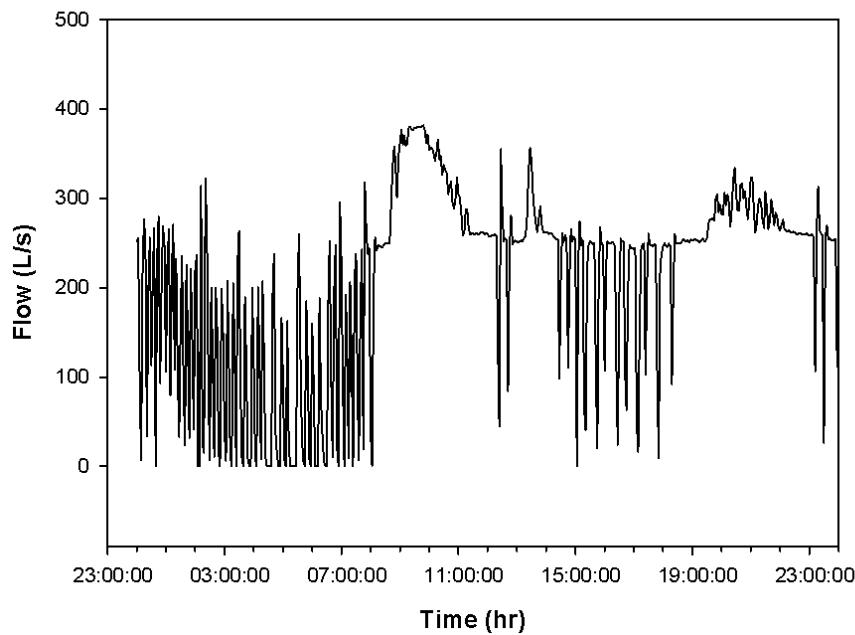


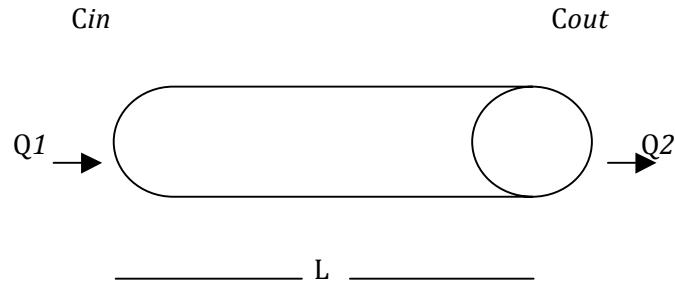
Fig. 50. Pumping events and HRT of Bellambi Pressure Main (a 24-hours period) on the sampling day (Sept 21st, 2011)

Table 5. Sampling collection designed

Sampling point	Chainage (m)	Diameter (mm)	Sampling time
SP0796 Wet Well	0	750	8:00:00
			9:00:00
			10:00:00
			11:00:00
Towradgi Ck (Lake PD)	2260	750	8:54:11
			9:45:42
			10:40:16
			11:47:39
Stuart Pk	6940	750	10:27:07
			11:07:37
			12:02:37
			13:27:06
Inlet to W'Gong STP	9894	750	11:39:24
			12:17:07
			13:25:19
			14:36:36



So far, the collected samples from the campaign have not fully compiled and analyzed. Once all the data is analyzed, a mass balance analysis will be calculated based on the following equations:



Chemical **consumption rates** for each pipe section

$$(Q1 \cdot Cin - Q2 \cdot Cout) = \frac{g}{day}$$

$$\frac{Q(Cin - Cout)}{2\pi r L} = \frac{g}{m^2 \cdot day}$$

The rates will then be identified to determine the inhibitory effects of FeCl₃ dosing in sewers. Due to the time constrain, the full analysis of this study will be carried out by the other sub project (SCORe-SP8).



5. FUTURE RECOMMENDATION STUDY

- FIELD OPTIMIZATION OF CAUSTIC SHOCK DOSING. The SCORe-SP6's field trials have demonstrated that caustic shock dosing has effectively repressed the reduction of sulfate and methane formation even with a short exposure time in both lab-scale and real systems. However, dosing optimization which includes the best combination of pH & exposure time in the field site has not been conducted. There is a need to find out the best dosing strategy (including the dosing frequency) before caustic is dosed in a real sewer system.
- FIELD APPLICATION OF SYNERGISTIC FNA & H₂O₂. A clear synergism between FNA and H₂O₂ in inactivating microorganisms residing in sewer biofilms has been demonstrated in one of the additional task of the project. The combination could be effectively used due to its high killing efficiency. This may also lead to the significant reduction of operating costs. However, due to the time and budget limitation, the field trial of the combined dosing is not yet performed. The field application is desired to confirm the findings from the lab-scale study.
- LIFE CYCLE ASSESSMENT (LCA). The potential of integrated management of sulfide and phosphate-related problems in sewer networks, wastewater treatment processes and anaerobic digesters has been highlighted in this project, which can substantially reduce the chemical consumption and costs and operating expenses. Nevertheless, a fair and holistic assessment at all the stages is required to avoid a narrow outlook on environmental concerns.



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