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# Growth kinetics of hydrogen sulfide oxidizing bacteria in corroded concrete from sewers

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ABSTRACT

Hydrogen sulfide oxidation by microbes present on concrete surfaces of sewer pipes is a key process in sewer corrosion. The growth of aerobic sulfur oxidizing bacteria from corroded concrete surfaces was studied in a batch reactor. Samples of corrosion products, containing sulfur oxidizing bacteria, were suspended in aqueous solution at pH similar to that of corroded concrete. Hydrogen sulfide was supplied to the reactor to provide the source of reduced sulfur. The removal of hydrogen sulfide and oxygen was monitored. The utilization rates of both hydrogen sulfide and oxygen suggested exponential bacterial growth with median growth rates of 1.25 $d^{-1}$ and 1.33 $d^{-1}$ as determined from the utilization rates of hydrogen sulfide and oxygen, respectively. Elemental sulfur was found to be the immediate product of the hydrogen sulfide oxidation. When exponential growth had been achieved, the addition of hydrogen sulfide was terminated leading to elemental sulfur oxidation. The ratio of consumed sulfur to consumed oxygen suggested that sulfuric acid was the ultimate oxidation product. To the knowledge of the authors, this is the first study to determine the growth rate of bacteria involved in concrete corrosion with hydrogen sulfide as source of reduced sulfur.



## 1. Introduction

Hydrogen sulfide induced concrete corrosion is a significant world-wide economic problem in many sewer networks due to not only the damage inflicted on concrete structures but also the costs of preventive measures [1,2]. Besides causing corrosion problems, hydrogen sulfide is malodorous and toxic, thus posing a threat to sewer workers at high concentration [3].

Hydrogen sulfide is formed under anaerobic conditions, mainly in the biofilms covering the submerged pipe surfaces. The concrete corrosion, however, takes place when hydrogen sulfide is released from the wastewater to the sewer atmosphere, where it subsequently absorbs to the concrete surface above the wastewater. Hydrogen sulfide is oxidized to sulfuric acid on the concrete surface. The sulfuric acid reacts with the alkaline components of the concrete to form gypsum. Since gypsum has little structural strength this process results in significant structural weakening of concrete pipes [4,5]. Temperature is an important parameter in the formation of hydrogen sulfide. Increasing temperatures result in increased rates of oxygen consumption, leading to anaerobic conditions, and increases the rate of hydrogen sulfide formation [5]. In temperate climate areas mainly gravity sewers immediately downstream of force mains are at risk of corrosion. Full-flowing force mains favor the formation of hydrogen sulfide, which is then released into the sewer atmosphere at the point where the force main discharges into the partly filled gravity sewer [6].

The corrosion of the concrete surfaces is initiated when the uptake of hydrogen sulfide, as well as carbonation, on concrete surfaces above the water line gradually lowers the surface pH [7,8]. When the pH of the concrete surface is lowered to approximately neutral, the conditions become favorable for biological processes and the oxidation of hydrogen sulfide transitions from being chemical to being mainly a biological process [4,9]. Different microorganisms are involved in the sulfide oxidation as the pH of the surface is further lowered from neutral to approximately pH 2 [9,10]. On heavily corroded concrete surfaces where the pH is below 2, *Acidithiobacillus thiooxidans* is generally accepted to dominate the bacterial population, although bacteria of the genus *Acidiphilium* have also been found in significant numbers [4,9,11]. Other autotrophic bacteria as well as heterotrophic bacteria and fungi have been identified on samples of corroded concrete

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**Table 1**
Specific growth rates for *Acidithiobacillus thiooxidans* with elemental sulfur as reduced sulfur source, as presented in the literature.

| pH | Specific growth rate ($d^{-1}$) | Reference |
|---|---|---|
| 1.5–2.5 | 2.874[a] | Chen et al. [14] |
| 0.9–1.0 | 0.324[a,b] | Rao and Berger [15] |
| 2.1–2.3 | 1.5[a,b] | |
| 3.0–3.2 | 1.2[a,b] | |
| 4.2–4.3 | 0.48[a,b] | |
| 1.5 | 2.58[a] | Konishi et al. [16] |
| 1–3.5 | 1.968[b,c] | Tichy et al. [17] |
| 1.5–3.5 | 1.920[a,b] | |

[a] Sulfur source: chemically produced elemental sulfur.
[b] Converted by the author from $h^{-1}$ to $d^{-1}$, which is the unit given in the cited reference.
[c] Sulfur source: biologically produced elemental sulfur.

[9,11,12]. However, the role and importance of these organisms in the corrosion process remains largely unknown.

The growth kinetics of *A. thiooxidans* have previously been studied, however, in the majority of the studies, elemental sulfur has been applied as source of reduced sulfur (Table 1). In general, the growth kinetics of *A. thiooxidans* with hydrogen sulfide as sulfur source is not well described in the literature. Hydrogen sulfide oxidation kinetics for *A. thiooxidans* have been reported [13]; but to the knowledge of the authors, no studies have reported growth kinetics using hydrogen sulfide as the electron donor.

The oxidation of hydrogen sulfide has been shown to be mainly a biological process [10,18]. In addition, the number of active hydrogen sulfide oxidizing bacteria, within the matrix of corroded concrete, has been identified as a key factor in predicting the corrosion rate of concrete exposed to hydrogen sulfide [19]. The objective of this study has therefore been to determine the growth kinetics of hydrogen sulfide oxidizing bacteria originating from corroded concrete when hydrogen sulfide is the source of reduced sulfur.

## 2. Materials and methods

### 2.1. Sample preparation

The growth kinetics were studied by suspending samples of concrete corrosion products in deionized water adjusted to pH 1 in order to mimic the acidic conditions found on corroded concrete [18]. The samples of corrosion products were collected from a pilot scale setup simulating a concrete gravity sewer with continuously dosing of hydrogen sulfide into the sewer atmosphere [20], and were thus expected to contain the microbial community responsible for the sulfuric acid production causing the corrosion. All samples of corroded concrete were kept at 4 °C after harvest and until usage as previous experiments has shown that the bacteria have a long survival time under these conditions [19].

Two types of experiments were carried out with five repetitions of each. In the first type, the pH was adjusted using 9 M sulfuric acid ($H_2SO_4$) whereas 6 M hydrochloric acid (HCl) was used in the other. The purpose of adjusting pH with hydrochloric acid was to reduce the background concentration of sulfate in order to facilitate the measurement of sulfate production by the bacteria. In order to further bring down the initial sulfate concentration in the samples adjusted with HCl, these were subjected to a number of washing steps. The washing consisted of suspension of the sample material in deionized water followed by centrifugation, decantation and finally resuspension of the pellet. This was repeated up to nine times. After washing, the concentration of sulfate in the suspension reached a concentration of approximately 2300–3000 g S $m^{-3}$. This is in the same order of magnitude as the solubility of gypsum under low pH according to the findings of Aagli et al. [21]. As the expected sulfate formation from the corresponding hydrogen sulfide consumption was significantly below this level, it was not possible to distinguish the formed sulfate from the background concentration and hence the results of the sulfate measurements are not presented. In the experiments with sulfuric acid for pH adjustment, approximately 15 g of sample material was used for each experiment. In the experiments with hydrochloric acid, approximately 22 g of sample material was used for each experiment to compensate for bacteria lost during the washing procedure.

The washed sample material as well as the unwashed sample material was suspended in deionized water to which 5 ml nutrient solution containing 20 g $NH_4Cl$ $l^{-1}$, 100 g $KH_2PO_4$ $l^{-1}$, 40 g $MgSO_4{\cdot}2H_2O$ $l^{-1}$, 1.5 g $CaCl_2$ $l^{-1}$, 1 g $FeCl_3{\cdot}6H_2O$ $l^{-1}$, and 1 g $MnSO_4{\cdot}H_2O$ $l^{-1}$. The pH of the solution was adjusted to 1 by the addition of hydrochloric acid. The nutrient solution was added to ensure that the growth of the bacteria was not limited by micro nutrients.

### 2.2. Experimental procedure

The sample suspension was kept in a closed batch reactor consisting of a 250 ml Erlenmeyer flask in which the oxygen and hydrogen sulfide concentration was measured continuously. The volume of the suspension was adjusted so that the reactor was without headspace. An expansion pipe in the stopper allowed the air to escape during aeration of the reactor. The reactor was filled so, that the solution was in the expansion pipe and level with the top of the rubber stopper. A water bath was used to buffer temperature changes, in this way a stable temperature could be maintained

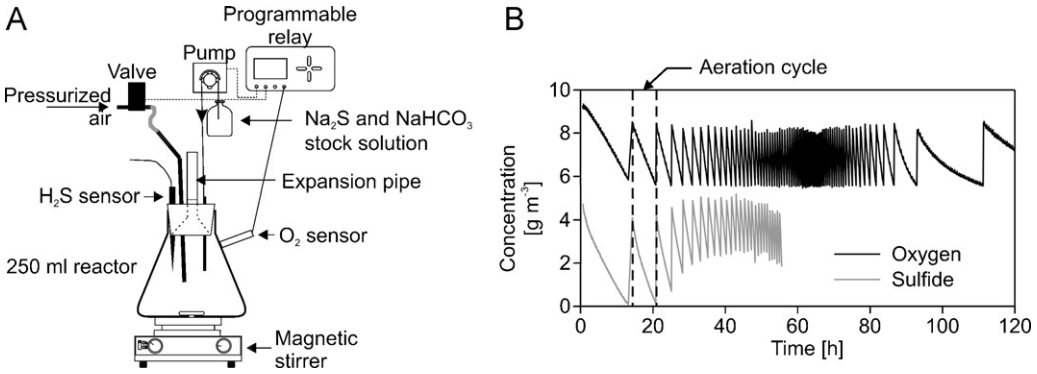


**Fig. 1.** Experimental setup (A) and an example of raw data from a growth experiment (B).

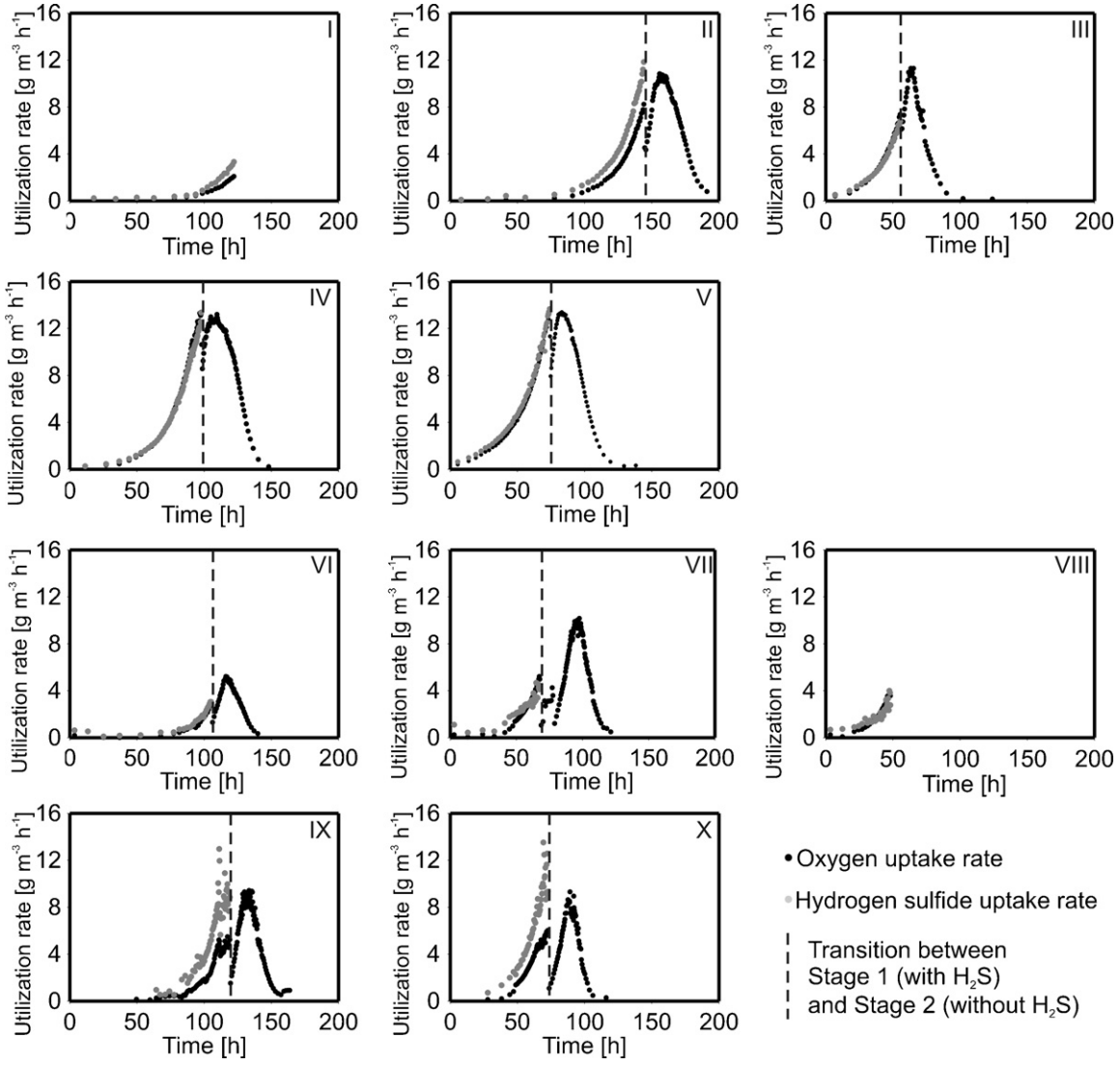


**Fig. 2.** Development in utilization rates for hydrogen sulfide and oxygen as function of time. Graphs I–V show results from experiments with sulfuric acid and graphs VI–X show results from experiments with hydrochloric acid.

through out each experiment (maximal standard deviation: 0.8 °C, generally the standard deviation was 0.4 °C). The average temperature was 22 °C. The water bath was made from a plastic box with water circulation provided using an aquarium pump. The reactor was operated in cycles using a programmable relay (Telemecanique SR3B101B, Schneider Electric Industries S.A.S., Rueil-Malmaison, France), which controlled aeration and addition of sulfide. In the beginning of each cycle, the suspension in the reactor was aerated with atmospheric air for 3 min to an oxygen concentration of approximately 80% air saturation to provide oxygen for the oxidation of hydrogen sulfide. After the aeration, a stock solution of 42 mM sodium sulfide ($Na_2S$) and 7 M sodium bicarbonate ($NaHCO_3$) in deionized water was pumped into the reactor. The sulfide concentration in the reactor corresponded to an equilibrium concentration with a gas phase concentration of 400–1000 ppm. This concentration range is not unrealistic to be found in a sewer atmosphere [22]. When the oxygen concentration dropped below 50% air saturation, a new cycle started. After establishment of sufficient microbial growth, the addition of sulfide was terminated and replaced with an addition of a stock solution containing 7 M sodium bicarbonate (Fig. 1). Sodium bicarbonate was added as source of carbon to compensate for the low solubility of $CO_2$ at the low pH.

The reactor was tested for diffusion of oxygen into the reactor as well as diffusion of hydrogen sulfide out of the reactor and auto-oxidation of hydrogen sulfide within the reactor. The diffusion of oxygen into the reactor was determined by filling the reactor with demineralised water which had been purged with nitrogen to an oxygen saturation of approximately 20%. The increase in the oxygen concentration was then monitored until it reached the level of 80% saturation, where the automatic aeration would start during an experiment. The loss of hydrogen sulfide due to auto-oxidation and diffusion was measured by filling the reactor with demineralised water with an oxygen saturation of about 80%. Hydrogen sulfide was then added and the decline in hydrogen sulfide concentration was monitored. The diffusion of oxygen into the reactor was 0.05 g $O_2$ $m^{-3}$ $h^{-1}$ within the oxygen concentration interval of the experiments. The loss of hydrogen sulfide due to diffusion and auto-oxidation was 0.2 g S $m^{-3}$ $h^{-1}$.

The rates of hydrogen sulfide and oxygen utilization showed the same pattern in all experiments (Fig. 2). Due to two sudden equipment failures, graphs I and VIII differ from the rest. The part of the data not affected by the failure is presented. In the following, the part of the experiments where hydrogen sulfide was present is designated Stage 1 and the part after depletion of hydrogen sulfide is designated Stage 2.

### 2.3. Analytical procedures

The concentration of hydrogen sulfide within the reactor was measured continuously using a hydrogen sulfide sensor (H2S-500, Unisense, Denmark). The oxygen concentration was likewise measured continuously using a fiber optic sensor with a sensor spot (Fibox 3, PreSens – Precision Sensing, GmbH, Germany). pH was measured using pH indicator strips (pH 0–2.5, Merck, Germany) 1–4 times a day.

In three of the experiments, where pH was adjusted using sulfuric acid, elemental sulfur was measured using a gas chromatograph with mass spectrometric detection (GCMS) (Thermo Finnigan Trace GC with a Trace MS detector, Thermo-Scientific, Denmark). The injection was performed with a split/splitless injector at 240 °C, the separation was performed using a Rxi-5 Sil MS capillary column (Restek, Bellefonte, PA, USA) (L 10 m, i.d. 0.18 mm, film 0.18 μm) in a temperature program (i.e. starting with 80 °C, keep for 2 min, ramping with 20 °C/min to 260 °C, hold for 5 min). Helium was used as carrier gas with 1.3 ml/min. Using these parameters, sulfur was eluted as one single symmetric peak with 2 s half width which was easy to integrate. The mass spectrometer was operated in selected ion monitoring mode (sulfur was analyzed with 56 ms dwelltime at $m/z$: 64, 96 and 256 atomic mass units (amu), respectively, while the musk xylene $D_{15}$ was monitored in a different retention time window with 167 ms dwell time at 294 and 312 amu). Cycle times were thus 0.5 s for the determination of both the internal standard and the sulfur. Source temperature was set to 180 °C. Each of the mass fragments was referred individually to the internal standard by the Xcalibur software. All samples were analyzed (injected) in duplicate. The final elemental sulfur concentration was calculated as an average of the results of the three mass fragments and also averaged over the two injections. Blank toluene runs have been performed five times in the series. When final sulfur concentration was calculated, the standard deviations on the averages ranged between 0.6 g S $m^{-3}$ and 5 g S $m^{-3}$. Samples were taken 1–4 times a day, the frequency increasing with increasing activity in the reactor. Elemental sulfur was extracted by shaking 0.5 ml of aqueous sample from the reactor with 0.5 ml of toluene (residue grade, VWR, Darmstadt, Germany) and the concentration of elemental sulfur was measured from the toluene phase using elemental sulfur as a calibrant. An internal standard calibration utilizing musk xylene $D_{15}$ (Ehrenstorfer, Germany) was used. For three samples, a second extraction was performed to control the extraction efficiency. This revealed extraction efficiencies of 60–70%. The limit of quantification was found to be approximately 6 g S $m^{-3}$. The results of the elemental sulfur analysis should therefore be considered as an indication for the formation and degradation of elemental sulfur rather as an exact representation of the concentration within the reactor.

In the experiments with washed sample and the pH adjusted by hydrochloric acid, samples for sulfate analysis were taken with the same frequency as the control of the pH. The samples were analyzed by ion chromatography (Dionex DX-600 model 50, USA) with an IonPac AS17 column. In the experiments where the pH was adjusted by sulfuric acid the sulfate concentration was not measured.

### 2.4. Calculation procedures

For each experiment, the sulfide utilization rate (SUR) and oxygen utilization rate (OUR) were calculated from the slope of the curves for each aeration cycle (Fig. 1), using the individual data points and the method of least squares. These rates were corrected to a temperature of 25 °C by an Arrhenius-type equation with a temperature coefficient of 1.07, typical for non-diffusion limited processes [5]. The loss of hydrogen sulfide and diffusion of oxygen into the reactor was small compared to the observed utilization rates and hence not accounted for in the calculation procedure.

The equations derived below were applied to calculate the growth rate from the SUR and OUR curves from each experiment. The determination of the growth rate was based on the assumption of exponential growth (Eq. (1)):

$$\frac{dX_{SOB}}{dt} = \mu X_{SOB} \tag{1}$$

where $X_{SOB}$ is the biomass concentration (g $m^{-3}$), $t$ is the time (h) and $\mu$ is the growth rate ($h^{-1}$).

Assuming that an increase in hydrogen sulfide oxidation rate is caused by growth of the hydrogen sulfide oxidizing biomass, Eq. (2) is derived for the description of the change in hydrogen sulfide oxidation rate:

$$\frac{dS_{H_2S}}{dt} = \mathrm{SUR} = \mu \frac{X_{SOB}}{Y_{H_2S}} \tag{2}$$

where $S_{H_2S}$ is the concentration of hydrogen sulfide (g S $m^{-3}$) and $Y_{H_2S}$ is the yield constant of the biomass with respect to hydrogen sulfide (g biomass $(g\,S)^{-1}$).

A similar equation can be written with respect to the oxygen consumption (Eq. (3))

$$\frac{dS_O}{dt} = \mathrm{OUR} = \mu \frac{X_{SOB}}{Y_O} \tag{3}$$

where $S_O$ is the concentration of dissolved oxygen (g $O_2$ $m^{-3}$) and $Y_O$ is the yield constant of the biomass with respect to oxygen (g biomass $(g\,O_2)^{-1}$).

The stoichiometry of the oxidation was calculated as the ratio between consumed oxygen and consumed hydrogen sulfide (Eq. (4))

$$\frac{\int_{t1}^{t2} \mathrm{OUR}\,dt}{\int_{t1}^{t2} \mathrm{SUR}\,dt} = \frac{\int_{t1}^{t2} \mu \frac{X_{SOB}}{Y_O}\,dt}{\int_{t1}^{t2} \mu \frac{X_{SOB}}{Y_{H_2S}}\,dt} = \frac{Y_{H_2S}}{Y_O} \tag{4}$$

where $t1$ and $t2$ are the start time and the end time of the experiments, respectively.

The calculations were done using the trapezoidal rule for numerical integration between the individual data points [23].

The data is represented by the population median rather than the average as not all data could be considered normally distributed according to the Lilliefors test of normal distribution [24]. The data scatter is illustrated by the 25% and 75% percentile of the data.

## 3. Results and discussion

The utilization rates for both hydrogen sulfide and oxygen for each experiment are shown in Fig. 2. The results from each of the experiments displayed a similar pattern. While hydrogen sulfide was present (Stage 1), an exponential increase in the utilization rate for both oxygen and hydrogen sulfide was observed (Fig. 2). After depletion of hydrogen sulfide (Stage 2), the oxygen utilization rate dropped immediately, but shortly here after it again increased sharply to form a second oxygen consumption peak (Fig. 2).

This pattern in oxygen utilization rate is most likely the result of first oxidation of hydrogen sulfide (Stage 1), followed by a secondary oxidation of the products from the hydrogen sulfide oxidation after hydrogen sulfide depletion (Stage 2).

### 3.1. Stoichiometry

The areas beneath the curves were used for the determination of the reaction stoichiometry. For the oxygen utilization rate, two areas were calculated (Table 2) under the curve corresponding to

**Table 2**
Stoichiometric coefficients for each part of the experiments as well as the total experiment. The data scatter is quantified as the 25% and 75% percentiles. OUR: oxygen utilization rate, SUR, sulfur utilization rate.

| | OUR/SUR Stage 1 | | | OUR/SUR Stage 2 | | | OUR/SUR total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Median | 25% Percentile | 75% Percentile | Median | 25% Percentile | 75% Percentile | Median | 25% Percentile | 75% Percentile |
| $H_2SO_4$ | 0.92 | 0.62 | 1.00 | 1.28 | 1.19 | 1.59 | 2.24 | 1.95 | 2.60 |
| HCl | 0.71 | 0.51 | 0.86 | 1.44 | 0.86 | 2.28 | 2.05 | 1.36 | 3.14 |
| Total | 0.75 | 0.62 | 0.97 | 1.28 | 1.05 | 1.86 | 2.24 | 1.62 | 2.71 |

Stage 1 and Stage 2, respectively. The area under the curve for the hydrogen sulfide utilization rate was likewise calculated. Based on these areas, the stoichiometric ratio of the oxygen and the hydrogen sulfide consumed was calculated according to Eq. (4). Using a Student's *t*-test, it was found that on a 95% level of significance, the ratios from experiments with sulfuric acid and experiments with hydrochloric acid were not statistically significant. Therefore, the median values calculated of the entire dataset are applied below.

The overall ratio between consumed oxygen and consumed hydrogen sulfide for all experiments had a median of 2.24 ($n = 8$; Table 2). In theory, the oxidation of hydrogen sulfide to elemental sulfur corresponds to a molar ratio between consumed oxygen and consumed hydrogen sulfide of 0.5 (Eq. (5)), whereas a ratio of 1 corresponds to the formation of thiosulfate (Eq. (6)), and a ratio of 2 corresponds to the formation of sulfate (Eq. (7)).

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O \quad (5)$$

$$H_2S + O_2 \rightarrow 0.5S_2O_3^{2-} + 0.5H_2O + H^+ \quad (6)$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (7)$$

The median of 2.24 is therefore too high to correspond to an overall formation of sulfate. The high ratio can partly be explained by the experiments being affected by the loss of hydrogen sulfide from the reactor and the diffusion of oxygen into the reactor which cause a slight overestimation of OUR and a slight underestimation of SUR. Loss and diffusion occurred during the entire experiment, however, their effect was most important in the initial part of the experiment, where the biological activity was low compared to these effects. For Stage 1, the ratio was higher than the ratio corresponding to the formation of elemental sulfur, however, not high enough to correspond to thiosulfate formation. This suggests the formation of a mixture of elemental sulfur and sulfur at a higher oxidation state than zero. This is in accordance with the findings of Jensen et al. [18].

The total amount of sulfur added as hydrogen sulfide was compared to the amount of elemental sulfur measured in three experiments (Fig. 3). These results support that elemental sulfur was first formed while hydrogen sulfide was supplied and then degraded after hydrogen sulfide had depleted. This is similar to the findings of [25], who proposed accumulation of elemental sulfur in bacterial cells to be storage of chemical energy for when external sulfide supply was absent.

The amount of elemental sulfur extracted from the suspension in the reactor during the experiments increased while hydrogen sulfide was supplied and decreased after the supply of hydrogen sulfide was terminated (Fig. 3). The difference between the added sulfur and the sulfur recovered as elemental sulfur could be explained as a combination of inefficient extraction of the sulfur and oxidation of the elemental sulfur while hydrogen sulfide was present. The latter was supported by the ratio between oxygen and hydrogen sulfide utilization, which was higher (0.75) than necessary for merely a formation of elemental sulfur (0.5) (Table 2).

### 3.2. Growth rates

The growth rate of the active biomass on hydrogen sulfide was evaluated using the data from Stage 1 of each experiment. Hence the increase in oxygen utilization rate and hydrogen sulfide utilization rate were simulated with Eqs. (2) and (3), i.e. the exponential equations describing the growth of hydrogen sulfide oxidizing biomass (Table 3). As for the stoichiometric coefficients

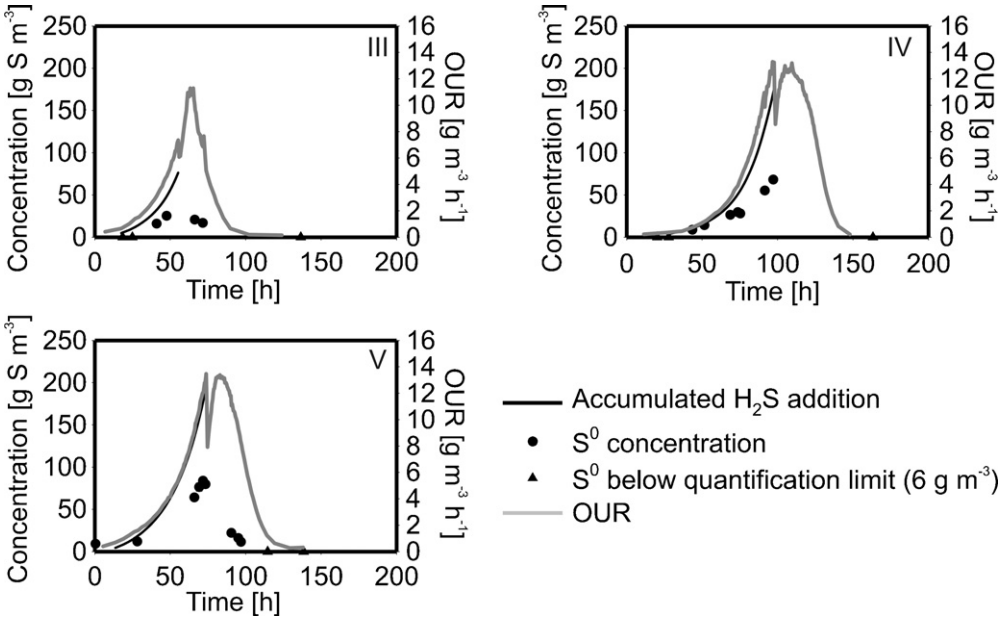


**Fig. 3.** Accumulated addition of hydrogen sulfide ($g\,H_2S\text{–}S\,m^{-3}$) and measured elemental sulfur concentration. The OUR curve illustrates the progress of the experiment.

**Table 3**
Median values for growth rates based on the hydrogen sulfide utilization rate (Eq. (2)) and the oxygen utilization rate (Eq. (3)). The data scatter was quantified as the 25% and 75% percentiles.

| | Growth rate, hydrogen sulfide ($d^{-1}$) | | | Growth rate, oxygen ($d^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Median | 25% Percentile | 75% Percentile | Median | 25% Percentile | 75% Percentile |
| $H_2SO_4$ | 1.22 | 1.12 | 1.30 | 1.26 | 1.19 | 1.35 |
| HCl | 1.32 | 1.13 | 1.51 | 1.77 | 1.33 | 1.99 |
| Total | 1.25 | 1.15 | 1.34 | 1.33 | 1.26 | 1.78 |

the difference between the results from the experiments using hydrochloric acid and the experiments using sulfuric acid for pH adjustment were not statistically significant at a 95% level of significance. Hence the use of hydrochloric acid for pH adjustment did not significantly alter the growth kinetics of the bacteria. It was also tested whether the rates based on the hydrogen sulfide utilization rates differed from the rates based on oxygen on a 95% level of significance, which was also not the case.

Comparing these growth rates (Table 3) to growth rates reported in the literature (Table 1), they are in the same order of magnitude as the specific growth rates reported for growth of *A. thiooxidans* with elemental sulfur as electron donor. The results of the present study indicate that the hydrogen sulfide oxidizing biomass on concrete surfaces in sewers can grow directly on hydrogen sulfide, i.e. in contrast to what has previously been reported [e.g. 7,26]. Thus it does not rely on chemical oxidation of hydrogen sulfide to elemental sulfur or thiosulfate, which is then oxidized for growth purposes, but also utilizes the energy released by the oxidation of hydrogen sulfide to elemental sulfur.

In order to predict the lifetime of concrete sewer pipes exposed to hydrogen sulfide, the linkage between the utilization of hydrogen sulfide on the surface and the production of sulfuric acid needs to be determined. This is also crucial for odor and health related prediction of the hydrogen sulfide distribution in sewer networks [20]. As the oxidation of hydrogen sulfide and sulfuric acid production are mediated by microbial processes, the prediction of the active biomass present on the surfaces is a key parameter. The growth kinetics of the hydrogen sulfide oxidizing bacteria when supplied with hydrogen sulfide as source of reduced sulfur is therefore essential. This study shows that under conditions where nutrients, hydrogen sulfide and oxygen are not limiting for the bacteria, the growth rate of the bacteria on hydrogen sulfide is comparable to the growth rate reported for pure cultures of acidophilic sulfur oxidizing bacteria with elemental sulfur as source of reduced sulfur. It therefore serves as a first step in predicting the biomass growth in the biofilm on the concrete surfaces based on the release of hydrogen sulfide from the wastewater.

## 4. Conclusion

Sulfur oxidizing bacteria originating from corroded concrete surfaces of a pilot scale sewer were grown in a batch reactor with hydrogen sulfide as the source of electron donor. The pH of corroded concrete material has been reported to be strongly acidophilic with pH 1 or less. Under such acidic conditions elemental sulfur was found to be produced when hydrogen sulfide was supplied as reduced sulfur source. The experiments showed exponential growth when hydrogen sulfide was supplied, with growth rates in the same order of magnitude as reported for pure cultures of *A. thiooxidans* when grown with elemental sulfur as the source of reduced sulfur under similar conditions. Upon depletion of hydrogen sulfide, the elemental sulfur produced was subsequently oxidized. The ratio between supplied sulfur and consumed oxygen suggested that sulfuric acid was the ultimate product of the oxidation process. The data obtained in this study can be used in sewer process modeling for the prediction of hydrogen sulfide induced concrete corrosion as well as the prediction of hydrogen sulfide concentration levels in sewer systems.

## Acknowledgement

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