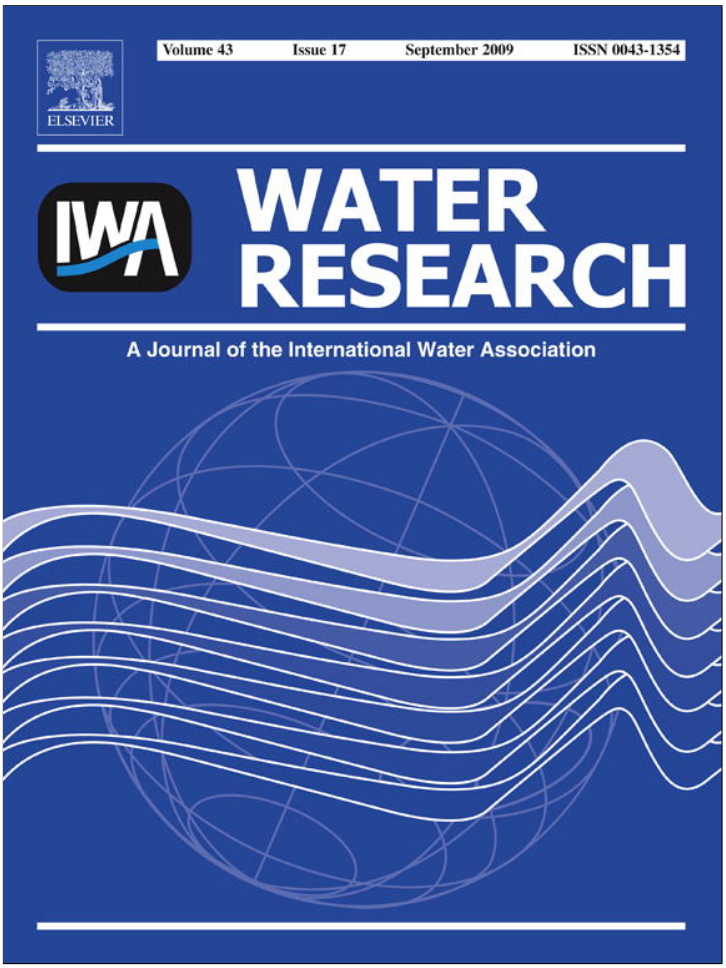

WATER RESEARCH 43 (2009) 4430–4440

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Sulfur transformation in rising main sewers receiving nitrate dosage

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ARTICLE INFO

Article history:
Received 27 April 2009
Received in revised form
30 June 2009
Accepted 2 July 2009
Published online 8 July 2009

Keywords:
Sulfide
Elemental sulfur
Sulfate
Sewer
Sulfur transformation
Nitrate

ABSTRACT

The anoxic and anaerobic sulfur transformation pathways in a laboratory-scale sewer receiving nitrate were investigated. Four reactors in series were employed to imitate a rising main sewer. The nitrate-dosing strategy was effective in controlling sulfide, as confirmed by the long-term sulfide measurements. Anoxic sulfide oxidation occurred in two sequential steps, namely the oxidation of sulfide to elemental sulfur (S^0) and the oxidation of S^0 to sulfate (SO_4^{2-}). The second oxidation step, which primarily occurred when the first step was completed, had a rate that is approximately 15% of the first step. When nitrate was depleted, sulfate and elemental sulfur were reduced simultaneously to sulfide. Sulfate reduction had a substantially higher rate (5 times) than S^0 reduction. The relatively slower S^0 oxidation and reduction rates implied that S^0 was an important intermediate during anoxic and anaerobic sulfur transformation. Electron microscopic studies indicated the presence of elemental sulfur, which was at a significant level of 9.9 and 16.7 mg-S/g-biomass in nitrate-free and nitrate-exposed sewer biofilms, respectively. A conceptual sulfur transformation model was established to characterize predominant sulfur transformations in rising main sewers receiving nitrate dosage. The findings are pertinent for optimizing nitrate dosing to control sulfide in rising main sewers.

1. Introduction

Centralization of sewage treatment has resulted in larger sewer networks, necessitating longer rising main sections in many cases. With increased retention time in long sewers, sewage turns into anaerobic when available oxygen and nitrate are consumed completely. Anaerobic condition in sewers causes the production of hydrogen sulfide (H_2S) in sewer biofilms and sediments. The buildup and subsequent emission of hydrogen sulfide to sewer atmosphere induces serious corrosion, malodor, and health problems (Thistlethwayte, 1972; US EPA, 1974; Pomeroy, 1990; US EPA, 1991; Hvitved-Jacobsen, 2002).

Injection of nitrate has been one of many chemical dosing strategies applied for sulfide control in sewers (Boon, 1995; Jefferson et al., 2002; Zhang et al., 2008). Over the last 70 years, control of hydrogen sulfide in sewers using various nitrate salts ($NaNO_3$, $Ca(NO_3)_2$, NH_4NO_3) was reported sparsely (Heukelekhm, 1943; Murray and Sims, 1979; Poduska and Anderson, 1981; Okabe et al., 2003). Nitrate, with concentration

Abbreviations: EDS, Energy dispersive spectroscopy; FIA, Flow-injection analyzer; HRT, Hydraulic retention time; IC, Ion chromatograph; NR-SOB, Nitrate-reducing sulfide-oxidizing bacteria; SAOB, Sulfide anti-oxidant buffer; SEM, Scanning electron microscopy; SOB, Sulfide-oxidizing bacteria; SRB, Sulfate-reducing bacteria; TDIS, Total dissolved inorganic sulfur; XRD, X-ray diffraction.

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doi:10.1016/j.watres.2009.07.001

ranging from 10 to 40 mg-N/L, was demonstrated to reduce sulfide concentration to 0.2–3 mg-S/L in rising main sewers of lengths ranging from 2.4 to 5 km (Bentzen et al., 1995; Saracevic et al., 2006). Rodriguez-Gomez et al. (2005) found that 5 mg-N/L of nitrate in sewage was capable to reduce sulfide production effectively in a 61 km long gravity sewer transporting reclaimed water. Another field trial conducted in a 6.7 km combined sewer network also proved the effectiveness of nitrate in controlling sulfide (Mathioudakis et al., 2006).

Nitrate did not have an immediate or long-lasting inhibitory/toxic effect on sulfate reduction by sewer biofilms (Mohanakrishnan et al., 2009). In the study, biofilms in a nitrate-receiving laboratory sewer system were found to fully maintain their sulfidogenic activity (sulfide production rate in the absence of nitrate) during several months of nitrate addition. Sulfide accumulation proceeded at normal rates even in the presence of nitrate at the beginning of nitrate addition. The accumulation did not stop until the third to fourth nitrate dose (30 mg-N/L). Anoxic sulfide oxidation by nitrate-reducing sulfide-oxidizing bacteria (NR-SOB) developed rapidly in the nitrate-receiving sewer biofilms, which was proposed as the primary mechanism for sulfide control in nitrate-receiving sewers (Mohanakrishnan et al., in press). The rapid proliferation of indigenous NR-SOB after the addition of nitrate has also been reported for several other sulfide-producing systems (Telang et al., 1999; Nemati et al., 2001; Haveman et al., 2005; Dunsmore et al., 2006; Garcia-de-Lomas et al., 2007; Kaster et al., 2007).

In a nitrate-receiving sewer, anoxic and anaerobic conditions may alternate depending on the wastewater flow rate (or hydraulic retention times – HRTs), the amount of nitrate added and its consumption rate. This is particularly the case in rising main sewers receiving limited amounts of nitrate, as wastewater could stay in pipes for hours during periods with low wastewater flows. An understanding of the detailed sulfur transformation processes under such conditions, which is lacking at present, is important for the optimal use of nitrate for sulfide control.

The aim of this work is to investigate the sulfur transformation pathways and intermediates by nitrate-acclimated sewer biofilms under alternating anoxic and anaerobic conditions. A reactor-based laboratory sewer system was used in the study. The sulfur transformation was investigated through long-term reactor performance monitoring, anoxic/anaerobic batch tests, and also the electron microscopic examination of the biofilms. A conceptual sulfur transformation model, describing predominant sulfur transformations and key intermediates, was established based on the findings of these experimental studies.

2. Materials and methods

2.1. Lab reactor system

The laboratory experimental system consisted of four air-tight reactors, namely R1–R4, connected in series. Each reactor had a volume of 0.75 L, with a diameter of 80 mm and a height of 149 mm (Fig. 1). Plastic carriers (Anox Kaldnes, Norway) of 1 cm diameter were clustered on four stainless-steel rods inside each reactor as biofilm samplers. The biofilm area, including reactor wall and carriers, was 0.05 m^2 (A/V = 70.9 m^2/m^3).

Domestic wastewater, collected weekly from a local wet well at the Robertson Park pump station, Indooroopilly, Brisbane (Australia), was used as the feed. The collection point locates in a predominantly domestic sewage catchment, collecting sewage flows from gravity sewers from the surrounding area. The wastewater composition had weekly variations in sulfate, volatile fatty acids (VFA), and chemical oxygen demand (COD) concentrations. Main parameters of the fresh sewage are listed in Table 1. Nitrate was not present in the fresh sewage. Sulfite and thiosulfate were present in negligible amounts (<1 mg-S/L). The sewage was stored in a cold room at 4 °C and heated up to 20 °C before being pumped into reactors.

The system was intermittently fed with sewage through a peristaltic pump (Masterflex 7520-47) following a typical pattern observed at a 1.1 km long rising main sewer (UC09) located at the Gold Coast, Australia (Guisasola et al., 2008). Sixteen feeding events occurred in every 24 h. Every feed pumping event lasted 2 min, transferring 0.75 L of sewage into the reactor system. The diurnal variation of sewage hydraulic retention times (HRTs) in the whole four-stage reactor system varied from 1.5 to 10.5 h. Nitrate solution (1500 mg-N/L) was dosed into R4 at a flow rate of 2.5 mL/min immediately after each pumping event for variable periods between 2 and 12.1 min. Nitrate dosages ranged from 10 to 60.4 mg-N/L, roughly proportional to the HRTs of the wastewater in R4, as shown in Fig. 1. A detailed description of the operation can be found in Gutierrez et al. (2009). To ensure homogeneous distribution in reactors, gentle mixing (240 rpm) was provided with magnetic stirrers (Heidolph MR3000) for feed or nitrate-dosing events.

2.2. Long-term performance monitoring

Anaerobic biofilms developed on the walls and carriers in the four reactors for over 6 months before the commencement of nitrate dosing to R4. Baseline measurements of sulfide in the system confirmed, at pseudo-steady state, the system displayed similar sulfide production capability (2.3 g-S/m^2-d) compared to that of UC09 sewer (2 g-S/m^2-d) (Gutierrez et al., 2009; Mohanakrishnan et al., 2009), and empirical values (0.48 ~ 2.4 g-S/m^2-d) (Hvitved-Jacobsen, 2002). The sulfide concentration in sewage at the end of UC09 was 5 ~ 15 mg-S/L.

Nitrate dosing was carried out in R4 continuously for over 12 months. To determine the long-term effect of nitrate dosing, wastewater samples were taken from R4 immediately after each nitrate-dosing event and immediately before the next feed pumping event. For pumping intervals longer than 2 h, extra samples were taken every 2 h. Sulfur species (sulfide, sulfite, thiosulfate, and sulfate) and nitrogen species (nitrate, nitrite, and ammonia) were analyzed as described in 2.4. Total dissolved inorganic sulfur (TDIS) was calculated as the sum of sulfide, sulfite, sulfate and thiosulfate.

To determine elemental sulfur concentration in the discharge, the whole volume of a feed pump event (750 mL)

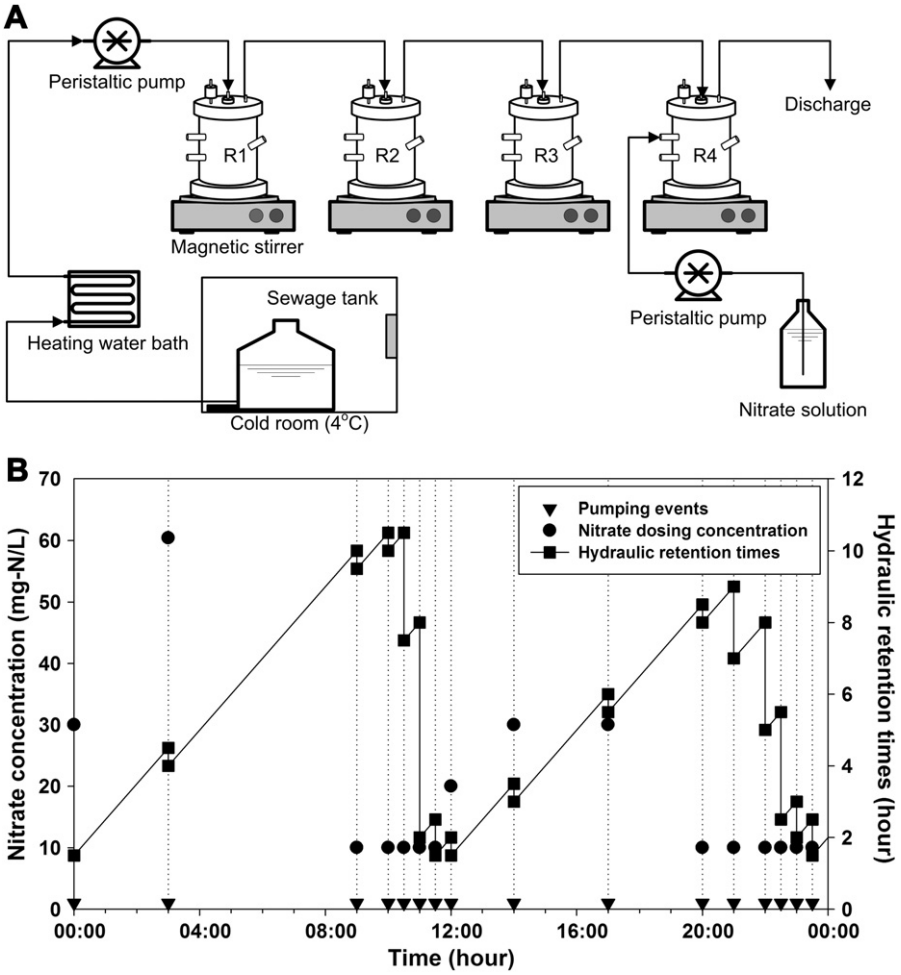

Fig. 1 – (A). Schematic of the laboratory-scale rising main sewer; (B). Operation conditions: feed pumping events (▼), nitrate-dosing concentration (●), and hydraulic retention times (■).

was collected from R4. The collected wastewater was centrifuged (Eppendorf Centrifuge 5810R) at 12 000 rpm for 10 min in batches of 50 mL. After each centrifugation, supernatant was discarded and more wastewater was centrifuged in the same tube. The pellets in the tube were analyzed for elemental sulfur, using a method described in Section 2.4. Fresh wastewater samples were analyzed for elemental sulfur with the same procedure.

Biofilm samples were collected from R1 to R4 for the determination of elemental sulfur concentration in biofilms. R1–R3 were grouped as nitrate-free biofilms, while R4 was classified as nitrate-exposed biofilm. To access the biofilms in the reactors, carrier-holding rods were screwed off. Two carriers were transferred into 50 mL conical centrifuge tubes after draining off attached water. Vigorous shaking at 900 rpm in an orbital mixer (Ratek Instruments Pty. Ltd., Australia) was employed to peel the biofilm off the plastic carriers (Gutierrez et al., 2008). The biofilm sample was mixed with 10 mL nitrogen-sparged milliQ water and centrifuged (Eppendorf Centrifuge 5810R) at 12 000 rpm for 10 min. The supernatant was discarded while the washed biofilm pellet was analyzed for elemental sulfur content as described in 2.4.

2.3. *Anoxic/anaerobic batch tests*

2.3.1. *Anoxic sulfur transformation*

The anoxic sulfide oxidation processes occurring in the R4 biofilm were investigated using batch tests. R4, fed with sewage from R3, was isolated from the reactor system. Each batch test lasted for eight hours. 30 mL of nitrate stock solution (1500 mg-N/L) was injected into R4, creating an initial nitrate concentration of 60 mg-N/L. Wastewater samples were taken at intervals ranging from 15 min (the first hour) to 2 h. To homogenize wastewater, gentle mixing was provided by magnetic stirrers (Heidolph MR3000) at 240 rpm for 1 min immediately before each sampling took place.

Biofilm samples were collected from R4 before and after the batch experiment. Sampling and analysis methods were described above in 2.2.

R3 was chosen as the reference reactor. R3 received wastewater from R2 with high sulfide and no sulfate, which

Table 1 – Main parameters of fresh sewage used in the sewer reactor experiments.

Parameter	Unit	Concentration
Sulfide	mg-S/L	2.8 ± 0.3
Sulfate	mg-S/L	10 ~ 25
VFA	mg-COD/L	50 ~ 100
Total COD	mg-O_2/L	469 ± 0.6
Soluble COD	mg-O_2/L	258 ± 5.3
Ammonium	mg-N/L	46.7 ± 0.5
pH	–	7.2 ~ 7.5

is the most similar situation among R1–R3 to that of R4. A reference experiment was carried out with the same level of nitrate dosing as applied to R4. The same sampling and measurement procedures were applied. These batch tests on R4 and R3 were repeated three times.

2.3.2. *Anaerobic sulfur transformation*

The activity of SRB and sulfur-reducing bacteria in R4 was investigated in a series of anaerobic batch tests. R4 was disconnected from R1 to R3 and flushed with fresh sewage for 8 min with stirring to remove residual nitrate. Biofilm samples from two carriers were taken for elemental sulfur analysis as described above. R4 was then filled with fresh sewage by turning on the feed pump for 2 min.

Wastewater samples were taken at a 2 h interval for analysis of sulfur species and the whole test lasted for 32–63 h. Two carriers were collected from the reactor at the end of each test for the analysis of elemental sulfur.

These tests were done three times. To investigate effects of high sulfate concentration on the anaerobic sulfur transformation, sulfate was spiked in R4 (48 mg-S/L) in one batch test. Above tests were also carried out in R1 as references.

2.4. *Chemical analysis*

2.4.1. *Dissolved sulfur and nitrogen compounds*

For the analysis of dissolved sulfur species (sulfide, sulfite, thiosulfate, sulfate), 1.5 mL wastewater was filtered (0.22 μm) into 0.5 mL preserving solution of sulfide anti-oxidant buffer (SAOB) (Keller-Lehmann et al., 2006). Samples were then analyzed within 24 h on an ion chromatograph (IC) with a UV and conductivity detector (Dionex ICS-2000). For the analysis of nitrogen species (nitrate, nitrite, ammonia), samples were filtered similarly, diluted 10 times and analyzed using Lachat QuikChem 8000 flow-injection analyzer (FIA).

2.4.2. *Elemental sulfur*

The analysis of elemental sulfur was developed based on the sulfite method (Janssen, 2008). The measurement employs the conversion of elemental sulfur and sulfite to thiosulfate at high pH (Goehring et al., 1949).

$$S^0 + SO_3^{2-} \rightarrow S_2O_3^{2-}$$

Centrifuged biofilm was resuspended in 10 mL nitrogen-sparged milliQ water. 1.5 mL of Na_2SO_3 solution (30% solution in 1 mM Na_2EDTA, made up with nitrogen-sparged milliQ water) and 0.15 mL of 1 M NaOH was added to the suspension. Samples were then incubated in an orbital shaker (120 rpm) at 60 °C for 12 h. After the incubation, samples were cooled down to room temperature. The suspension was centrifuged again at 12 000 rpm for 10 min. 1 mL of the supernatant was transferred into air-tight vial, which was then analyzed for thiosulfate by ion chromatograph. The final concentration of elemental sulfur was calculated from the thiosulfate concentration according to the reaction stoichiometry.

2.5. *Electron microscopic examinations*

To prepare biofilm samples for electron microscopy, one carrier with biofilm was immediately pre-fixed with 2.5% glutaraldehyde with 75 mM lysine in 0.1 M cacodylate buffer for 11 min. Samples were then fixed using 3% glutaraldehyde in 0.1 M cacodylate buffer. The fixation process included incubation in microwave oven (Biowave) at 150 W with vacuum. Fixed biofilms were washed with 0.1 M cacodylate buffer immediately. Post-fixation consisted of vacuum incubation in microwave at 80 W with 1% osmium tetroxide in 0.1 M cacodylate buffer. Samples were then dehydrated with increased concentration of ethanol, i.e. 50–100% with a step size of 10%. Fixed samples were frozen in liquid nitrogen and small particles showing inner structure were fractured, thawed in 100% ethanol. These particles were mounted using carbon tab on stainless-steel stubs and sputter coated with platinum.

Mounted samples were examined using JEOL 6300F or 6400F to obtain scanning electron microscope (SEM) images. Analysis of elemental composition was conducted using energy dispersive spectroscopy (EDS) on JEOL JSM-6460.

For X-ray diffraction (XRD) analysis, freeze-dried biofilms were processed using the Diffrac$^+$ Evaluation Package Release 2007 and PDF-2 2007. The presence of elemental sulfur in biofilms was examined using Bruker AXS D8 Advance X-Ray diffractometer equipped with a SOL-X detector and copper target. The following conditions were used: 2–90 degrees for 2-Theta; step size of 0.02 degree; 1.2 s per step; 40 kV; 30 mA.

3. Results and discussion

3.1. *Long-term performance of nitrate dosing*

Fig. 2 shows a typical profile corresponding to R4, where nitrate concentration fluctuated between 4 and 100 mg-N/L due to nitrate accumulation and consumption. It is evident that R4 was always under anoxic conditions while receiving nitrate. Nitrite, an intermediate product of denitrification, was present at a level of 0.2–14 mg-N/L.

Sulfide concentration in the effluent (measured immediately before a pumping event) was below 2 mg-S/L for most of the time over the 24 h period. The removal of sulfide was due to sulfide oxidation with nitrate (Mohanakrishnan et al., 2009). However, the effluent sulfide concentration was approximately 5 mg-S/L in the periods of 10 AM to 12 PM and 10 PM to 12 AM. In these periods, feed pumping interval was 0.5 h, inadequate for complete sulfide oxidation. The results show that the dosing strategy was reasonably effective in controlling sulfide in the discharge.

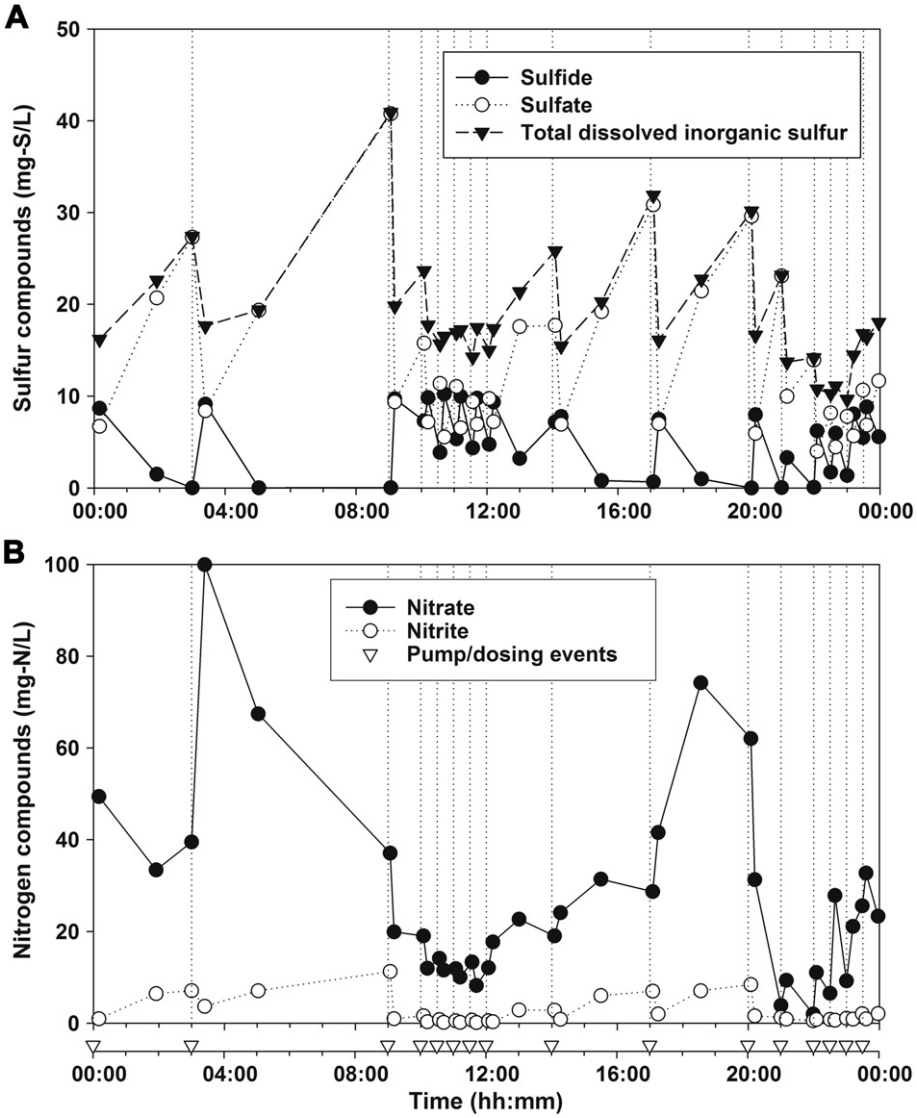

Fig. 2 – 24-h profiles of (A). Main sulfur compounds: sulfide (●), sulfate (○) and total dissolved inorganic sulfur (▼), and (B). Nitrogen compounds: nitrate (●), nitrite (○) in R4. Vertical dotted lines indicate pumping events (▽). Sulfite and thiosulfate in R4 were negligible, and hence not shown in the figure but included in the calculation of total dissolved inorganic sulfur.

Sulfate was observed to increase along with the decrease of sulfide. However, the amount of increase was not equal to the sulfide decrease. This is clearly shown by the TDIS concentration, which fluctuated from *ca.* 10 mg-S/L (10 AM to 12 PM, 10 PM to 12 AM) to *ca.* 40 mg-S/L (9 AM). The TDIS concentration in the feed was constant over the day at *ca.* 15 mg-S/L.

The TDIS profile suggests that insoluble intermediates were formed during the sulfur transformation. There was a net accumulation of these intermediates in periods with frequent pumping (e.g. 10 AM to 12 PM, 10 PM to 12 AM). In these periods, the TDIS concentration in R4 decreased between pumping cycles. However, these intermediates were consumed (oxidized to sulfate) in other periods with infrequent pumping, resulting in substantially higher TDIS concentrations. Biological sulfide oxidation was reported to start with the formation of polysulfide (S_x^{2-}), which was protonated to produce elemental sulfur (Brune, 1995; Steudel, 1996; van der Zee et al., 2007). We hypothesize that the intermediate was elemental sulfur, which will be further demonstrated in 3.2.

3.2. *Elemental sulfur as an intermediate*

Cell morphology and biofilm structure were examined using SEM before being further analyzed with EDS. Spot EDS analysis (spot size: *ca.* 1 μm) of R4 cryo-fractured biofilm (one example is shown in Fig. 3) demonstrated strong sulfur peaks, along with some other peaks (insignificant amounts of iron, barium, and calcium). The carbon and oxygen peaks originated from the organic biofilm matrix, while osmium and platinum were introduced during the biofilm fixation and coating procedures, respectively. No discernable sulfur peak

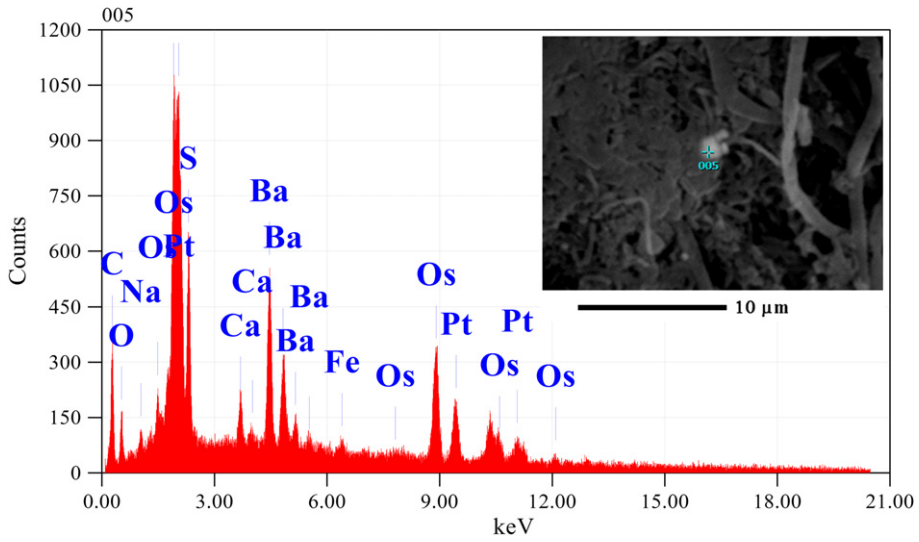

Fig. 3 – A representative EDS spectrum of the cryo-fractured biofilm. Acquisition parameters: Acceleration Voltage: 30.0 kV; Probe Current: 1.0 nA; PHA mode: T3.

was observed from control reactor biofilm samples (data not shown).

EDS is not capable of determining the oxidation states of sulfur. The high sulfur peak detected by EDS could be either crystalline sulfuric salts or elemental sulfur. However, XRD analysis of biofilm showed no presence of any crystals (Appendix Fig. A-1). This observation excluded the possibility of crystalline sulfuric salts. The sulfur observed with EDS was likely elemental sulfur granules, in an amorphous form. Indeed, biologically produced sulfur granules have previously been reported to be amorphous (Hageage et al., 1970) or partly consisting of orthorhombic crystal S^8 in a sulfide oxidation bioreactor (Janssen et al., 1999). Sizes of elemental sulfur granules observed were found to be in the range of sub-micrometer to micrometer, consistent with the literature observation (Kleinjan et al., 2006).

Fig. 4 shows the S^0 contents in biomass sampled from nitrate-free (R1–R3), and nitrate-exposed biofilms (R4). Biofilm in R4 (mean = 16.7 mg-S^0/g-biomass) had significantly higher S^0 content ($P = 0.0001$) than untreated biofilm from R1 to R3 (mean = 9.9 mg-S^0/g-biomass). This indicates that higher S^0 content in the R4 biofilm was due to nitrate dosing.

Elemental sulfur (S^0) in the R4 discharge was measured to contain S^0 at 0.94 ± 0.2 mg-S/L, which was significantly ($P = 0.04$) higher than the S^0 concentration in the sewage fed to the system (0.25 ± 0.1 mg-S/L). These results confirm that S^0 was produced in the system.

3.3. *Anoxic sulfur transformation*

Batch studies in R4, with R3 as reference, were conducted to investigate the anoxic oxidation of sulfide in the presence of nitrate. The results from one such batch test are shown in Fig. 5. Sulfide was found to be oxidized completely within 20 min. However, sulfate did not increase accordingly. The TDIS concentration decreased in this period (Fig. 5A inset), which suggests that sulfide was oxidized to other undissolved sulfur species rather than SO_4^{2-} (thiosulfate and sulfite were negligible throughout the batch tests). Following the complete removal of sulfide, sulfate and TDIS gradually increased. TDIS reached 40 mg-S/L at the end of the test. Fig. 5B shows that nitrate consumption occurred during the entire test, but at a much higher rate in the first 20 min, corresponding to the fast consumption of sulfide. Nitrite accumulation was below 3.7 mg-N/L at all times. As discussed in 3.1 & 3.2, the undissolved intermediate here was most likely elemental sulfur. The measurement of elemental sulfur in biofilms at the beginning and the end of the test confirmed the loss of elemental sulfur (3.5 ± 0.02 mg-S^0/g-biomass). Similar results were obtained in all repeated tests.

In the reference reactor (R3), batch tests indicated that the TDIS concentrations remained nearly constant throughout

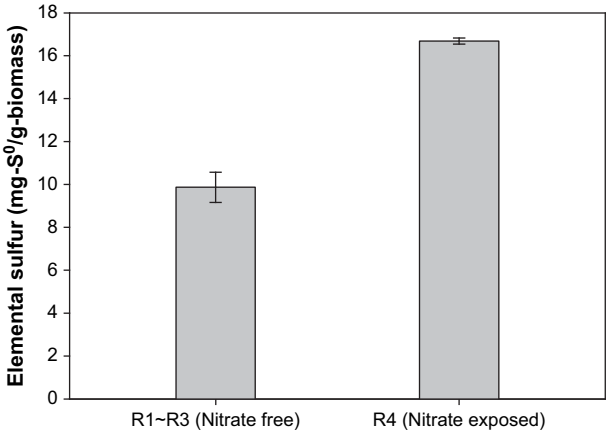

Fig. 4 – Elemental sulfur contents in nitrate-free and nitrate-exposed biofilms. Error bars indicate standard errors.

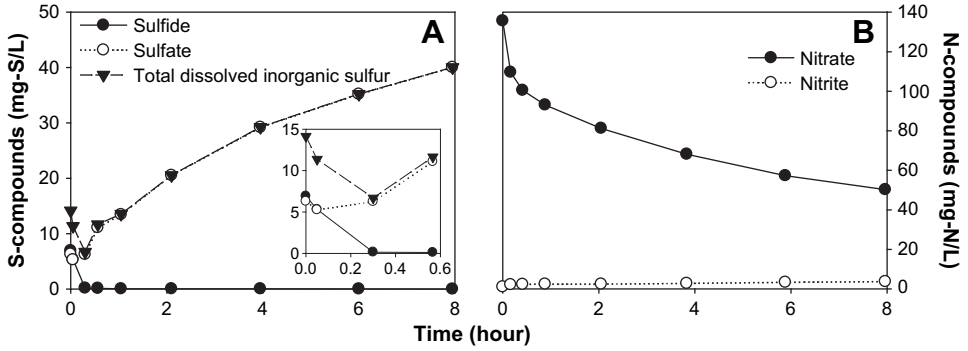

Fig. 5 – Anoxic sulfide oxidation by nitrate in R4. (A) Sulfur compounds: sulfide (●), sulfate (○), and total dissolved inorganic sulfur (▼); (B) Nitrogen compounds: nitrate (●), and nitrite (○). Inset in plot A shows an enlargement of the first 0.6 h.

the 8 h period (data not shown). This suggests that the increase of sulfate observed in the R4 test was not due to the oxidation of certain unknown sulfur compounds (e.g. organic sulfur compounds) contained in the wastewater.

The results show that anoxic sulfide oxidation by sewer biofilms using nitrate can be described as a two-step process, namely the oxidation of sulfide to elemental sulfur, and oxidation of elemental sulfur to sulfate. These reactions are described below (Li et al., 2008):

$$5S^{2-} + 2NO_3^- + 12H^+ \rightarrow 5S^0 + N_2 + 6H_2O$$
$$\Delta G^\theta = -955 \text{ kJ/reaction}$$

$$5S^0 + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+$$
$$\Delta G^\theta = -2738 \text{ kJ/reaction}$$

The sulfur profiles presented in Fig. 5A indicate that sulfide oxidation is substantially faster than the oxidation of elemental sulfur. This is reasonable considering the fact that sulfide is soluble while elemental sulfur is insoluble. Two types of sulfide-oxidizing bacteria (SOB), i.e. sulfate producers and sulfur producers, developed at different sulfide loading rates (Buisman et al., 1991). Sulfide loading and nitrate-dosing rates varied with HRTs in the sewer reactors. Both sulfate and sulfur producers were possibly present in R4.

3.4. *Anaerobic sulfur transformation after the depletion of nitrate*

Batch studies were conducted in R4 and R1 without nitrate (anaerobic condition). No effects of VFA on elemental sulfur and sulfate reduction were observed because VFA (20–100 mg-COD/L) was not a limiting factor for the biological process. The replicates of batch studies produced similar results, indicated by sulfur transformation rates shown in Table 2. Fig. 6A shows sulfate consumption and sulfide production in a typical test. The initial sulfate reduction rate was *ca.* 20.9 mg-S/m^2-h. This rate was lower than the sulfide production rate, which was 26.3 mg-S/m^2-h. The difference between the two rates was 5.4 mg-S/m^2-h. The sulfide concentration kept on rising after the depletion of sulfate. Fig. 6B shows the TDIS concentration profile during the test. It was observed that the TDIS concentration was increasing at a constant rate of 5.4 mg-S/m^2-h during the entire 35 h experiment.

In contrast, in R1, sulfate reduction was approximately equal to sulfide production, and the TDIS remained approximately constant (Fig. 6C). The R1 result suggests that sulfide production from the breakdown of organic materials contained in wastewater (e.g. proteins) was negligible, and therefore, sulfide production in excess of sulfate reduction observed in R4 was likely due to the anaerobic reduction of elemental sulfur presented in sewer biofilms (formed during anoxic sulfide oxidation, as discussed above). Indeed, a decrease of 1.2 mg-S^0/g-biomass for the duration of 35 h was observed in the R4 biofilm. The reduction rate of elemental sulfur should be equal to the increasing rate of TDIS (Fig. 6B).

The presence or absence of sulfate was found to have no effect on the elemental sulfur reduction rate. Also, elevated sulfate (48 mg-S/L) did not change this rate. It can be speculated that the reduction of sulfate and elemental sulfur in R4 was carried out by two different groups of bacteria, namely the sulfate-reducing bacteria (SRB), and the sulfur (S^0)-reducing bacteria. It appears that SRB did not use elemental sulfur even after sulfate depletion, and the sulfur-reducing bacteria did not use sulfate throughout the anaerobic batch tests. It has been previously reported that dissimilatory sulfur-reducing bacteria were unable to reduce sulfate or other

Table 2 – Reaction rates of main sulfur transformation processes under anoxic and anaerobic conditions.

Conditions	Biological processes	Transformation rates (g-S/m^2-d, average ± standard error)
Anoxic	Sulfide oxidation	17.1 ± 2.3
	Elemental sulfur oxidation[a]	2.2 ± 0.4
Anaerobic	Sulfate reduction	0.57 ± 0.02
	Elemental sulfur reduction[b]	0.1 ± 0.01

a This rate decreased slightly towards the end of batch tests because of lower concentration of elemental sulfur.
b Elemental sulfur reduction rate was calculated as the difference between sulfide production and sulfate reduction.

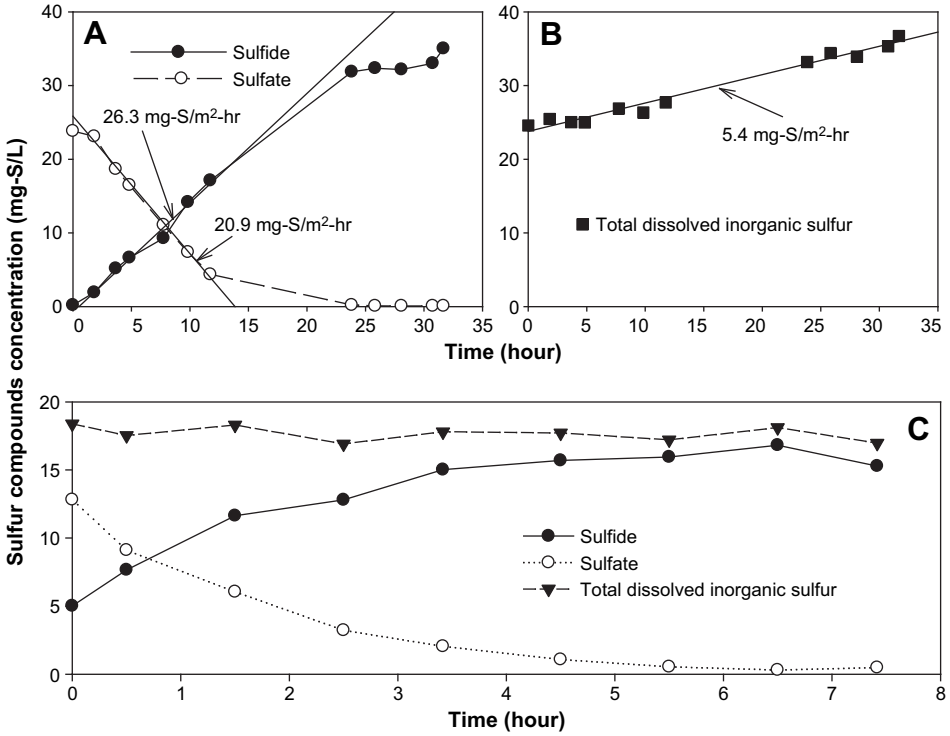

Fig. 6 – Sulfur transformation in R4 (A and B) and R1 (C) under anaerobic condition. (A) Sulfide (●) and sulfate (○); (B) total dissolved inorganic sulfur (■); (C) Sulfide (●), sulfate (○), and total dissolved inorganic sulfur (▼).

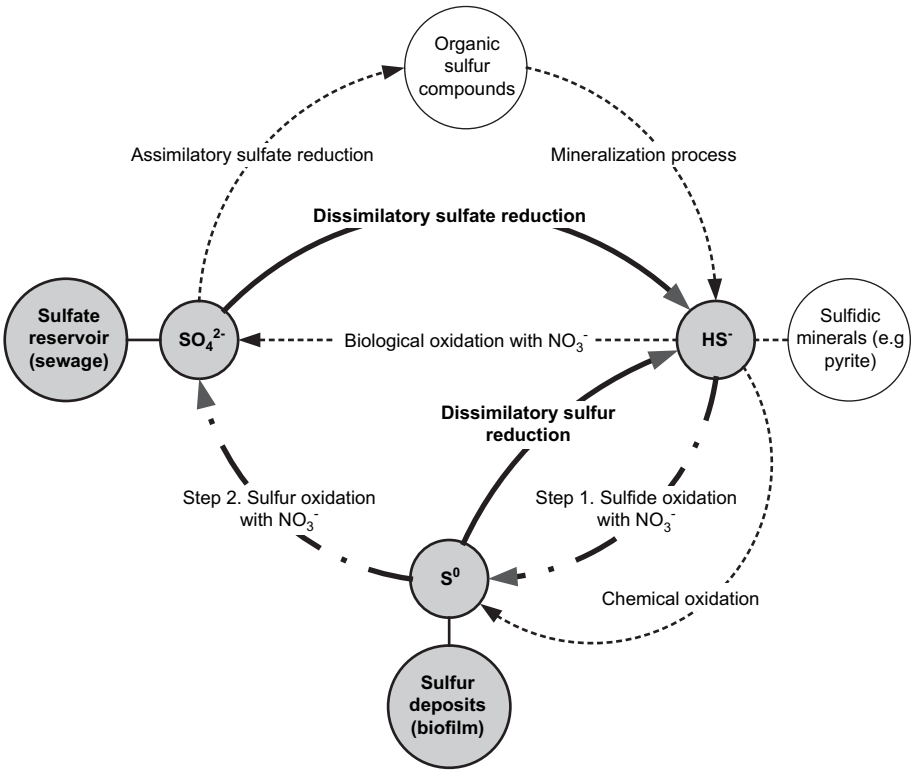

Fig. 7 – A conceptual model for anoxic and anaerobic sulfur transformation by nitrate-acclimated sewer biofilms. Bold lines indicate simultaneous sulfate and elemental sulfur reduction. Dot-dashed bold lines indicate sequential oxidation of sulfide and elemental sulfur. Dotted thin lines are processes that could be excluded from the simplified conceptual model.

oxyanions of sulfur, and sulfate-reducing bacteria could only reduce sulfur in a few cases (Bergey and Holt, 1994). The coexistence of SRB and sulfur-reducing bacteria in R4 and their preference for different sulfur compounds can explain our findings about simultaneous and independent reduction of sulfate and elemental sulfur.

3.5. *A conceptual model for anoxic and anaerobic sulfur transformation by nitrate-acclimated sewer biofilms*

Main sulfur species involved in both anoxic and anaerobic sulfur transformations by nitrate-acclimated sewer biofilms were found to be sulfate, sulfide, and elemental sulfur. Contributions from other sulfur species (thiosulfate and sulfite) to the total sulfur balance were negligible in both cases.

The predominant sulfur transformation processes performed by nitrate-acclimated sewer biofilms are summarized in Fig. 7. Under anoxic conditions, sewer biofilms oxidises sulfide to elemental sulfur, which is subsequently oxidized to sulfate. Under anaerobic conditions, sulfate and elemental sulfur are reduced to sulfide simultaneously. Fig. 7 also presents some other potential reactions (Hao et al., 1996; Kleinjan et al., 2003), which were demonstrated to be insignificant in this study.

The measured reaction rates (per biofilm surface area) of the sulfur transformation processes are listed in Table 2. Note that these values could be specific to the biofilm studied. However, the comparison of these rates show the capabilities of the same biofilm. It is important to observe that the anoxic sulfide and elemental sulfur oxidation rates are substantially higher (20–30 times) than their reduction rates. Other researchers have also reported that the reduction rate of oxidized sulfur was slower than sulfide oxidation rate (van der Zee et al., 2007; Yavuz et al., 2007). These differences in rates are desirable for sulfide control in sewers, as they indicate that sulfide formed under anaerobic sections of sewer pipes could be rapidly oxidized once nitrate is made available.

Previous research has shown the production of elemental sulfur as an intermediate for sulfide oxidation carried out by various aerobic sulfide-oxidizing bacteria (SOB) (Chan and Suzuki, 1993; Fuseler and Cypionka, 1995; Basu et al., 1996). More recently, some research has reported the production of elemental sulfur through sulfide oxidation with oxygen in wastewater (Janssen et al., 1999; Nielsen et al., 2003; Nielsen et al., 2005). In an anaerobic digester, elemental sulfur was reported to be produced due to sulfide oxidation by nitrate (Sher et al., 2008).

The experimental results, for the first time, confirmed the production of elemental sulfur as an important intermediate for sulfide oxidation by nitrate in sewer wastewater. Furthermore, this intermediate was found to be either further oxidized to sulfate, or reduced to sulfide depending on the conditions (anoxic or anaerobic). The findings could be used to optimize nitrate-dosing strategy in real sewer networks.

4. Conclusions

The anoxic and anaerobic sulfur transformation performed by sewer biofilms acclimated to nitrate dosing was investigated using a laboratory-scale sewer system. The main findings are:

- Elemental sulfur is an important intermediate product during anoxic and anaerobic sulfur transformation by sewer biofilms acclimated to nitrate. Elemental sulfur produced can be transported with sewage into the discharge. Part of the elemental sulfur is accumulated in biofilms, and can be further oxidized to sulfate in the presence of nitrate, or reduced to sulfide in the absence of nitrate.
- Under anoxic conditions (nitrate dosing), sulfide is oxidized in two steps, namely the oxidation of sulfide to elemental sulfur and then the further oxidation of elemental sulfur to sulfate. The two steps occur sequentially, with the second step occurring primarily after sulfide depletion. The first step is considerably faster than the second one.
- Under anaerobic conditions, the reduction of sulfate and elemental sulfur occur simultaneously, with sulfate reduction much faster than elemental sulfur reduction. Sulfate reduction and elemental sulfur reduction processes are independent of each other, likely conducted by S^0-dedicated (sulfur-reducing bacteria) and SO_4^{2-}-dedicated (sulfate-reducing bacteria) bacteria respectively.

Acknowledgements

The research was supported by the Australian Research Council through Projects LP0454182 and LP0882016. Guangming Jiang is grateful to the scholarships: Endeavour International Postgraduate Research Scholarship (IPRS) and University of Queensland International Living Allowance Scholarship (UQILAS). Prof. dr. Albert Janssen from Sr. Technologist Water and Waste Services, Shell Global Solutions International BV, The Netherlands kindly provided the method for the analysis of elemental sulfur. The authors acknowledge Dr. Beatrice Keller-Lehmann for her helpful discussion and expertise about analysis methods. We also thank Ms. Ying Yu and Anya Yago from Centre of Microscopy and Microanalysis at UQ for their help with the SEM, EDS, and XRD.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version at doi:10.1016/j.watres.2009.07.001.

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