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Evaluation of oxygen injection as a means of controlling sulfide production in a sewer system

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ABSTRACT

Oxygen injection is often used to control biogenic production of hydrogen sulfide in sewers. Experiments were carried out on a laboratory system mimicking a rising main to investigate the impact of oxygen injection on anaerobic sewer biofilm activities. Oxygen injection (15–25 mg O_2/L per pump event) to the inlet of the system decreased the overall sulfide discharge levels by 65%. Oxygen was an effective chemical and biological oxidant of sulfide but did not cause a cessation in sulfide production, which continued in the deeper layers of the biofilm irrespective of the oxygen concentration in the bulk. Sulfide accumulation resumed instantaneously on depletion of the oxygen. Oxygen did not exhibit any toxic effect on sulfate reducing bacteria (SRB) in the biofilm. It further stimulated SRB growth and increased SRB activity in downstream biofilms due to increased availability of sulfate at these locations as the result of oxic conditions upstream. The oxygen uptake rate of the system increased with repeated exposure to oxygen, with concomitant consumption of organic carbon in the wastewater. These results suggest that optimization of oxygen injection is necessary for maximum effectiveness in controlling sulfide concentrations in sewers.

1. Introduction

Sewers have long been recognized as sites of biological and chemical transformations (Thistlethwayte, 1972; Hvitved-Jacobsen, 2002a; Zhang et al., 2008). One biotransformation with serious implications for wastewater authorities is the reduction of oxidized sulfur species, leading to the formation and release of hydrogen sulfide gas. The bacteria responsible for sulfide generation, the sulfate reducing bacteria (SRB), are located in biofilms of anaerobic sewer sections. The availability of sulfate and organic substrates in domestic sewage provides SRB with optimal conditions for growth and respiration (Hvitved-Jacobsen, 2002a). Emission of sulfide from the liquid to the gas phase in subsequent gravity sewers results in corrosion and damage to sewer infrastructure and is also the cause of odour and health problems (US EPA, 1974).

A number of operational strategies have been employed to control sulfide production in sewer networks (Boon, 1995; Boon

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et al., 1998). These generally involve the prevention of anaerobic conditions by the addition of oxidants (oxygen, nitrate, nitrite), the precipitation of formed sulfide with iron salts, and/or the elimination of SRB populations with biocides (chlorine, hydrogen peroxide, ozone). Deciding which treatment to use is far from straightforward. Oxygen injection is often an attractive option as it is relatively inexpensive and targets rising mains, where SRB activity is highest (Hvitved-Jacobsen, 2002a). Oxygen is thought to both inhibit SRB activity (Marschall et al., 1993) and oxidize the sulfide that has already formed (O'Brien and Birkner, 1977; Nielsen et al., 2003). However, various operational constraints could lower the effectiveness of oxygen injection in practice, and a full assessment of this strategy with detailed investigations of the impact of oxygen injection on anaerobic biofilm activities is still lacking.

Several major challenges need to be addressed to successfully implement oxygen injection as a sulfide control strategy. Firstly, to maximize its transfer through rising main pipes, oxygen can only be injected when wastewater pumps are running. The solubility of pure oxygen in water is limited to 45–50 mg O_2/L in pressure mains (Hvitved-Jacobsen, 2002a), and therefore oxic conditions may not be maintained continually during periods of infrequent pump operation. Secondly, research has shown that certain SRB can tolerate high levels of oxygen by either (i) detoxifying reactive oxygen species like hydrogen peroxide and superoxide, (ii) positioning themselves in anoxic microniches where their activity can continue unhindered, and/or (iii) utilizing oxygen as a terminal electron acceptor for carbon or reduced sulfur metabolism (Coulter and Kurtz, 2001; Dolla et al., 2006; Hardy and Hamilton, 1981; Marschall et al., 1993; Santegoeds et al., 1998; Sass et al., 1998). Some SRB species are also able to continue sulfate reduction in oxic environments, and to oxidize sulfide aerobically (Canfield and Des Marais, 1991; Fuseler et al., 1996), although no clear evidence exists for the aerobic growth of SRB under these conditions. These investigations were carried out on a wide range of SRB, from pure cultures to aerobic wastewater biofilm, marine sediments, and hypersaline microbial mats, and illustrate the metabolic adaptability of SRB in response to oxygen. Similar studies testing the effects of oxygen on SRB activity in rising main sewer biofilm are needed. Lastly, oxygen injection to sewers can negatively impact on downstream biological nutrient removal due to the consumption of valuable and often limiting volatile fatty acids (VFA) (Hvitved-Jacobsen et al., 1995), and this ideally should be minimised.

The aim of this study is therefore to comprehensively evaluate the effectiveness of oxygen injection on sulfide production in a simulated rising main sewer. The impacts of oxygen injection on anaerobic sewer biofilm structure and activities are investigated using online performance monitoring, batch tests, microsensor measurements and molecular microbial tools.

2. Material and methods

2.1. *Laboratory system design and operation*

Difficulties associated in working directly with real rising main sewers (low accessibility) were overcome in this study by the installation of a laboratory reactor system, designed to simulate a real rising main in terms of operation and performance. The laboratory reactor system consisted of two parallel lines, control and experimental, each with four completely sealed mixed reactors (named rising main control 1–4; RMC1–4, and rising main experimental 1–4, RME1–4, respectively) connected in series (Fig. 1). Each reactor had a volume of 0.75 L and an inner diameter of 80 mm. The reactors were made of Perspex™, an acrylic polyvinyl chloride sheet widely used due to its long-term durability. The reactors were completely covered with aluminium foil to avoid exposing the sewage and biofilm to light. Each reactor lid was equipped with a small container with a volume of 70 mL. This container was also filled with the same wastewater as in the reactors, so as to prevent any vacuum and oxygen entry during wastewater displacement. Plastic Kaldnes carriers (circular, 1 cm diameter; Anox Kaldnes, Norway) were placed on rods inside the reactors in order to obtain easily extractable biofilm samples for further detailed analyses.

The system was intermittently fed with sewage through a peristaltic pump (Masterflex model 7520-47) following a typical pattern observed at the UC09 rising main (Guisasola et al., 2008). The system was exposed to 16 sewage pump cycles on a daily basis and a diurnal variation of sewage hydraulic retention time (HRT) was maintained, with minimum and maximum HRTs of 2–10.5 h, respectively. The system was maintained under quiescent conditions except during pumping events when the wastewater was mixed, similar to that in a real rising main. To simulate this effect sufficiently, the reactors were additionally stirred by magnetic stirrers during pumping events. Fresh domestic wastewater was collected weekly from a local wet well in Brisbane, Australia and transported to the laboratory immediately. The sewage typically contained sulfide concentrations of <3 mg S/L, sulfate concentrations between 10 and 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). The analytical methods used for the measurement of sulfur and VFA species are described in Section 2.6. pH values of 7.6 ± 0.1 were measured in the inlet feed. The sewage was stored at 4 °C to minimize biological transformations, and heated to 20 °C before it was pumped into the laboratory sewers.

The system was operated for several months to establish pseudo steady-state conditions and to develop mature anaerobic biofilm on the walls of the reactor and the carriers. Despite having a different biofilm surface area to volume ratio (57 m^2/m^3) when compared to UC09 (26 m^2/m^3), the simulated laboratory rising main achieved similar sulfide production per area as the real rising main (approximately 2.0 g S/m^2 day). The trends in sulfide concentrations over HRT of both systems are presented in Guisasola et al. (2008) and Sharma et al. (2008a). Oxygen injection was then started in the experimental line for 120 days. An oxygenation chamber (1.5 L) was set up before RME1 (Fig. 1), where fresh wastewater was pumped and pure oxygen was injected into the wastewater. Oxygenation occurred for 2 min before every pump event. The wastewater was allowed to settle for 1 min to release any oxygen bubbles present before it was injected into RME1.

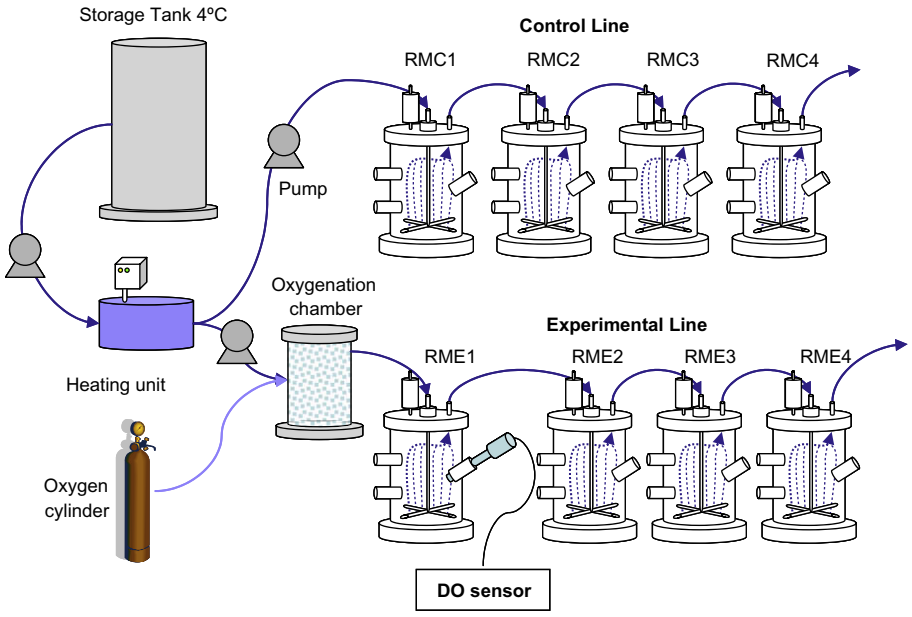

Fig. 1 – Schematic of the laboratory reactor system.

Initial oxygen concentrations of 15–25 mg O_2/L were measured in RME1 after every pump cycle with fresh sewage. This range of oxygen concentration was chosen based on the experience of wastewater authorities like Gold Coast Water (personal communication), who observed that oxygen injectors in the field routinely work at <40% of their capability, and hence a much lower dose than the theoretical 40–50 mg O_2/L was achieved in reality.

The operation of the reactor system is divided into three periods: Period 1: pre-oxygen injection/baseline (Days −90 to 0), Period 2: oxygen injection to the experimental line (Days 0–120), and Period 3: post-oxygen injection/recovery of the experimental line (Days 120–200). Day 0 represents the start of oxygen injection in the experimental line. No changes were made to the routine operation of the control line in Periods 1–3. Detailed tests were done in all periods to determine the impacts of oxygen on biofilm activities and composition. All tests were carried out in a temperature-controlled lab (20 ± 1 °C).

2.2. *Monitoring of system*

The effectiveness of oxygen injection on sulfide concentrations was determined by monitoring changes in wastewater characteristics as it travelled through the control and experimental lines in Period 2. Wastewater slugs were captured at the inlet and the outlets of the control (RMC4) and experimental (RME4) lines over a wide range of HRT and analyzed for dissolved sulfur species, VFA, and soluble and total chemical oxygen demand (COD) using the analytical techniques described below. The HRT of a wastewater slug was defined as the time the sample had spent in the reactor system at the time of sampling and was calculated from the operational data of the pump. The changes in wastewater dissolved oxygen (DO) levels as it travelled through the experimental line were recorded using DO probes in the reactors.

2.3. *Aerobic biochemical transformations: batch tests in RME1*

The biochemical sulfur and carbon transformations in the presence of oxygen in RME1 were quantified by measuring changes in the wastewater composition over time during a number of batch tests. The experiments were started immediately after fresh, oxygenated wastewater was pumped into the reactor. The continuous operation of the system was temporarily halted for the duration of each batch test. The reactor was kept stirred at 240 rpm and liquid samples were drawn every 20 min for the first 2 h and hourly thereafter through a sampling port fitted with Tygon® tubing 06419-16 into a 5 mL syringe. The samples were immediately filtered using 0.22 μm filters (Millipore, Millex GP) into vials containing sulfide anti-oxidant buffer (SAOB) or vials without buffer and analyzed for dissolved sulfur species and VFA, respectively, using methods described in Section 2.6. During sampling, the volume of liquid removed from the reactor was replaced by sewage of equivalent HRT present in the storage container on top of the reactor lid. This avoided any vacuum and re-aeration issues. Dilution caused by the volume displacement per sample was approximately 0.67% of the total reactor volume and thus assumed negligible.

DO values in the reactor were recorded using a DO probe. Rates were calculated using linear regression of the data points obtained. Eleven tests were conducted in Period 2. These tests were also conducted in Periods 1 (four tests) and 3 (eight tests), where oxygen was supplied to the reactor only during the test.

2.4. *Anaerobic biochemical transformations: batch tests in RME1–4, RMC1–4*

Batch tests were conducted in all the reactors in both lines to observe the impact of oxygen injection on the anaerobic

biofilm activities. The reactors were filled with (non-oxygenated) fresh sewage. Liquid samples were then drawn at regular intervals for 3 h under stirring conditions (240 rpm) and analyzed for dissolved sulfur species and VFA. Sulfide production rates were calculated by linear regression. Three tests were carried out in Period 1, 11 tests in Period 2 and seven tests in Period 3.

2.5. Chemical and biological aerobic sulfide oxidation

Chemical sulfide oxidation with oxygen was tested in a biofilm-free reactor with 0.22 μm filtered wastewater that was aerated to produce a high DO concentration (20 mg O_2/L). Aeration was then stopped and sulfide was added to produce a concentration of 15 mg S/L. Liquid samples were drawn at regular intervals for 40 min and analyzed for dissolved sulfur species. In Period 2, the combined processes of chemical and biological sulfide oxidation with oxygen were tested in the RME1 biofilm reactor, which was exposed to fresh wastewater and similar DO and sulfide levels as the biofilm-free reactor and also sampled in a similar manner. Both the reactors were continuously stirred at 240 rpm. Sulfide oxidation and DO consumption rates were calculated by linear regression.

2.6. Analytical methods

Filtered samples were injected into vials (with or without preservative) and stored in ice till the entire batch test was completed. Samples were then taken directly to the analytical lab for immediate analysis. The time between sampling and chemical analysis thus varied between 0.5 and 8 h. Liquid samples for analyses of dissolved sulfur species (SO_4^{2-}, HS^-, $S_2O_3^-$ and SO_3^{2-}) were measured using a compact Dionex ICS-2000 ion chromatograph with an AD25 absorbance (230 nm) and a DS6 heated conductivity detector (35 °C) in series. Preceding the conductivity detector a Dionex ASRS-ULTRA II 4-mm suppressor (131 mA) was attached. Samples were injected with a Dionex AS50 autosampler. Liquid samples were filtered through a 0.22 μm pore diameter unit and stored in 2 mL IC Dionex sample vials PTFE/silicon septa (No. 055427) with 0.5 mL of SAOB solution. Extreme care was taken to avoid contact with air during sampling.

SAOB was made up as follows: 0.8 g NaOH and 0.7 g ascorbic acid were dissolved in a 250 mL volumetric flask with helium sparged and filtered distilled, deionised water. SAOB solution prevented oxidation of sulfur species in the liquid sample and the release of sulfide to the gas phase. Independent studies comparing stability of sulfur species with and without SAOB solution conclusively demonstrated that the use of SAOB solution allowed storing the samples for at least 4 days at 4 °C without any appreciable deterioration in the concentration of significant measured sulfur species deterioration (Keller-Lehmann et al., 2006). This was therefore used as a preservation method for the entire study.

VFA were measured by Perkin–Elmer gas chromatography with column DB-FFAP 15 m × 0.53 mm × 1.0 μm (length × ID × film) at 140 °C, while the injector and FID detector were operated at 220 and 250 °C, respectively. High purity helium was used as carrier gas at a flow rate of 17 mL/min. Filtered sample (0.9 mL) was transferred into a GC vial to which 0.1 mL of formic acid was added. For the analysis, a volume of 1 μL of sample was injected in splitless mode. The VFA species analyzed included acetic, propionic, butyric, isobutyric, valeric, isovaleric and hexanoic acids. The measured values were converted to COD using the theoretical chemical oxygen demands of these chemicals. Soluble and total COD were determined following the closed reflux colorimetric method 5220D of standard methods (APHA, 1998) using a spectrophotometer Merck CSB spectroquant model SQ300 (25–1000 mg COD/L range). Soluble COD was measured in 0.22 μm pore filtered samples. Oxygen measurements were made using DO probes (YSI InPro 6050 connected to a 4100 Mettler Toledo O_2 transmitter). pH was measured with a TPS minichem-pH controller (Version 2.1.1).

2.7. Microelectrode measurements: RME1

Biofilm carriers were removed from the reactor rods in parallel with the batch experiments using tweezers and gently placed into a container previously filled with wastewater from the same reactor that it was taken from. Care was taken not to touch the surface of the biofilm where the measurements were eventually made. The carrier was then mounted in a PVC self-made flow cell (Fig. i, Supplementary material), containing 0.22 μm filtered wastewater mixed with 300 mM phosphate buffer in the ratio of 7:1 to ensure a stable pH of 7.0–7.5. The wastewater was filtered weekly from fresh wastewater, stored at −20 °C and thawed to room temperature before the start of every experiment. The wastewater was bubbled with nitrogen gas (99.99% of purity) during experiments investigating anaerobic sulfide production, or with a mixture of air and nitrogen for investigation of sulfide production under aerobic conditions. In the latter case, a biofilm carrier was exposed in separate experiments over the course of 2 days, to oxygen concentrations of 0.6, 0.9, 3.1 and 6.5 mg O_2/L. The initial wastewater concentrations of sulfate (13 mg S/L) and VFA (30 mg COD/L) in these experiments were similar, but decreased over the course of each experiment, and in the experiment with the highest oxygen concentrations (8.3 mg O_2/L), the VFA dropped to <1.1 mg COD/L. Microsensors were mounted on micromanipulators and positioned on the surface of the biofilm using a dissection microscope. Profiles were recorded by penetrating the biofilm in increments of 100 μm. The thickness of the biofilm was roughly estimated using an eyepiece micrometer and the depth of penetration adjusted accordingly. Three to six steady-state replicate profiles were obtained per experiment.

Microsensors for oxygen were constructed according to Revsbech (1989) and sensors for hydrogen sulfide (Kuhl et al., 1998) and pH (Revsbech and Jorgensen, 1986) were purchased from Unisense A/S (Denmark). The tip diameters of all sensors were between 10 and 15 μm. Standard calibration of the sensors was done before every experiment (Mohanakrishnan, 2008; Revsbech, 1989; Revsbech and Jorgensen, 1986). Total sulfide profiles were calculated from the hydrogen sulfide and pH profiles as described in Kuhl et al. (1998). Sulfide production zones within the biofilm were calculated using the diffusion reaction model developed by Berg et al. (1998). Diffusion coefficients used for total sulfide and oxygen in the biofilm were 1.39×10^{-5} (Kuhl and Jorgensen, 1992) and

2.12×10^{-5} cm^2/s (Broecker and Peng, 1974), respectively, at 20 °C. Adjustments were made to the diffusion coefficients depending on the liquid temperature. The porosity was assumed to be close to unity and constant with depth in the biofilm.

2.8. Biofilm composition, structure and physical characteristics

2.8.1. Denaturing gradient gel electrophoresis (DGGE)

The changes in microbial community composition in RME1 biofilm as a result of continuous oxygen injection were monitored using DGGE. DNA was extracted from biofilm on Day −2 (Period 1), Days 29, 71, 110 (Period 2) and Day 157 (Period 3). DNA extraction and polymerase chain reaction (PCR) amplification of the 16S gene were performed as described in Mohanakrishnan (2008). In PCR amplification of the *dsr* gene, a nested approach was used, where the *dsrAB* gene was initially amplified using the forward primer DSR1Fmix (DSR1F, DSR1Fa and DSR1Fb in equimolar concentrations) and the reverse primer DSR4R mix (DSR4R, DSR4a, DSR4b and DSR4c in equimolar concentrations) (Loy et al., 2004; Wagner et al., 1998). A mixture of the primer variants ensured maximum coverage of *dsrAB* gene fragments. Each 50 μl reaction tube contained 25 μl HotStar Taq MasterMix (Qiagen, Inc.), 0.5 μl (or 2 μM) forward primer, 0.5 μl (or 2 μM) reverse primer, 1 μl (or 0.016 μg/μl) bovine serum albumin, 22 μl dH_2O and 1 μl (or approximately 8.5 ng/μl) DNA template. DNA concentrations were measured using the nanodrop (Model ND-1000). Thermal cycling was carried out by using an initial denaturation step of 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 52 °C for 40 s, and elongation at 72 °C for 1 min 30 s. A final elongation step at 72 °C for 10 min completed the PCR. No visually obvious bands other than that of the desired PCR product appeared on the agarose gel. Further, we also checked different DGGE banding patterns obtained from the same sample (i) without first round purification and (ii) with first round purification. This did not differ; hence purification of the first round PCR product was not carried out in the remaining samples.

The amplified *dsrAB* gene was used as the template for a subsequent PCR reaction, in which the *dsrB* gene was amplified using the forward primer 2060F-GC and the reverse primer DSR4R (Loy et al., 2004; Wagner et al., 1998) and otherwise similar reaction mix as above. Thermal cycling consisted of an initial denaturation step of 94 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s, and elongation at 72 °C for 1 min. The final elongation step was done at 72 °C for 30 min.

Positive (*Desulfotomaculum* clone) and negative (no template) controls were included in all reactions, and sizes of the PCR products were verified on a 1.5% agarose gel. Samples were then run on an 8% acrylamide gel with a denaturing gradient of 30–70% using a procedure described in Mohanakrishnan (2008). The DGGE marker used consisted of amplified *amoA* gene products from *Nitrosomonas europaea* and *Nitrospira multiformis* isolates, and four other environmental clones (Nicolaisen and Ramsing, 2002). Selected 16S and *dsr* bands were excised from the gels and sequenced (Macrogen, Inc.). The partial sequences were submitted to GenBank (Acc. Nos. EU440737–EU440746 (16S) and EU426863–EU426874 (*dsr*)) and similarity analysis was done using the BLASTN Search program (http://www.ncbi.nlm.nih.gov/BLAST). Alignment of 16S-DGGE partial sequences and phylogenetic analysis was done using the ARB software (Ludwig et al., 2004). Closely related sequences identified from the BLAST searches were added to the database. Phylogenetic trees were created using the maximum-likelihood algorithm (AxML) and bootstrap analysis was done using maximum parsimony with 1000 replicates.

2.8.2. Scanning electron microscopy

The surface of RME1 biofilm was analyzed using scanning electron microscopy in Periods 1 and 2. The biofilm carrier was pre-fixed in 2.5% glutaraldehyde and 75 mM lysine in 0.1 M cacodylate buffer for 11 min, and vacuum fixed in a 3% glutaraldehyde in 0.1 M cacodylate buffer at 150 W. Post fixation consisted of vacuum treatment with 1% osmium tetroxide in 0.1 M cacodylate buffer at 80 W and dehydration of the sample using an increasing gradient of ethanol washes from 50 to 100%. The sample was also dried with hexamethyldisilazane to preserve biofilm formation. Processed samples were mounted on carbon tabs and sputter coated with platinum. The samples were viewed using a JEOL 6300F scanning electron microscope operated at 5–10 kV.

2.8.3. Solids analysis

Biofilm samples over the course of the continuous oxygen injection study were analyzed for solids. The biofilm attached to the carrier was suspended in filtered wastewater by rapid shaking at >900 rpm in an orbital mixer (Ratek Instruments Pty. Ltd., Australia) till the biofilm had visually detached from the carrier (approximately 4 min). It was then analyzed for volatile suspended solid content as per Standard Methods 2540D (APHA, 1998). To validate the method, the amount of biomass retained on the carrier by the shaking process was estimated by comparing the COD of clean carriers ($n = 3$), carriers with the biofilm removed ($n = 3$) and carriers with biofilm attached ($n = 8$) using a standard photometric test kit with commercially available reagents (CSB Spectroquant, Merck). Absorbance readings were obtained using Method 237 on the SQ118 spectrophotometer (Merck, Inc.). The samples of carriers with biofilm were diluted five times in MilliQ water to be within the selected analysis range of 100–1500 mg COD/L. Less than 3.5% of the biomass was retained on the carrier (Fig. ii, Supplementary material), and this technique of detaching biofilm from the carrier was therefore deemed acceptable.

3. Results and discussion

3.1. Effectiveness of oxygen on sulfide discharge levels

The effectiveness of oxygen injection on sulfide discharge was evaluated by comparing the concentration of sulfide in the outlets of the control and experimental lines. A clear correlation ($R^2 > 0.94$) was observed between sulfide concentrations and HRT of the discharged wastewater (Fig. 2), and higher

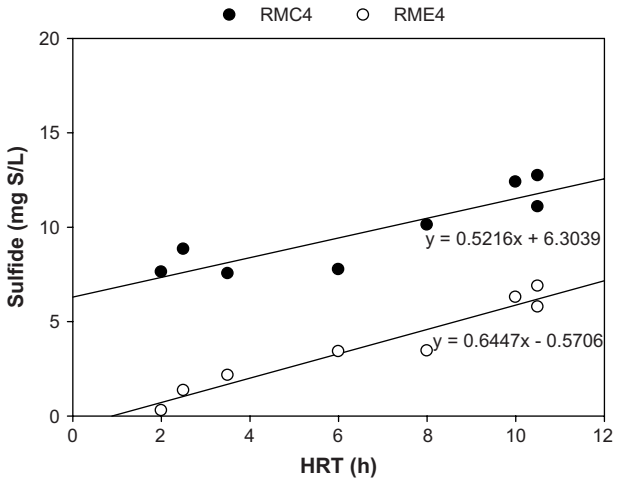

Fig. 2 – Sulfide concentrations at the outlets of RMC4 and RME4 as a function of HRT. Linear regression lines and corresponding equations are presented.

sulfide concentrations were observed at higher HRTs. Correlation between HRT and wastewater sulfide concentrations has also been demonstrated in field studies on rising mains (Sharma et al., 2008a). Our lab studies revealed a similar dependency even in the presence of oxygen injection.

The sulfide concentration in wastewater discharged from the experimental line was lower than the control line at all HRT (Fig. 2), but there was no significant difference in the rate of net increase in sulfide (the difference of production and loss due to consumption and oxidation) with HRT. This suggests that oxygen did not impact on the sulfide production capability of the biofilm and was only effective in delaying sulfide accumulation in the wastewater by the period of time where oxic conditions persisted. During the periods of frequent pumping (HRT = 2 h), the sulfide concentration in the effluent from the experimental line was reduced by 95%. However, in periods of less frequent pumping (HRT > 5 h) this figure was only 50%. Fig. 3 shows that oxygen levels decreased as the wastewater travelled through the reactor system and oxygen only reached RME4 during periods of frequent pumping (7 am–9 am and 7 pm–9 pm). During longer HRT, the oxygen supplied was not sufficient to maintain oxic conditions for the entire journey of the wastewater through the reactor system. Consequently, sulfide accumulated before the wastewater was discharged, and the effectiveness of oxygen injection was reduced.

The daily variation in the sulfide concentrations of discharged wastewater in both lines is shown in Fig. 4, together with the estimated concentrations calculated from the HRT using the correlation obtained from Fig. 2. According to these estimations, 53 mg S/day dissolved sulfide was discharged from the experimental line while 151 mg S/day was discharged from the control line. Oxygen injection to the inlet of the experimental reactor system thus resulted in 65% reduction in the amount of sulfide discharged. The effectiveness obtained when injecting oxygen depended on the HRT of the wastewater which is controlled by the pumping pattern. Oxygen injection is therefore likely to be less effective in rising mains with high retention of wastewater, or on days with fewer pump cycles due to low flow. Sulfide accumulation on depletion of oxygen further reflects the need, with the current method of dosing, to maintain oxic conditions at all times in all parts of the system to eliminate sulfide build-up.

Oxygen injection also affected the concentrations of carbon in the wastewater (Table 1). The soluble and total COD in wastewater discharged from the experimental line were approximately 10–15% lower than in the control line. The increase in carbon consumption in response to oxygen injection is likely due to aerobic bacterial respiration. Aerobic COD removal has been reviewed in gravity sewers (Hvitved-Jacobsen, 2002b). The processes responsible for aerobic COD transformation in oxygenated rising mains are likely to be similar to that in aerobic gravity sewers. A negative consequence of increased carbon consumption in oxygenated rising mains is the possible hindrance of denitrification and phosphorus removal in downstream wastewater treatment plants due to lack of readily biodegradable substrates (Hvitved-Jacobsen et al., 1995). Some carbon consumption was also observed in the control line and is attributed to the combined effects of sulfate reduction and methane production (Guisasola et al., 2008) recently reported in rising main sewers.

3.2. *Impact of oxygen on biofilm activities*

Batch tests investigating the activities of biofilm in RME1 showed that the biochemical transformations in the wastewater were very different in the presence and absence of oxygen (Fig. 5A). After injection of oxygen, sulfide was rapidly oxidized to thiosulfate and sulfate, and sulfide did not accumulate as long as oxygen was present in the bulk. The total dissolved sulfur concentration (sum of sulfide, sulfite, sulfate and thiosulfate) decreased in the presence of oxygen, and this was likely due to the formation of elemental sulfur or similar intermediates during the sulfide oxidation process (Nielsen et al., 2003), which were not directly measurable with the methods applied. Rapid oxygen consumption was observed and the VFA concentration decreased substantially under oxic conditions as expected from aerobic bacterial respiration. When anaerobic conditions resumed after 45 min, the sulfide concentration increased immediately, indicating that oxygen exposure had no lasting inhibitory effect on SRB activity. This observation supports the conclusions made from Figs. 2 and 3. The total dissolved sulfur level increased in this period, indicating that the intermediate compounds, accumulated in the biofilm under oxic conditions, were now being reduced to sulfide. These trends in the wastewater characteristics in the presence of oxygen were consistent in all the batch tests.

Fig. 5B presents the sulfate reduction capability of the biofilm, measured in RME1 with fresh sewage and under anaerobic conditions. Oxygen injection did not affect the capability of the biofilm to transform sulfate, even after 120 days of oxygen exposure. Diffusive fluxes of sulfide out of the biofilm, calculated from microsensor profiles, revealed similar trends and confirmed the findings of these batch tests (Fig. iii, Supplementary material). Reasons for the lack of SRB inhibition and toxicity by oxygen in sewer biofilm were studied using microsensor experiments on biofilm from the experimental line (Fig. 6). Since the activity of the biofilm in the

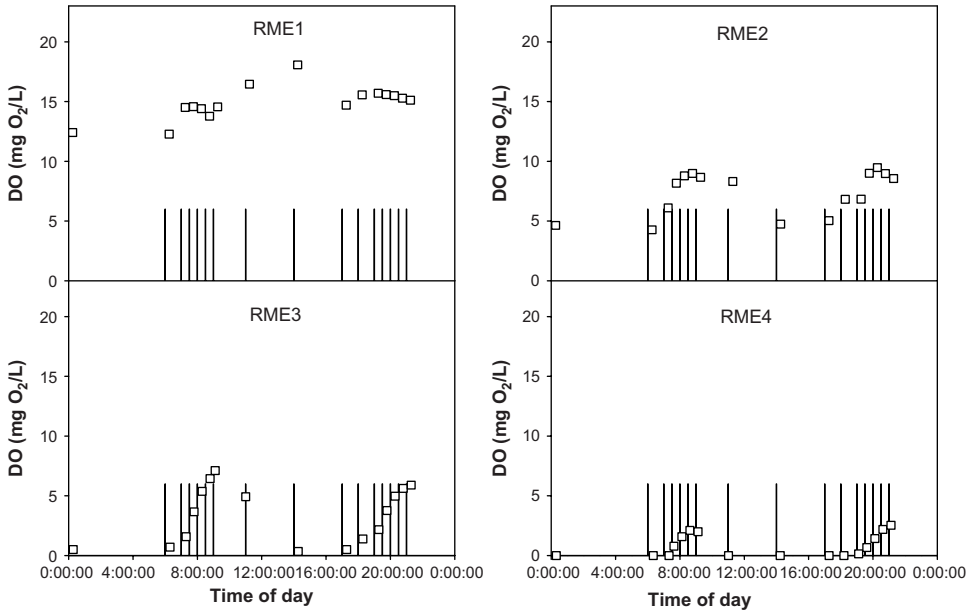

Fig. 3 – DO concentrations in RME1–RME4 over the course of a day, measured at these locations immediately after each pumping event. The vertical lines indicate pumping events.

control reactor was stable (confirmed by batch tests), the biofilms were not further investigated using microsensor and the focus was mainly on the experimental line. It was evident that mass transfer limitations and rapid consumption of oxygen in the biofilm play a prominent role in sheltering SRB from oxygen. Oxygen did not penetrate beyond 150 μm, in spite of high bulk concentrations, as long as VFA was available in the wastewater (Fig. 6B and C). The lower layers of the biofilm were thus anaerobic and sulfide production continued unhindered in this part of the biofilm, which explains why oxygen did not inhibit SRB capability (Fig. 5B), and sulfide accumulation resumed immediately on depletion of DO (Fig. 5A). Similar results were observed in aerobic wastewater biofilm by Kuhl and Jorgensen (1992), who showed that sulfate reduction continued in the anaerobic part of the biofilm, which shifted spatially within the biofilm in response to oxygen. Increasing the bulk oxygen in our system from 3.1 to 6.5 mg O_2/L did lead to a decreased flux of sulfide out of the biofilm (from 54.4 to 39.9 mg S/m^2 h). This was presumably caused by sulfide oxidation within the aerobic upper layers of the biofilm.

Complete oxygen penetration through the biofilm was observed only when VFA had been depleted from the wastewater (Fig. 6D). The net consumption of sulfide under these conditions confirms the biofilm sulfide oxidation capability. A mean sulfide oxidation rate of 25.3 mg S/m^2 h (0.8 mmoles S/m^2 h) and a mean oxygen consumption rate of 52.9 mg O_2/m^2 h (3.8 mmoles O_2/m^2 h) was observed, suggesting conditions were present for complete sulfide oxidation according to

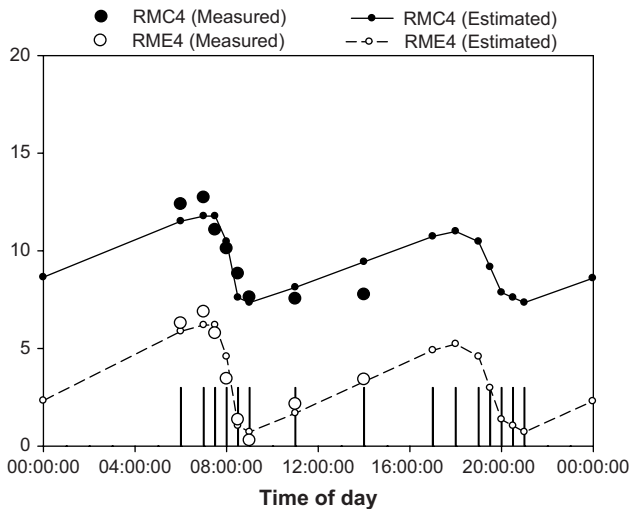

Fig. 4 – Measured (circles) and estimated (lines) sulfide concentrations at the outlets of RMC4 and RME4 over the duration of a day. The vertical lines indicate pumping events.

Table 1 – Summary of the inlet and outlet VFA, soluble and total COD concentrations in the control and experimental lines in Period 2

Parameters (Mean ± SE), $n = 12$	Sampling location		
	Inlet of system	Outlet of RMC4	Outlet of RME4
VFA: concentration (mg COD/L)	60.21 ± 3.82	1.42 ± 0.35	0.86 ± 0.40
Soluble COD: concentration (mg COD/L)	250.67 ± 14.33	165.94 ± 10.12	141.72 ± 11.75
Total COD: concentration (mg COD/L)	455.33 ± 13.67	337.74 ± 16.81	305.14 ± 21.00
% Consumption	–	25.8	33.0

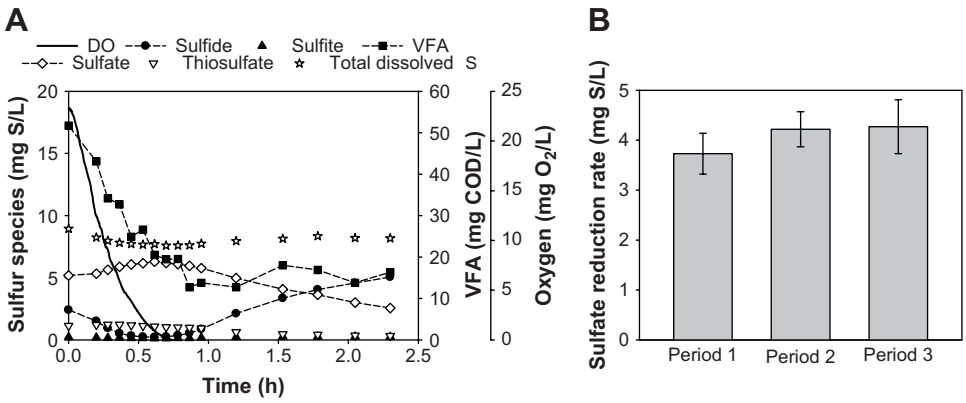

Fig. 5 – (A) A typical oxygen injection batch test in RME1 from Period 2 (Day 86) showing changes in DO, dissolved sulfur species and VFA. (B) Average sulfate consumption rates in anaerobic batch tests in RME1, with standard errors; $n = 3; 11; 7$ in Periods 1–3, respectively.

the equation $H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$. Full penetration of the biofilm with oxygen for 3–4 h did not affect SRB activity irreversibly, and sulfide production resumed upon return of anaerobic conditions and addition of VFA (Fig. 6E). This is consistent with the studies that have demonstrated the survival of SRB when exposed to oxygen sporadically or at low concentrations (Kjeldsen et al., 2004; Okabe et al., 1998; Santegoeds et al., 1998). The microsensor results collectively demonstrate that SRB capability in sewer biofilm does not change due to restricted contact with oxygen, and that SRB activity continues in the presence of bulk oxygen. It further reveals that SRB are capable of surviving short periods of oxygen exposure.

The role of chemical versus biological sulfide oxidation under oxic conditions was studied in batch experiments presented in Fig. 7. A linear relationship was observed between the oxygen consumption and sulfide oxidation in both tests. The amount of sulfide oxidized chemically in 0.6 h was 8.6 mg S/L (Fig. 7A) while the amount of sulfide oxidized in the same time due to a combination of chemical and biological sulfide oxidation was 14.5 mg S/L (Fig. 7B). This suggests approximately similar contributions of chemical and biological sulfide oxidation in this reactor, which had a high biofilm surface area to bulk water volume ratio of 57/m. The relative contributions of chemical and biological sulfide oxidation are not fixed, and in pipes with large diameters (i.e. larger volume to biofilm surface area ratio) chemical sulfide oxidation likely exceeds biological sulfide oxidation. Many studies have reported on the kinetics and stoichiometry of chemical sulfide oxidation (Chen, 1972; Nielsen et al., 2003; O'Brien and Birkner, 1977) and it is well understood that chemical sulfide oxidation is a complex process that results in the formation of various products and intermediates. Thiosulfate was the predominant end product of chemical sulfide oxidation in our reactor, formed at a rate of 6.9 mg S/L h compared with sulfate production rate of 0.9 mg S/L h. The low sulfate production rate is attributed to the stable nature of thiosulfate, and the substantially lower rate at which further oxidation to sulfate proceeds (Nielsen et al., 2003). Higher thiosulfate (9.7 mg S/L h) and sulfate (3.1 mg S/L h) were produced with combined chemical and biological sulfide oxidation. These higher rates reflect the contributions of biological sulfide oxidation. The total dissolved sulfur decreased at a rate of 11.9 mg S/L h with combined chemical and biological sulfide oxidation and a rate of 5.2 mg S/L h from pure chemical sulfide oxidation. This indicates the formation of intermediate sulfur products like polysulfides and elemental sulfur from the partial oxidation of sulfide and confirms related observations in Fig. 5A. These intermediate sulfur products were not measurable using our analytical techniques, and further research is needed to investigate their exact composition and content.

Repeated exposure to oxygen altered the oxygen uptake rates (OUR) and VFA consumption rate in RME1 (Fig. 8). The OUR in RME1 substantially increased from 11.5 ± 1.2 mg O_2/L h (Period 1) to 50.8 ± 4.7 mg O_2/L h (Period 2) (Fig. 8A). This was presumably a result of heterotrophic bacterial growth in RME1 biofilm and is supported by the measured increase in the amount of biofilm biomass during oxygen injection (Fig. iv, Supplementary material). No significant decrease was observed in the amount of biomass on stopping oxygen injection. A follow-on effect of increased oxygen uptake in the sewer would thus be a progressive increase in the oxygen uptake capability of sewer biofilm and faster re-establishment of anaerobic conditions between pump events. Hence the impact of oxygen initially observed after commencing oxygen injection may diminish over time. Note that the volumetric oxygen consumption rate is dependent on the A/V ratio of the pipes. The OUR in a real sewer pipe with, for example, a diameter of 1 m (A/V ratio = 4 m^2/m^3) is expected to be an order of magnitude lower than that measured in this study (A/V ratio = 57 m^2/m^3). The VFA consumption rate in RME1 increased from 11.3 ± 1.1 mg COD/L (Period 1) to 59.3 ± 3.6 mg COD/L (Period 2), revealing a strong correlation between the OUR and VFA consumption rate. Like OUR, the VFA consumption rate decreased after stopping oxygen injection (Fig. 8B), but was still higher than in Period 1, even after 80 days. Re-starting oxygen injection in oxygen-acclimated rising mains will therefore have progressively lesser impact compared with non-oxygen-acclimated mains.

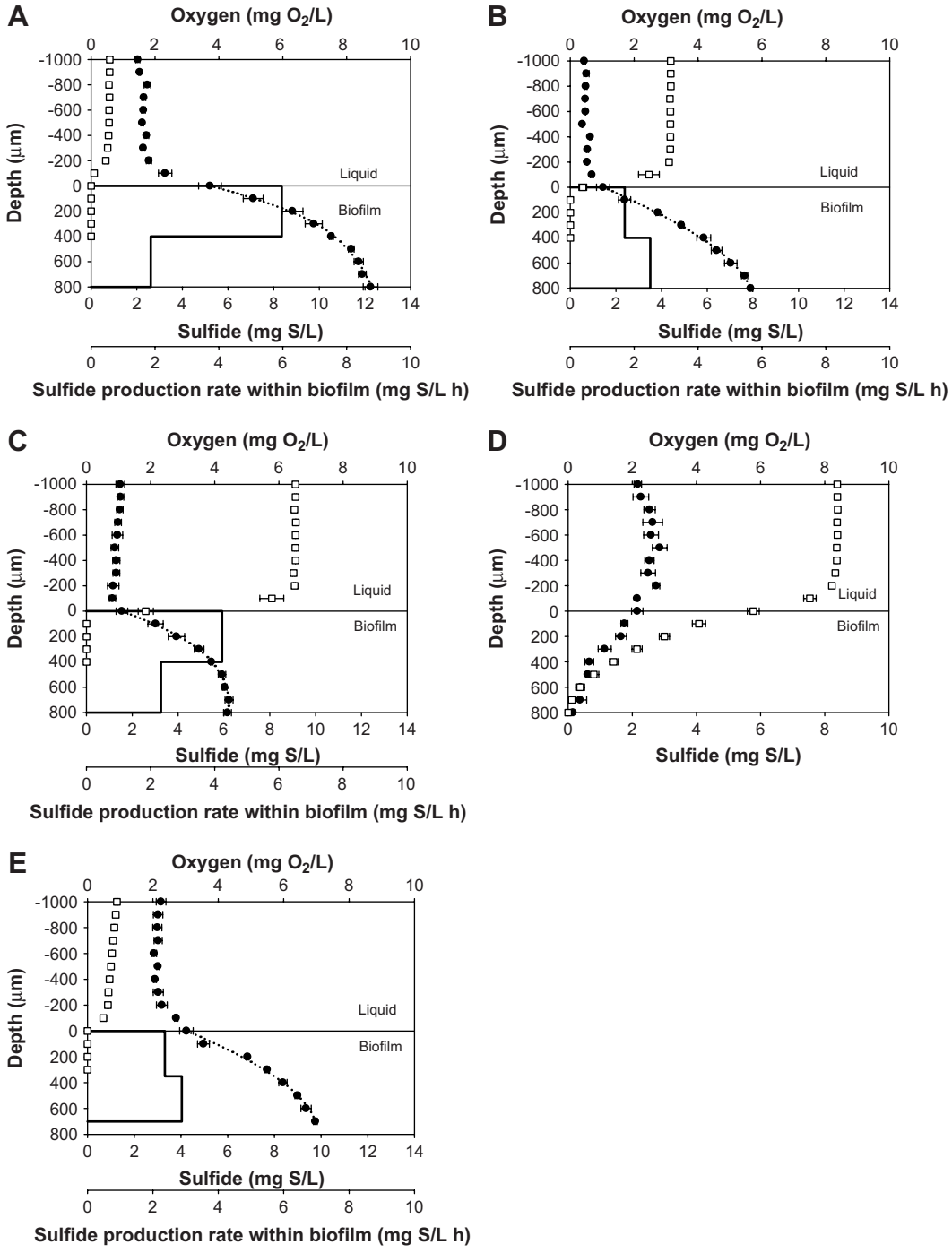

Fig. 6 – Oxygen (□) and total dissolved sulfide (●) microprofiles in carrier biofilm under different bulk oxygen and VFA concentrations (A) 0.6 mg O_2/L, 34.57 mg COD/L; (B) 3.1 mg O_2/L, 48.41 mg COD/L; (C) 6.5 mg O_2/L, 32.31 mg COD/L; (D) 8.3 mg O_2/L, < 1.1 mg COD/L; and (E) 0.9 mg O_2/L, 28.81 mg COD/L. The surface of the biofilm was defined as depth 0 μm. Dotted lines are model predictions of sulfide concentration gradients within the biofilm. Full lines are sulfide production profiles.

3.3. *Impact of oxygen on biofilm composition, structure, and physical characteristics*

RME1 biofilm was analyzed for the presence of SRB populations using *dsr*-DGGE and revealed that dominant SRB populations were not affected by oxygen injection (Fig. 9), which fits well with the limited change in SRB capability observed in Fig. 5B. A number of bands from the gel were sequenced and found to be similar in sequence (>83%) to *Desulfovibrio fructosovorans* (GenBank Acc. No. AF418187, Lane A), *Desulfobulbus rhabdoformis* (GenBank Acc. No. AJ250473, Lane B), an uncultured *Desulfotomaculum* clone (GenBank Acc.

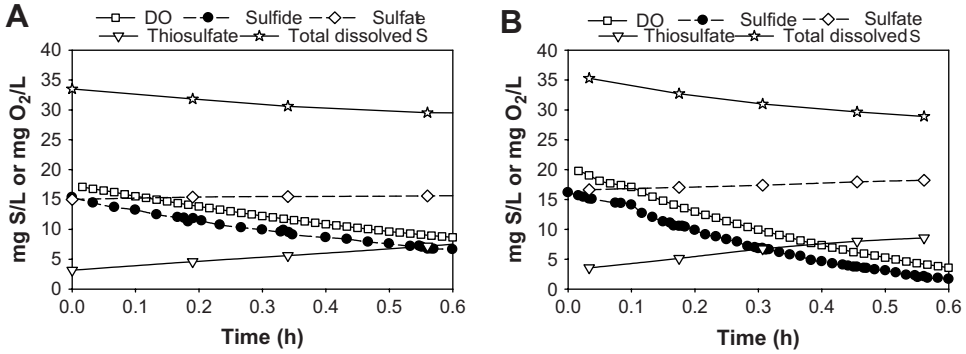

Fig. 7 – Batch tests measuring (A) chemical sulfide oxidation with oxygen and (B) the sum of chemical and biological sulfide oxidation with oxygen.

No. DQ415718, Lane C), uncultured SRB clones (GenBank Acc. Nos. DQ250775, Lane D; AM181120, Lane E; EU258818, Lane F), *Syntrophobacter fumaroxidans* (GenBank Acc. No. CP000478, Lane G), *Desulforhabdus amnigena* (GenBank Acc. No. AF337901, Lane H), uncultured SRB clones (GenBank Acc. Nos. AM181124, Lane I; AM181111, Lane J), *Desulfomicrobium escambiense* (GenBank Acc. No. AB061531, Lane K) and an uncultured SRB isolate (GenBank Acc. No. EF065047, Lane L). Bacteria from *Desulfovibrio*, *Desulfobulbus* and uncultured *Desulfotomaculum* genera found in this study have previously been identified in wastewater systems and are known to be versatile in their electron acceptor requirements and to survive in the continued presence of oxygen for up to few days without loss of viability (Cypionka et al., 1985; Widdel, 1998), and could have contributed to the immediate recovery in activity observed in Fig. 6E.

16S-DGGE analysis of the bacterial community also showed no substantial change in response to oxygen injection, suggesting that the growth of biomass (Fig. iv, Supplementary material) observed in response to oxygen injection was from bacterial populations already abundant in the biofilm. Thirteen bands were present on Day −2 and 14 bands on Day 29. Fourteen bands were still visible on Day 157 (gel not shown). Bands consistently present in the biofilm on all days were excised and sequenced. They corresponded to bacteria from 10 diverse phylogenetic groups, including Gram-positive heterotrophic bacteria and Gram-negative *Proteobacteria*. More information on these sequences and the maximum-likelihood phylogenetic tree with bootstraps can be obtained from Fig. v (Supplementary material). The high bacterial diversity observed in RME1 biofilm was visible when the biofilm surfaces at the end of Periods 1 and 2 were examined with scanning electron microscopy (Fig. vi, Supplementary material). Morphotypes detected included rods, cocci and vibrios. The SEM micrographs revealed an increase in what was interpreted to be extracellular polymer substances (EPS) in the biofilm exposed to oxygen. The color and texture of the biofilm visually changed as a result of oxygen addition from a dark green-brown compact biofilm under the microscope to one with two layers, an inner dark green-brown layer and an outer light brown surface layer with irregular protrusions. It was considered sufficient for the purposes of this study to limit the microbial community analysis to the reactor most likely to be impacted by oxygen (RME1). More comprehensive tests demonstrating the impact of oxygenation on microbial community dynamics and

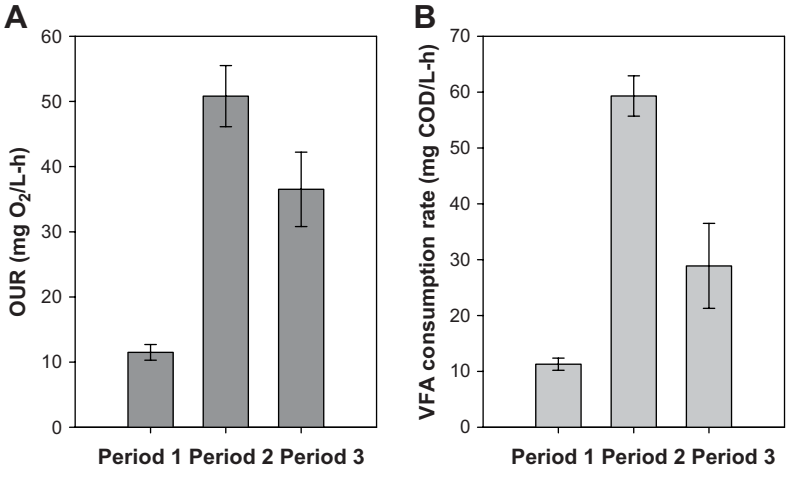

Fig. 8 – Averages of (A) OUR and (B) VFA consumption rates measured in aerobic batch tests in RME1, with standard errors; $n = 4$; 11; 8 in Periods 1–3, respectively.

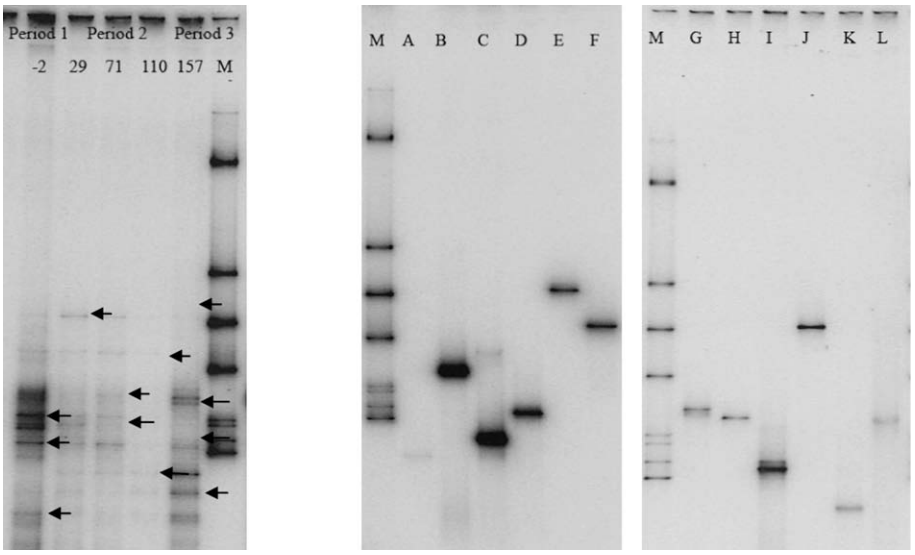

Fig. 9 – Gel 1: *dsr*-DGGE of biofilm over the course of the oxygen injection study. Arrows indicate bands that were excised from this gel. The lane numbers indicate sampling days. M = Marker. Gel 2: reamplification of excised bands. Descriptions of organisms similar in sequence to the bands in Lanes A–L are described in Section 3.3.

reproducibility of biofilm communities would have been possible by comparing biofilm compositions of both the experimental and control lines. Further research is also needed to examine the effect of physical characteristics like porosity, macro-porosity and density variations, which might play a role in biofilm activities.

3.4. *Effect of oxygen on downstream SRB activity*

Fig. 10 summarizes the sulfide production rates, measured under non-limiting substrate conditions and in the absence of oxygen, in each of the four reactors of the control and experimental lines on Day 100 of Period 2. A *p*-test with a null hypothesis of no significant difference was used to compare the calculated sulfide production rate in the control and experimental lines. The sulfide production capability of RME1 and RME2 was unchanged due to oxygen addition (p value > 0.05); however, significant increases (p value < 0.05) of 41 and 45% were observed in the average sulfide production rate of RME3 and RME4 in comparison with RMC3 and RMC4, respectively. The increase in sulfide production capability in downstream sections is thought to be a result of changes in wastewater characteristics induced by oxygen injection. In the control line, most of the sulfate present in incoming sewage was reduced to sulfide in RME1 and RME2, and the last two reactors received much less sulfate (1–3 mg S/L), expect during periods of frequent pumping. With the injection of oxygenated sewage in the first reactor, sulfide present in the system was oxidized to sulfate (Fig. 5A), and RME3 and RME4 consistently received wastewater with higher sulfate concentrations (3–7 mg S/L). It is thus likely that exposure to higher sulfate enabled the growth of SRB populations in downstream biofilm, and consequently these biofilms were capable of higher sulfide production than prior to oxygen injection. Spatial variation in biofilms activities along the length of rising mains has previously been demonstrated (Mohanakrishnan, 2008), and similar shifts in SRB activity due to oxygen injection are expected in the field. It is interesting to note that because the sulfide production capability in RME1 and RME2 did not decrease as a result of oxygen injection, the overall capability of the system to produce sulfide increased.

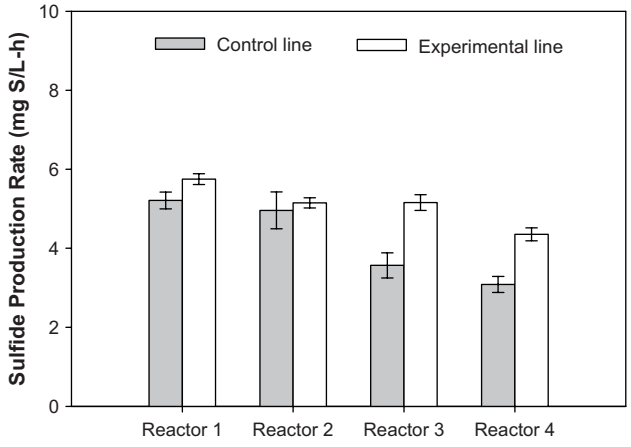

Fig. 10 – Sulfide production rates in the control (grey columns) and experimental (white columns) lines on Day 100 of Period 2 measured with fresh sewage.

3.5. *Oxygen injection as a sulfide control option*

Oxygen has widely been used as a sulfide oxidant in sewers without a clear understanding of its impacts on wastewater characteristics and sewer biofilm activities. Consequently, wastewater authorities have been perplexed when faced with on-going odour emission and corrosion issues in spite of oxygen dosing (personal communication with Gold Coast Water, Australia). The reason behind this, as revealed in this

study, is that oxygen does not prevent SRB activity and growth within biofilm (Figs. 5B and 9). This is mainly caused by the limited oxygen exposure, as oxygen only partially penetrates sewer biofilm (Fig. 6). Sulfide production is observed in lower layers of the biofilm even when oxygen is present in the bulk, and when oxygen is depleted, sulfide instantly accumulates at a similar rate prior to oxygen injection (Fig. 5A). Additionally, the intermittent oxygen exposure does not have a lasting effect on SRB that are exposed to oxygen (Fig. 6E). Oxygen injection furthermore increases the overall capability of sewers for sulfide production (Fig. 10) by increasing SRB activity in downstream biofilm.

Reduction in overall sulfide discharges at the outlets of the sewers (Fig. 4) with the current mode of oxygen injection (i.e. to the inlet of the rising main) is the result of effective chemical (Fig. 7A) and biological sulfide (Fig. 7B) oxidation. The end products of sulfide oxidation include thiosulfate, sulfate and intermediate sulfur compounds. Some of these intermediate sulfur compounds formed during sulfide oxidation are retained in the biofilm and contribute to 'additional' sulfide production in the absence of oxygen (Fig. 5A). Complete reduction of sulfide discharges would require the entirety of the pipe to be oxic, which is not possible with the existing method of dosing (i.e. into wastewater when the pump is running). For a given sewer system, the rate at which the injected oxygen is consumed is constant. This means, with the amount of oxygen dosed during the pumping event, it would be impossible to keep the pipe oxic at a long HRT (Fig. 2). Rapid oxygen consumption in the biofilm (Fig. 5A) and increases in oxygen uptake (Fig. 8A) due to the likely growth of heterotrophic bacteria (Figs. iv and v, Supplementary material), also suggested by increases in VFA consumption rates (Fig. 8B), prevent continued maintenance of oxic conditions. This is reflected in the reduction in oxygen concentrations along the length of the sewer (Fig. 3).

The location of injection and the oxygen dosing rate are therefore important factors in the optimization process. The oxygenated wastewater needs to be retained in the sewer for the time it takes to achieve complete sulfide oxidation and should not be depleted before the wastewater is discharged from the main. Shifting oxygen dosing to nearer the end of rising mains could allow for this. Another possibility is to use multiple or alternate injection stations in sewer networks, at locations where oxygen can substantially reduce overall sulfide discharges. Sharma et al. (2008b) have recently applied a dynamic rising model capable of predicting sulfide concentrations to determine the optimal location of the point of oxygen injection in a sewer network. Optimization of oxygen injection needs to be studied further and detailed practical and financial feasibility analyses are essential to facilitate effective decision making. The depletion of wastewater carbon content due to oxygen injection (Table 1) also needs to be assessed, if the sewers discharge to a wastewater treatment plant performing biological nutrient removal, as costs may be incurred for additional carbon dosing. It should be noted that this study was conducted on a laboratory reactor system rather than on a real rising main sewer. While the lab system mimicked a real sewer system on many aspects including the areal sulfide production rate, it is different from a real system on many other aspects. For example, its A/V ratio is substantially higher than that of a typical sewer. The physical and microbial structures of the biofilms could also be different due to different hydraulic flow giving rise to different shear stresses. Therefore, field studies are necessary to verify the results obtained from the lab systems.

4. Conclusions

While oxygen continues to be widely used by wastewater authorities to mitigate the sulfide problem in sewers, results in this study demonstrate that optimization is necessary for maximum effectiveness. The following conclusions are made:

- Oxygen injection decreases sulfide discharges in rising mains by chemical and biological sulfide oxidation. The efficiency of oxygen is related to its presence in the bulk which is controlled by the HRT of the wastewater.
- Oxygen does not affect SRB community composition or activity. Rapid consumption of oxygen by the upper layers of the biofilm allows sulfide production to continue in the deeper layers even when oxygen is present in the bulk. Oxygen furthermore promotes SRB activity in downstream biofilm, increasing the overall sulfide production capability of sewers.
- The oxygen uptake rate of the biofilm increases with repeated exposure to oxygen. A substantial increase is also observed in the wastewater carbon consumption due to oxygen injection.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2008.07.042.

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