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Hydrogen Sulfide Oxidation and Sewer Corrosion

Including the articles:

- Description of the hydrogen sulfide induced corrosion of concrete sewer systems - a new approach.
- Hydrogen sulfide uptake rates and biomass concentration of microorganisms from corroded concrete

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Preface

This Master's thesis is produced at Aalborg University and consists of 2 articles in the area of hydrogen sulfide oxidation and sewer corrosion. The study was written with supervisors Associate Professor Asbjørn Haaning Nielsen and Professor, with special responsibilities, Jes Vollertsen, at the section of Environmental Engineering, from Department of Biotechnology, Chemistry and Environmental Engineering at Aalborg University in Denmark.

The 1st article, "Description of the hydrogen sulfide induced corrosion of concrete sewer systems - a new approach ", concerns corrosion of concrete sewers, parameters for estimation of annual corrosion rates and influence of sewer atmosphere velocity on hydrogen sulfide removal rates.

The 2nd article, "Hydrogen sulfide uptake rates and biomass concentration of microorganisms from corroded concrete", concerns the relationship between the hydrogen sulfide removal rate and the biomass of hydrogen sulfide oxidizing microorganisms, measured in a suspension bioreactor. Furthermore, it concerns methyl mercaptane oxidation kinetics.

I would like to dedicate a special thanks to:

- Post-doctoral Researcher Henriette Stokbro Jensen for her help with the reactor setup, used in article 2.
- Associate Professor Jeppe Lund Nielsen for help with determination of the microbial diversity of reactor samples, used in article 2.
- Laboratory Technician Jytte Dencker for help in the laboratory.
- Associate Professor Peter Roslev for help with selection of method for biomass determination, used in article 2.
- Assistant Professor Kai Bester for general counseling.
- Line Winther, Helene Winther and Mia Busch Agergaard who contributed in various ways.

I would furthermore like to thank all the people associated to institute 18, Department of Biotechnology, Chemistry and Environmental Engineering at Aalborg University, for many experiences that implicit has contributed to this thesis.

The results of this thesis are accessible by contact to e-mail markkalhoej@msn.com and will be applied to www.kalhoej.com.

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Description of the hydrogen sulfide induced corrosion of concrete sewer systems - a new approach

Abstract

Hydrogen sulfide is removed from the sewer gas phase by absorption and oxidized on the exposed concrete surface, which results in concrete corrosion. The relation between actual and potential corrosion was investigated and described as a corrosion reduction factor. A study on the fate of partly oxidized hydrogen sulfide that is immobilized in corroded concrete was performed. This determined that the sulfur compounds immobilized in the corroded matrix has a significant corrosive potential. The immobilized sulfur compounds were oxidized to sulfuric acid and corroded the sewer, when a lack of hydrogen sulfide occurred. Furthermore, an investigation of the influence of atmosphere velocity on hydrogen sulfide removal rate in sewer systems was performed. This investigation indicated a significant increase in hydrogen sulfide removal with increasing sewer atmosphere velocities.

Introduction

Hydrogen sulfide formation in drainage systems often is a result of the wastewater transport to large centralized treatment plants. This results in long transport distances and requires pumping the wastewater through pressure pipes. In the pressure pipes, there is no reaeration of the wastewater as in the gravity pipes, which lead to anaerobic conditions and sulfide production because of microbial activity [Danish Environmental Protection Agency, 1988].

Sulfide is primarily produced in the sewer biofilm covering the submerged sewer walls. In a gravity sewer, where oxygen is present in the wastewater, the sulfide is oxidized back to sulfate, and usually does not cause any problems. Figure 1 illustrates the reduction of sulfate in the biofilm and the oxidation of sulfide back into sulfate. This process contributes to the oxygen consumption of the biofilm, thus a fall in DO concentration when it penetrates the biofilm.

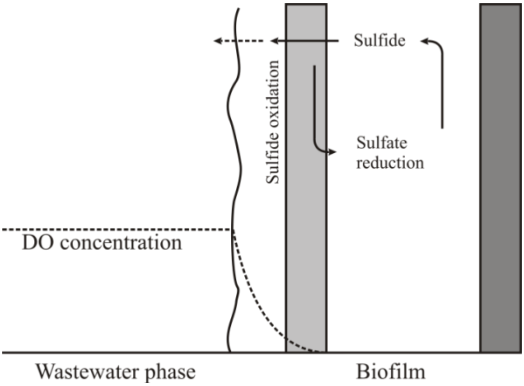

Figure 1. Sulfate cycle in submerged sewer biofilms and the DO concentration.

In pressure pipes where the DO concentration is zero in the wastewater phase, due to lack of reaeration, the sulfide cannot be oxidized and it diffuses from the biofilm into the liquid phase, as illustrated by the dotted arrow in Figure 1. At the outlet of a pressure pipe, where it becomes a gravity sewer again, the sulfide is partly released to the gas phase as hydrogen sulfide [Hvitved-Jacobsen, 2002].

From a facility standpoint, hydrogen sulfide corrodes the concrete pipes and wells of up to 5 mm per year [Mori *et. al.*, 1991]. This means that parts of the drainage systems must be replaced earlier than expected [Hvitved-Jacobsen, 2002]. The magnitude of the corrosion problems are concentration dependent and can be displayed in a simplified manner, as shown in Table 2.

Table 1. The facility consequences caused by different total sulfide concentrations in the wastewater [Hvitved-Jacobsen, 2002].

Sulfide concentration [mg S · L^{-1}]	**Corrosion problems**
<0,5	Small
0,5-2	Medium
>2	Significant

Low concentrations of sulfide in wastewater are not very problematic because it normally contains metals that will precipitate with the sulfide as relatively inert metal-sulfides. Total sulfide concentrations of 0.5 [mg S · L^{-1}] does not necessarily give significant problems in the sewers, but should be monitored. Serious problems can occur when sulfide concentrations exceed 2 [mg S · L^{-1}], and depending on the individual situation, a reduction of the hydrogen sulfide quantities should be considered.

The traditional approach for predicting H_2S related concrete corrosion is base on the postulate that all the hydrogen sulfide that can be released, is released into gas phase when the wastewater is exiting a pressurized pipe [US EPA, 1974]. Furthermore, it is assumed that all hydrogen sulfide is oxidized on the concrete surface and reacts with alkaline components of concrete. This does not correspond with what actually is observed, because moisture droplets on the concrete surface are assumed to drip into the wastewater, thus all the hydrogen sulfide does not react with the concrete. Even more significant is the fact that not all hydrogen sulfide is completely oxidized, but just partly oxidized, and ends up as immobilized elemental sulfur on the sewer wall [Parker, 1945; Jensen *et. al.*, in preparation]. These conditions were described as a corrosion reduction factor for the first time by US EPA [1974]. The factor was suggested to be between 0.3 and 1 depending on the hydrogen sulfide load, but without any references [Hvitved-Jacobsen, 2002; US EPA, 1974]. This hydrogen sulfide load dependency is not fully understood and is mentioned in a new report from the Water Environment Research Foundations as one of the largest research gaps of sewer processes right now [WERF, 2007]. The value of the corrosion reduction factor is actually unknown and so is the role of elemental sulfur in the corroded concrete matrix.

The Water Environment Research Foundation, WERF, has made a profound literature study [WERF, 2007] of the research on odors and corrosion in sewer systems, which resulted in, inter alia, a list of research gaps, where 2 of the 4 top-priority research gaps are:

- Improving understanding of the nature of sewer system ventilation.
- Improving understanding of the relationship between H_2S gas phase concentrations and corrosion.

There are three main objectives of this study; the first is to describe the relation between actual and potential corrosion in a corroded concrete sewer matrix. The second is to investigate the fate of the immobilized sulfur compounds in the corroded matrix. This pool of sulfur compounds, which is stored by the microorganisms in the corroded concrete on the sewer wall, is potentially corrosive and this study will investigate the actual corrosive effect. The third objective is to describe the relation between sewer atmosphere velocity and the rate of hydrogen sulfide removal on the sewer wall.

Materials and Methods

Hydrogen sulfide oxidation in sewer systems is investigated in a pilot scale setup at a sewer research and monitoring station in the town of Frejlev, a few kilometers west of Aalborg, Denmark [Vollertsen *et. al.*, 2008]. The Frejlev sewer monitoring station is located below the ground with easy access to a continuous supply of wastewater from a purely residential catchment of approximately 2000 inhabitants.

The experiments of this study are performed in 2 different pilot scale setups. The first setup is described right below and the second setup is described later. The first setup is used to investigate the relation between actual and potential corrosion of concrete sewers when exposed to hydrogen sulfide. The same setup is used to describe the actual corrosive effects of immobilized sulfur compounds, which is performed by comparing the corrosion caused during hydrogen sulfide addition and the corrosion by a period after hydrogen sulfide addition, referred to as post exposure corrosion. In order to describe these relationships, a setup of concrete sewers is constructed; in this case an already existing setup is modified to the purpose of this experiment. The setup is in a pilot scale, with 6 separate sewer reactors of concrete pipe segments that were cut from a standard Portland cement concrete pipe. Each reactor consists of 10 concrete pipe segments with an individual length of 0.2 meters, which gives the reactor a total length of approximately 2 meters. To make the reactor as similar as possible to real sewer pipes, it has an air circulation system, a wastewater circulation system, a wastewater renewal system, a wastewater depth of 0.05 m and a hydrogen sulfide gas injector. The air circulation system consisted of a 3.0 m PVC pipe with an inner diameter of 22 mm and a fan blower. The resulting gas velocity inside the reaction chamber was 0.053±0.007 $[m \cdot s^{-1}]$ measured with a flow-meter at the outlet of the PVC pipe [Nielsen, *et. al.*, 2008]. This result in a circulation time of approximately 40 s and is comparable to velocities measured in real sewers [Madsen *et. al.*, 2006]. The wastewater circulation is done by a centrifugal pump with capacity of pumping more than 1 ls^{-1}, but due to hydrostatic reduction in the system, the flow is approximately 0.3 ls^{-1}, which is a lower velocity than found in a real sewer of this size. The same applies for the wastewater renewal system where there is a renewal pumping session every third hour that exchanges 20% of the total volume, which means that the wastewater has an average cycling time of 15 hours. The wastewater parameters should not affect oxidation of hydrogen sulfide on the pipe wall significantly, because the oxidation in the water phase is a small fraction of the total oxidation. The hydrogen sulfide oxidation primarily takes place on concrete wall in contact with the gas phase [Vollertsen *et. al.*, 2008]. The two wastewater parameters, velocity and renewal, has a more significant influence on the hydrogen sulfide production under anaerobic conditions, but this reactor setup receives hydrogen sulfide from an external source to ensure a consistent gas phase concentration throughout the experiment. In order to ensure complete mixing of injected hydrogen sulfide in the sewer atmosphere and to avoid jet streams from the air circulation, a diffuser that covers both outlets is installed. The system is air and watertight and to avert a pressure buildup, a 1 mm pressure equalizer drilling is made in the back PVC plate.

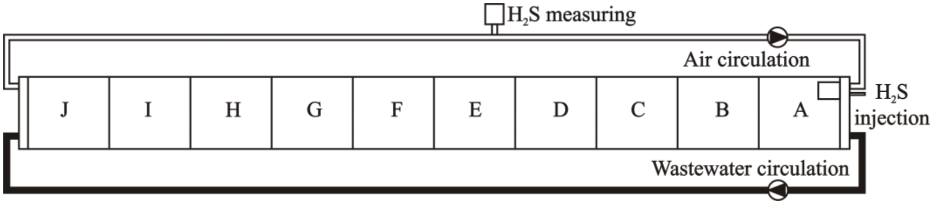

Figure 2. A principal figure of a reactor used to simulate corrosion process in sewers.

The whole system of each reactor is controlled by a Programmable Logic Controller (PLC) that operates the components of the system; this is an indispensable part of the reactor setup, which enables the operator to run the system with many different configurations. In this setup, the parameter configuration results in a hydrogen sulfide injection every hour and every injection makes the hydrogen sulfide gas phase concentration increase to an average of approximately 1100 ppm_v. Injecting every hour ensures that all hydrogen sulfide is absorbed before the next injection, which prevents a buildup of hydrogen sulfide gas [Nielsen, *et. al.*, 2008]. To document the absorption of hydrogen sulfide, the removal rate is measured 15 days into and throughout the experiment. According to Nielsen *et. al.* (2008), an increase in hydrogen sulfide removal rate to a range of 100 – 500 [mg S m^{-2} h^{-1}] at 100 ppm_v is expected. In order to document this, an Odalog gas detector (App-Tek International Pty Ltd, Brendale, Australia) was installed in the air circulation system. To ensure a precise measurement, the gas detector was calibrated using 100 ppm_v H_2S calibration gas (Euro-Gas Management Services Ltd, Plymouth, UK). The Odalog gas detector measures the hydrogen sulfide concentration in the atmosphere and stores it every 15 seconds. There was no temperature control and no continuous temperature measurements, but the pilot scale structure is placed underground and random temperature measurements indicate an average of 10ºC. The alkalinity of a concrete pipe was analyzed according to Snell *et. al.* (1966–1974). The method was modified for larger samples of approximately 5 g concrete and resulted in $CaCO_3$ equivalents of 0.181 g $CaCO_3$ / g concrete [Vollertsen et. al, 2008].

The experiment consists of concrete reactors that are paired up, Table 1. Reactor pair A consists of reactors I and IV, both reactors were exposed to H_2S for 48 days. After 48 days of exposure, half of the concrete segments from each reactor were washed and weighed. In order to compensate for H_2S injection point related complications, mentioned by Vollertsen *et. al.* (2008), the segments washed are a, c, e, g and i of reactor I and b, d, f, h and j of reactor IV, shown in Figure 3.

Table 1. Parameters describing the reactor setup.

Pipe pairs	A		B		C	
	I	IV	II	V	III	VI
Period of H_2S exposure	28-08-08 – 15-10-08		28-08-08 – 12-11-08		28-08-08 – 15-12-08	
Exposure time [days]	48		76		109	
Period of post exposure	15-10-08 – 20-12-08		12-11-08 – 17-01-09		15-12-08 – 19-02-09	
Post exposure time [days]	66		66		66	

When the start weight is subtracted the end-weight, the amount of corroded concrete is determined. Then, the reactors are reassembled and run for another 66 days without hydrogen sulfide addition, which should enable the bacteria to fully oxidize the sulfur to sulfuric acid on the undisturbed concrete segments [Jensen *et. al.*, in preparation]. After 66 days, the other half of the reactors are washed and weighed. The amount of corroded concrete is again determined by subtracting the end-weight from the start weight. This also accounts for the other reactors. The only difference is that the pair, II and V, is exposed to H_2S in 76 days, and the pair, III and VI, is exposed to H_2S in 109 days. All reactors have a post exposure period of 66 days after the period of hydrogen sulfide exposure.

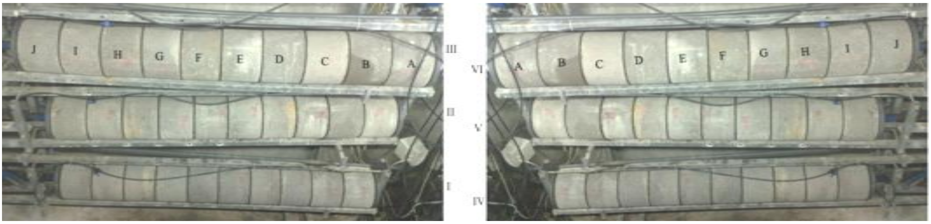

Figure 3. The experiment setup with six reactors consisting of 10 concrete segments each.

Figure 3 shows the reactor setup with the 6 reactors and the concrete segment distribution. The hydrogen sulfide injection point is at concrete segment A.

The second experiment setup is used to investigate the relationship between sewer atmosphere velocity and hydrogen sulfide removal rate. The first setup was reorganized to the purpose of ventilation experiment after the corrosion reduction factor and post exposure corrosion experiment was performed. The new setup is performed under the same circumstances as mentioned in the first setup, with two major changes; the first is that the setup was reorganized into two pipes, each consisting of 23 concrete segments instead of 6 pipes consisting of 10 segments each. The second is the air circulation system. The two pipes are connected in each end by two 3.5” rubber tubes and in one of the pipe ends, a centrifugal fan blower is fitted (ebm-papst Mulfingen GmbH & Co., Mulfingen, Germany). The blower is regulated by a volt signal 0 – 10 volt and is capable of generating a sewer atmosphere velocity of 1.9 [$m \cdot s^{-1}$]. The ventilation setup is illustrated in Figure 4.

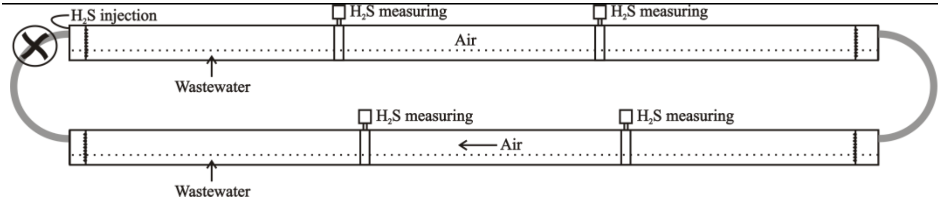

Figure 4. The ventilation setup consists of 2 pipe section of 4.6 meters each, a centrifugal fan blower and 4 hydrogen sulfide measuring points.

The ventilation setup has 9.2 meters of concrete segments, which results in 3.9 m^2 of hydrogen sulfide exposed area. 4 hydrogen sulfide measuring points are fitted into the setup and in each end of the concrete pipes; a sewer atmosphere mixing plate is fitted to avoid jet streams and ensure full mixing of the sewer atmosphere when it is entering and exiting the 3.5” rubber tubes. The experiment was performed at an average temperature of 20ºC and a wastewater depth of 0.05 m. A picture of the reorganized setup is shown in Figure 5.

Figure 5. Picture of the ventilation experiment setup.

The ventilation experiment is executed by injecting hydrogen sulfide every 20 minutes, which results in an instantaneous increase of the hydrogen sulfide concentration to approximately 200 ppm$_v$. This was performed at five different sewer atmosphere velocities, whilst measuring the hydrogen sulfide concentration with Odaloggers. The five sewer atmosphere velocities used in the experiment are shown in Table 2. The velocities were measured with a flow meter at the outlet of the rubber tube in the opposite end of the fan blower (Windmaster 2, Kaindl electronic, Germany). The measurements were converted to velocities in the concrete reactor before being displayed in Table 2. The optimal determination of the sewer atmosphere velocity would be a measurement inside the concrete reactor with the recirculation system fully closed. This was not possible due to limitations of the flow meter, regarding low velocities, but the conversion confirmed by a measurement at maximum velocity.

Table 2. Sewer atmosphere velocities investigated during ventilation experiments.

No.	Velocity [m s^{-1}]
1	0.1
2	0.5
3	0.7
4	1.1
5	1.9

In order to achieve steady state in the reactor, it has been running for 50 days before starting the experiment, which ensures that a corroded matrix is established. Furthermore, the experiments were executed in one day.

Calculations

During the experiments, the hydrogen sulfide concentration is measured in order to document the removal. The data is used to calculate a removal rate of hydrogen sulfide.

When calculating the total removal, it is divided into two processes, the atmosphere–concrete wall mass transfer and the atmosphere–wastewater mass transfer. According to Vollertsen *et. al.* [2008], the atmosphere–wastewater mass transfer is insignificant in concrete sewers due to the high removal rate on the concrete sewer wall, thus it was neglected in the calculation of the hydrogen sulfide removal, which then is described by an n-order kinetics equation:

$$\frac{dp_{H2S}}{dt} = c \cdot p_{H2S}^{n} \qquad (1)$$

where p_{H2S} is the partial pressure of H_2S (ppm_v), t is time in hours, c is the reaction constant ($\mathrm{ppm_v^{-n} h^{-1}}$) and n is the reaction order (-).

The removal of hydrogen sulfide is described as rate of hydrogen sulfide absorption dependent of the time:

$$f = c \cdot p_{H2S}^{n} \cdot \frac{V}{A} \cdot \frac{P}{RT} \cdot 10^{-3} \cdot M_w \qquad (2)$$

where f is the surface-specific hydrogen sulfide absorption rate (mg S m^{-2} h^{-1}), V is the volume of the sewer atmosphere (m^3), A is the pipe wall area exposed to the gas phase (m^2), P is the pressure (atm), R is the gas constant (m^3 atm K^{-1} mol^{-1}), T is the temperature (K), and M_w is the molar weight of sulfur (g mol^{-1}).

The hydrogen sulfide removal described in Equation 2 is used to describe the potential corrosion by converting it into a corrosion rate, r_{corr} [USEPA, 1974].

$$r_{corr} = \frac{100}{32} \cdot \frac{f}{A} \qquad (3)$$

where r_{corr} is the corrosion rate of the concrete surface (g m^{-2} h^{-1}), *f* is the hydrogen sulfide absorption rate on the concrete wall and A is the alkalinity of the concrete in units of $CaCO_3$. The corrosion rate describes the ratio between H_2S-S and $CaCO_3$, which potentially could react in a ratio of respectively 32 grams and 100 grams.

The equation can be reformed by dividing with the density of concrete, $\rho_{concrete} = 2.4 * 10^6$ g m^{-3}; the corrosion rate is transformed to corrosion depth per time:

$$c_{potential} = 11.4 \cdot \frac{f}{A} \qquad (4)$$

where $c_{potential}$ is the corrosion depth [mm y^{-1}]. The equation describes the corrosion depth in relation to a total oxidation of all hydrogen sulfide to sulfuric acid and a total reaction with the concrete. This is not the case, due to intermediates and runoff with condensed water, why the equation is expressed:

$$c_{actual} = k \cdot 11.4 \cdot \frac{f}{A} \qquad (5)$$

where c_{actual} is the actual corrosion depth [mm y^{-1}] and k is the corrosion reduction factor (-). The correction factor is usually between 0.3 – 0.4 under conditions where the concrete is exposed to high amounts of hydrogen sulfide, but at low exposure the factor approaches 1.0.

k, the corrosion reduction factor is calculated as:

$$k = \frac{Actual\ corrosion\ depth}{Potential\ corrosion\ depth} \qquad (6)$$

where the actual corrosion depth [mm] is the observed corrosion depth and the potential corrosion depth is theoretical corrosion depth based on the hydrogen sulfide load.

The actual corrosion depth must be determined, in order to determine k. the actual corrosion is calculated as:

$$Actual\ corrosion\ depth = \frac{m_{corroded\ concrete}}{\rho_{concrete} \cdot A_{H2S}} \qquad (7)$$

where $m_{corroded\ concrete}$ is the weight loss of the concrete due to corrosion and A_{H2S} is the area exposed to hydrogen sulfide.

Results and Discussion

The hydrogen sulfide addition results in an instantaneous increase in hydrogen sulfide concentration in the reactor. As mentioned in the method, the hydrogen sulfide is added every hour, which is substantiated by the Odalog measurements of Figure 6.

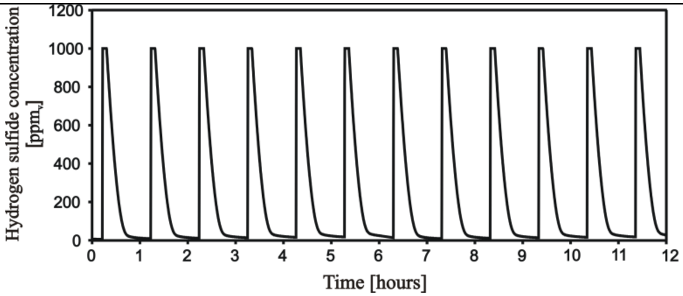

Figure 6. A concentration measurement of hydrogen sulfide injection and removal in a reactor. This is a cut from a larger datasheet of 24 hours, which contributed to the determination of one point in the hydrogen sulfide oxidation development in Figure 7.

The hydrogen sulfide addition in Figure 6 illustrates the general hydrogen sulfide load and removal. These measurements have been executed 3 times in each of the 6 reactors: the first 15 days into the experiment, the second after 34 days and the third after 72 days. The measuring cycles of hydrogen sulfide each consist of at least 24 hours of measuring that contribute to determination of the hydrogen sulfide removal rate.

The hydrogen sulfide removal rate in the six reactors is shown in Figure 7. The reactors have been running with different periods of hydrogen sulfide addition, as mentioned in Table 1, and are divided into three reactor pairs A, B and C. The hydrogen sulfide removal rate is documented to be increasing significantly during the first couple of months in concrete pipe reactors [Nielsen *et. al.*, 2008]. The increasing hydrogen sulfide removal is caused by growth of hydrogen sulfide oxidizing bacteria on the hydrogen sulfide exposed surface [Jensen *et. al.*, in preparation]. Figure 7 illustrates a general hydrogen sulfide removal during the hydrogen sulfide addition. The first measurement of hydrogen sulfide concentrations, thus removal rates, was made 15 days after the hydrogen sulfide addition began. The hydrogen sulfide removal rate after 15 days indicates that the activity was in the same order of magnitude reported by Nielsen *et. al.* (2008). Therefore, this should provide the same corrosive properties.

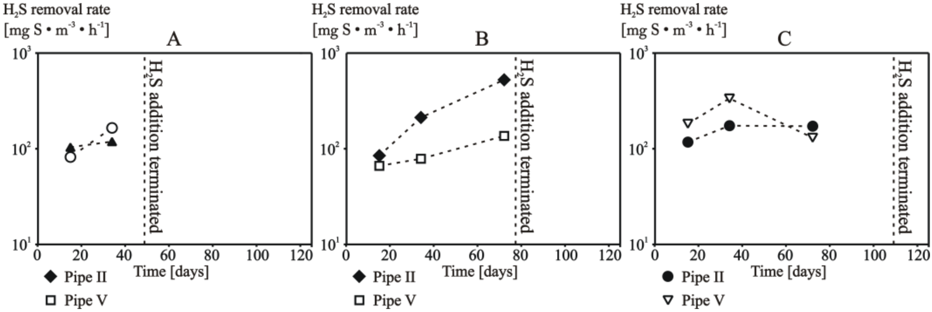

Figure 7. Hydrogen sulfide removal rate in the six reactors. Reactor pair A has been exposed to hydrogen sulfide for 48, reactor pair B has been exposed to hydrogen sulfide for 76 and reactor pair C has been exposed to hydrogen sulfide for 109.

The corrosion of the reactor pipes was visible at the point of washing, although not as much at the first washing step as later. The washing resulted in aggregates and gypsum to fall off, but before the washing, the concrete segments were visually inspected. The inspection revealed a significant difference between the concrete segments that was washed immediately after the termination of hydrogen sulfide addition and the segments with 66 days postponed washing. The segments washed immediately after the termination of hydrogen sulfide addition had small yellow grains, which is often seen in corroded concrete and is assumed to be deposits of elemental sulfur [Parker, 1945; Islander *et. al.*, 1991]. The concrete segments with 66 days postponed washing had significantly less yellow grains, which indicate a further oxidation of the sulfur compounds [Jensen *et. al.*, in preparation].

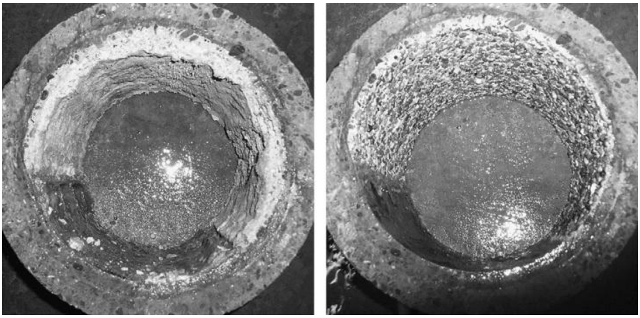

Figure 8. The hydrogen sulfide causes the concrete to corrode and swell up as white gypsum, as shown in the left picture. The washing of the concrete segments causes aggregates and gypsum to fall off.

The reactor segments was, as previously mentioned, washed in two stages to create a post exposure corrosion. The first washing of each reactor is shown in Figure 9. The average weight loss of the segments during hydrogen sulfide addition is shown with standard deviation. Simultaneously, as three steps, the development of the corrosion reduction factor is illustrated. In graph A, each of the 3 steps represents the corrosion reduction factor of the added hydrogen sulfide respective to each of the 3 periods. This means that the first step is calculated with basis in the first 48 days of hydrogen sulfide addition, the second step with basis in the next 28 days and third with basis in the last 33 days. In graph B, each of the 3 steps represents the corrosion reduction factor of the total amount of added hydrogen sulfide respective to each of the 3 periods. This means that the first step is calculated with basis in the first 48 days of hydrogen sulfide addition, the second step with basis in 76 days of hydrogen sulfide addition and third with basis in 109 days of hydrogen sulfide addition.

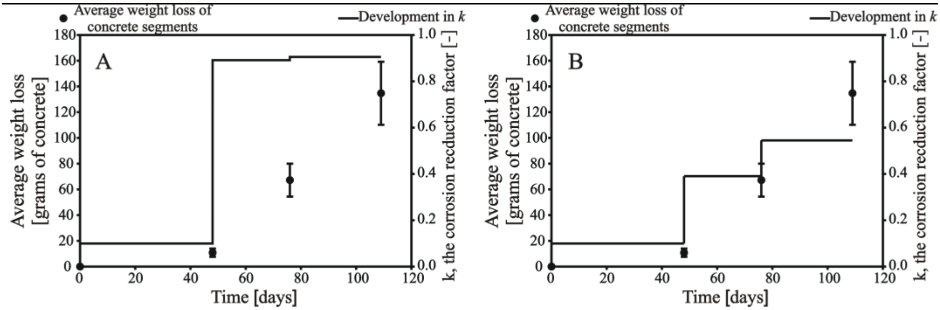

Figure 9. The average weight loss of the segments during hydrogen sulfide addition is shown with standard deviation. The development of the corrosion reduction factor throughout the hydrogen sulfide addition is shown as 3 steps that each represent the hydrogen sulfide addition of a period of time. In graph A it is the period between the weight loss measurements and in graph B it is the period from the beginning to the individual weight loss measurement.

When observing graph A, it indicates that the corrosion reduction factor becomes stationary, around 0.9, after 48 days with undisturbed conditions. When observing graph B, it indicates that the total corrosion reduction factor is increasing. This tendency indicates that the factor is high when a corroded matrix is established and that the total corrosion reduction factor is increasing when the corroded matrix is not removed. A removal of the corroded matrix could be causes by a full running sewer, for instance due to heavy rain. It seems that the hydrogen sulfide is stored in the corroded matrix when this is thin and maybe totally penetrated by the hydrogen sulfide. When the hydrogen sulfide is observed as a substrate, the hydrogen sulfide oxidation to elemental sulfur might be the easy oxidation, and the oxidation of elemental sulfur to sulfate might be the harder oxidation [Jensen *et. al.,* in preparation]. When the corroded matrix develops, the penetration becomes partial; the microorganisms begin to oxidize hydrogen sulfide fully into sulfate.

The indication of the sulfur compounds being further oxidized is documented by the average weight loss of the concrete segments, shown in Figure 10. The total oxidation of hydrogen sulfide to sulfuric acid has a corrosive property by sulfuric acid reacting with the alkaline components of the concrete [Hvitved-Jacobsen, 2002]. The concrete segments with 66 days postponed washing have a significantly increase in average weight loss, which is ascribe to oxidation of sulfur compound stored in the corroded matrix [Jensen *et. al.,* in preparation].

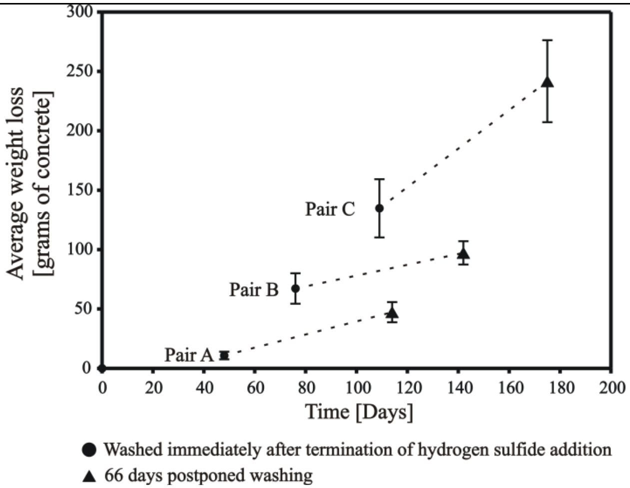

Figure 10. The average weight loss of the concrete segments with immediately and 66 days postponed washing.

Figure 10 shows significant differences in weight loss between immediate and postponed washing. This indicates more total oxidation of hydrogen sulfide, and therefore, more sulfuric acid that reacts with the alkaline components of the concrete, when the washing is postponed. As mentioned in Table 1, each reactor has been exposed to hydrogen sulfide for different periods. This might have an influence on the corrosion, as the activity is low in the startup phase of the reactors and increases due to development of the hydrogen sulfide oxidizing matrix on the sewer wall. The average weight loss of the concrete segments, when they are exposed to hydrogen sulfide, has a tendency of linearity with a lag phase in the start period.

The development in the average weight loss in Figure 10 indicates a significant further oxidation of the added hydrogen sulfide in the postponed washing segments. This tendency is also illustrated by the corrosion depths of Table 3. The corrosion depth of the immediately washed concrete segments, that has been exposed in 48 days, is significantly less than the corrosion depth of the 66 days postponed washing concrete segments. This is also indicated by the ratio between immediately and postponed washed segments.

Table 3. The hydrogen sulfide induced corrosion depths

Days of hydrogen sulfide addition	Corrosion depth of the hydrogen sulfide exposed area of the reactor [mm]		Ratio [Immediately / Postponed]
	Immediately washed	66 days postponed	
48	0.078	0.341	4.37
79	0.484	0.700	1.44
109	0.970	1.736	1.78

The concrete segments that have been exposed to hydrogen sulfide for 48 days have a much higher ratio between immediately and postponed washing than the ones that has been exposed for 79 and 109 days. This indicates that a large percentage of the added hydrogen sulfide is accumulated as elemental sulfur when the corroded matrix just has been removed. Furthermore, it indicates that when the corroded matrix has been left undisturbed for a longer period of time, the ratio between immediately oxidized and accumulated hydrogen sulfide decreases. This indicates that the hydrogen sulfide oxidizing microorganisms produce larger amounts of elemental sulfur in the development of in the corroded matrix than when a certain amount of biomass in the matrix is established. This might be caused by the diffusion boundary into the corroded concrete matrix on the sewer wall, which is created by the increasing oxidation capability of the biomass. The diffusion boundary might be forcing the microorganisms located deep inside the matrix to oxidize the elemental sulfur in greater extent than when all the microorganisms are exposed to high amount of hydrogen sulfide. Another way of describing this is that the microbial activity increases, and at some point, it is so high that the microorganisms is in a state of starvation and degrade all accessible hydrogen sulfide.

In order to assess the effect of the hydrogen sulfide in the reactors, the efficiency of the added hydrogen sulfide to each reactor is calculated, which is expressed as an observed corrosion reduction factor, k-factor, shown in Figure 11. The observed corrosion reduction factors in Figure 11 are calculated on basis of the total amount of hydrogen sulfide added to the reactor.

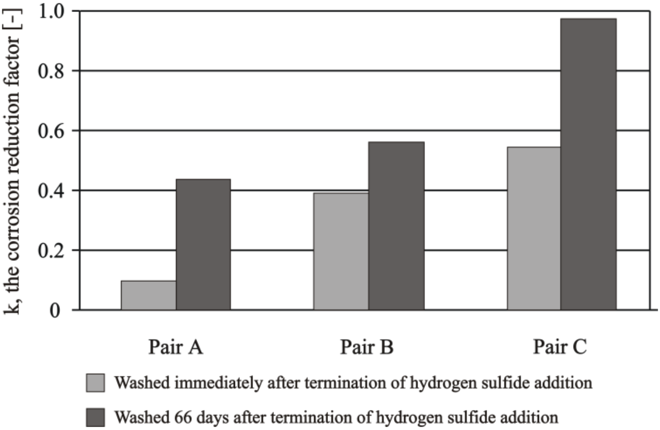

Figure 11. The corrosion reduction factors of the 3 reactor pairs are shown. The columns illustrate the actual corrosion of the pipe segments as a fraction of the potential corrosion that the amount of hydrogen sulfide could cause.

The tendency of Figure 11, where the actual corrosion of the 3 reactor pairs is shown as a ratio of the potential corrosion cause by the added amount of hydrogen sulfide, is a significant corrosion efficiency of postponed washed segments. This is illustrated by a significant increase in the corrosion reduction factor. The corrosion reduction factor is nearly 1 after postponed washing in reactor pair C, which indicates that the actual corrosion is nearly 100% of the potential corrosion. In this case, the post exposure is responsible of 44% of the corrosion. The corrosion reduction factor varies from 10% to 98% in efficiency throughout the experiment, which indicates that different parameters affect the corrosion efficiency strongly. The experiments and the literature indicate that the three significant parameters are the growth and establishment of hydrogen sulfide oxidizing matrix, the hydrogen sulfide load, and post exposure periods.

These parameters should be considered and a corrosion equation should be expressed, but the amount of parameters would complicate this too much. This could only be executed a full modeling of the sewers. Even so the parameter can be used separately in order to enable a wise choice of corrosion reduction factor.

The second reactor setup was, as mentioned, used to perform a ventilation experiment. The ventilation experiment was performed at 5 sewer atmosphere velocities and in an interval of 0 – 200 ppm_v of hydrogen sulfide. This is represented by the hydrogen sulfide removal rate at 10 ppm_v with the velocity as a variable, Figure 12.

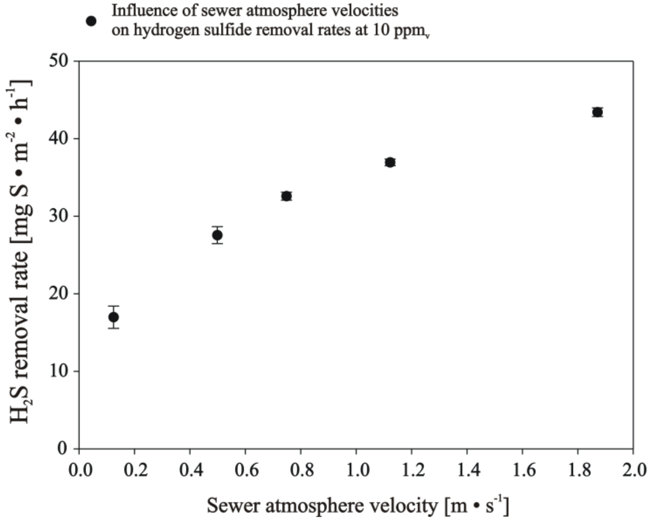

Figure 12. The influence of sewer atmosphere velocity on the hydrogen sulfide removal rate at 10 ppm_v

The relation between the hydrogen sulfide removal rate and the sewer atmosphere velocity in Figure 12 indicates that the absorption of hydrogen sulfide into the corroded matrix is dependent on the sewer atmosphere velocity. It is not linearly dependent, but has a tendency of going towards a maximum oxidation rate as the velocity increases. It could be an n-order dependency, with $n < 1$, in this and then a max as when velocity goes towards ∞. When the velocity dependency is considered, the corrosion of the sewer could be distributed to either a small section of a sewer or to a much bigger part of the sewer by ventilating the sewer at a high velocity, which is due to the non linear dependency of velocity.

Conclusion

The investigation of the relation between actual and potential corrosion, by hydrogen sulfide oxidation in concrete sewer, indicates a significant loss of corrosion potential when the corroded matrix is largely removed. The corrosion was reduced to 10% of the potential in a period of 48 days after the corroded matrix was largely removed. When the matrix was established again, it results in a much higher corrosion reduction factor, thus an actual corrosion of approximately 90% of the potential corrosion. The experiment showed that the actual corrosion remained at 90% when the corroded matrix was undisturbed. This knowledge could be implemented as a corrosion prevention method, as it indicates that the intermediates from hydrogen sulfide oxidation are stored in the corroded matrix, for a period of time and could be washed off.

The consequences of hydrogen sulfide oxidation were studied, with the post exposure corrosion as one of the main objectives. The results of the experiments indicate that the post exposure has a significant influence on the total corrosion of concrete sewers. The original assumption that the hydrogen sulfide, that did not react with the concrete, ran off the sewer walls, is very uncertain according to these results. The actual corrosion was nearly 100% of the total potential corrosion after 109 days of hydrogen sulfide exposure and 66 days post exposure. In this case, the post exposure was responsible of 44% of the corrosion.

This is the first time the corrosion reduction factor, k-factor, actually has been determined and documented. The general perspective is that the k-factor is low, $k \approx 0.1$, in a period of 1-2 months after the corroded matrix is largely removed, thus the corrosion is insignificant despite a large potential. The k-factor is increasing linearly after 1-2 months and becomes more and more significant. Furthermore, it is indicated that a post exposure period of 2 months enables a very high k-factor, $k > 0.9$. This indicates a significant corrosion potential of concrete sewer when the corroded matrix is undisturbed.

The dependency of sewer atmosphere velocity in relation to the hydrogen sulfide removal rate is relevant, in order to predict where the hydrogen sulfide oxidation is occurring. In addition, it is relevant in relation to modeling the hydrogen sulfide oxidation in sewers. The results of the experiment indicate that the hydrogen sulfide removal on the corroded sewer surface is velocity dependent and that it is increasing towards a maximum hydrogen sulfide removal rate at high velocities. It is the first time that a sewer atmosphere velocity dependency of the hydrogen sulfide removal rate has been determined and it seems to be a significant parameter in the description of the hydrogen sulfide removal rate. The hydrogen sulfide removal rate dependency of sewer atmosphere velocity was investigated in a velocity interval of 0.1 – 2 $[\mathrm{m} \cdot \mathrm{s}^{-1}]$ and the hydrogen sulfide removal rate increased 260% within this interval.

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Hydrogen sulfide uptake rates and biomass concentration of microorganisms from corroded concrete

Abstract

Hydrogen sulfide oxidation in sewer systems was investigated in a bench scale bioreactor, with corroded concrete in suspension. The hydrogen sulfide concentration and the oxygen concentration were measured continuously during the run of the bioreactor. Samples for protein analysis were taken throughout the experiments. The results of these experiments indicate a variation in activity between different hydrogen sulfide oxidizing microorganisms. As an attempt to achieve a monoculture of the most active hydrogen sulfide oxidizing microorganisms, the reactor experiment was started by inoculating from a running reactor. This resulted in a better relation between hydrogen sulfide removal rates and protein concentration, as the bacterial diversity approached a culture of Acidithiobacillus.

As a sub study, the kinetics of methyl mercaptane oxidation in sewer systems was investigated in the same bioreactor. The results of the methyl mercaptane oxidation showed that the diversity of microorganisms in corroded concrete enabled oxidation of methyl mercaptane. Furthermore, the results indicate that the culture of Acidithiobacillus that were achieved by inoculation, were unable to oxidize methyl mercaptane.

Introduction

Hydrogen sulfide causes many problems in sewer systems. These problems have origin in sulfide formation. Sulfide is primarily formed by sulfate respiration where heterotrophic anaerobic bacteria oxidize organic compounds with sulfate as terminal electron donor. Carbohydrates, amino acids and alcohols are good substrates for sulfur-reducing bacteria **[**Tanaka and Hvitved-Jacobsen, 2001; Nielsen *et. al.*, 1998]. Apart from sulfate, other sulfur compounds like thiosulfate, sulfite and elemental sulfur are available to be reduced to sulfide [Tchobanoglous, 1981]. The term “hydrogen sulfide” describes the substance in the gas phase $H_2S_{(g)}$ and dissolved phase $H_2S_{(aq)}$, whereas sulfide describes sulfide ions in the liquid phase, all shown in Equation 8. The equation illustrates the relationship of hydrogen sulfide in gas phase and aqueous dissolved phase, which can be expressed by Henry's Law. Furthermore, it describes the distribution of aqueous dissolved sulfide that is evenly distributed between H_2S and HS^- at pH 7. S^{2-} is only found in measurable quantities at pH above 12 [Hvitved-Jacobsen, 2002].

Equation 8. The equilibrium equation between sulfide and hydrogen sulfide gas.

$$H_2S_{(g)} \overset{\text{Water-air transfer}}{\leftrightarrow} H_2S_{(aq)} \overset{pKa1=7.0}{\leftrightarrow} HS^- \overset{pKa2=14}{\leftrightarrow} S^{2-}$$

Hydrogen sulfide is problematic in several ways because it has an unpleasant smell, even at low concentrations, which can be annoying for residents in the vicinity of a pressure pipe outlet [Hvitved-Jacobsen, 2002]. Higher concentrations of hydrogen sulfide are toxic. Concentrations and effects of hydrogen sulfide are shown in Table 4.

Table 4. Odor and health effects of hydrogen sulfide in the atmosphere, recording to U.S. National Research Council, 1979 and ASCE, 1989.

Effect	**Concentration in the atmosphere [ppm_v]**
Limit for odor detection	0.0001-0.0002
Unpleasant smell	0.5-30
Headache, nausea and also eye, nose and throat irritations	10-50
Inactivation of the sense of smell	≈50
Serious eye and respiration problems	50-300
Life threatening	300-500
Instant death	>700

Furthermore, hydrogen sulfide is a compound with corrosive properties when it is oxidized and converted to sulfuric acid [Hvitved-Jacobsen, 2002]. This happens through the oxidation of emitted hydrogen sulfide on the sewer wall, where it is absorbed in the humid film on the concrete wall, and oxidized either chemically or biologically by aerobic autotrophic bacteria to sulfuric acid [Vincke *et. al.*, 2000]. Sulfuric acid is neutralized by the concrete alkalinity, which degrades the concrete surface to gypsum, whereby the concrete loses its physical strength [Hvitved-Jacobsen, 2002; Parker, 1945]. A simple stoichiometric example of concrete corrosion is shown below.

Equation 9. Simplified concrete corrosion [Hvitved-Jacobsen, 2002].

$$H_2SO_4 + CaCO_3 \rightarrow H_2O + CO_2 + CaSO_4$$

Usually, the worst corroded areas are just above the water level and at the sewer crown [Danish Environmental Protection Agency, 1988]. This might be due to the higher humidity just above the water level and at the sewer crown, why sulfuric acid reacts easier. Hydrogen sulfide is not the only compound produced under anaerobic conditions that causes problems in the sewers. There are many compounds with vast odorous complications; one is methyl mercaptane, an organic sulfur compound, which is produced under anaerobic conditions in the sewer. Methyl mercaptane is sometimes observed in higher concentrations than hydrogen sulfide [Hwang, Y, 1995]. The growth parameters of hydrogen sulfide oxidizing microorganisms are relevant in order to describe the corrosion of sewers in further detail. This study will contribute to a better understanding of the hydrogen sulfide oxidation kinetics, which is a one of the controlling parameters in modeling concrete sewer corrosion [Article 1], thus this study will contribute to a better prediction of concrete sewer corrosion.

The growth characteristics of hydrogen sulfide oxidizing microorganisms from corroded concrete, with the hydrogen sulfide uptake rate as growth parameter, were determined in Jensen *et. al.* [In preparation], but that study does not relate the growth activity to the actual biomass concentration. The objective of the present study is to investigate the relationship between the biomass and the hydrogen sulfide uptake rate with protein analysis throughout a growth experiment. As an addendum, a sub-study of methyl mercaptane oxidation is performed. This has an objective of investigating the oxidation of other odorous sulfur compounds by hydrogen sulfide oxidizing microorganisms from corroded sewer concrete.

Materials and Methods

The oxidation of hydrogen sulfide is measured in a batch reactor. Two types of experiments have been made in the reactor, one where corrosion product is suspended in a sulfuric acid solution and another where an inoculation from an active reactor was made to isolate the active microorganisms. The corrosion product is collected from the pilot scale experimental setup used in the first article [Article 1], thus it should contain hydrogen sulfide oxidizing microorganisms [Parker, 1945]. It is unknown if the corrosion product contains any methyl mercaptane oxidizing microorganisms or if the hydrogen sulfide oxidizing microorganisms also oxidize methyl mercaptane; however, no methyl mercaptane was added during the growth experiments. The reactor is fitted with different kinds of equipment to document the activity. The reactor is shown in Figure 13 and consists of a 250 ml conic glass wessel, which contains an electrochemical hydrogen sulfide electrode to measure the sulfide concentration in the reactor (Unisense, Aarhus, Denmark).

The electrochemical hydrogen sulfide electrode is connected to a PA2000 picoamperemeter that detects the current through the electrode, which increases with the H_2S concentration (Unisense, Aarhus, Denmark). The picoamperemeter is connected to a computer through an IQ VmA-4 data logger that sends the data to a computer through an USB cable (ComputerBoard, USA). When the reactor is running, it is controlled by a PLC that controls the time of aeration through a voltage threshold. When the oxygen saturation decreases to 60% of the oxygen concentration at total air saturation, the aeration is activated, and when 90% saturation is reached, the aeration inactivates. The oxygen concentration is measured with an oxygen sensitive sensor spot that is placed inside the reactor and the oxygen concentration is measured through an optic fiber. The oxygen sensor is connected Fibox that is connected to a computer with an USB connection (PreSens, Germany). The reactor is aerated with pressurized air, the pH of the reactor is kept at 1 during the experiment by addition of sulfuric acid and the experiments is performed in a temperature regulated container at 20ºC.

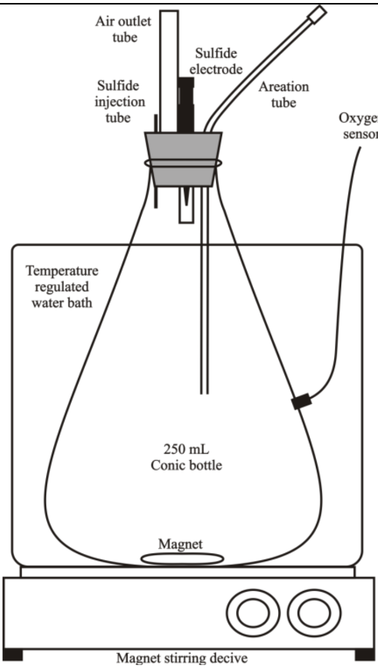

Figure 13. Reactor setup for the hydrogen sulfide oxidation experiment with different equipment fitted in a 250 ml glass wessel.

When a reactor cycle is started, a series of calibrations and solutions are made. At first, the oxygen sensor and the hydrogen sulfide electrode are calibrated. Then, a nutrient solution is prepared by defrosting a premixed nutrient solution that consists of 6 different nutrient salts, which were selected with reference to Jensen *et. al.* [In preparation]. The concentration of nutrient salts is described in Table 5.

Table 5. The concentration of nutrient salts in the stock solution and reactor with 5 ml nutrient solution.

Nutrient salt [-]	**Stock concentration [g/l]**	**Reactor concentration [g/l]**
NH_4Cl	20	0.4
KH_2PO_4	100	2
$MgSO_4 \cdot 2H_2O$	40	0.8
$CaCl_2$	1.5	0.03
$FeCl_3 \cdot 6H_2O$	1	0.02
$MnSO_4 \cdot H_2O$	1	0.02

The nutrient salts were used in other similar projects and selected with consideration of microbial growth [Jensen *et. al.*, in preparation]. In order to grow, the hydrogen sulfide oxidizing microorganisms need an electron donor. In this case, sulfide is used and it is added as disodium sulfide in a water solution, which is an alkaline solution, pH > 12. The sodium sulfide solution contains approximately 1% w/v sodium sulfide and when this alkaline solution is injected into the reactor that has pH of 1, a new pH equilibrium is reached. However, due to the strong acidity of the reactor, the pH of 1 is maintained. The low pH makes hydrogen sulfide available for the hydrogen sulfide oxidizing microorganisms, Equation 8. The hydrogen sulfide is added after aeration of the reactor using a peristaltic pump, where approximately 2 ml of the solution is added.

Consumption of oxygen is primarily caused by hydrogen sulfide oxidation. Consequently, both oxygen and hydrogen sulfide concentrations are measured throughout the experiment with an interval of 15 seconds. The hydrogen sulfide oxidation has different reaction pathways [Brüser *et. al.*, 2000]. The hydrogen sulfide oxidation to elemental sulfur, the further oxidation of elemental sulfur to sulfate and the direct oxidation of hydrogen sulfide to sulfate are described in Table 6. Sulfur has different oxidation stages in these reaction pathways, and when the hydrogen sulfide is fully oxidized to sulfate, the O_2/S ratio is 2.

Table 6. Different oxidation pathways of hydrogen sulfide, and the appertaining ratio between oxygen and hydrogen sulfide.

Reaction	O_2/S [mol/mol]
$2H_2S + O_2 \rightarrow 2S^0 + 2H_2O$	0.5
$2S^0 + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+$	1.5
$2H_2S + 4O_2 \rightarrow 2SO_4^{2-} + 4H^+$	2

In order to describe the uptake of hydrogen sulfide performed by microorganisms, the abiotic hydrogen sulfide removal processes are determined. These processes are the diffusion out of the reactor and chemical hydrogen sulfide oxidation, which is subtracted from the total oxidation of hydrogen sulfide. Furthermore, the reoxygenation of the reactor is determined and is added to the oxygen uptake rate. The determination of these parameters is done in two different experiments. First, a continuous measurement of reoxygenation in the reactor, and then, a linear regression of the data in the interval used in the reactor experiments, which is 60 - 90% of the oxygen concentration at total air saturation. Second, a continuous measurement of hydrogen sulfide being removed from the reactor, followed by a linear regression of the data in the interval used in the reactor experiments, which is 0 – 0.5 mg S / l. The regression results are shown in Table 7.

Table 7. The chemical oxidation and diffusion of sulfide as hydrogen sulfide removal rate and the reoxygenation rate.

Sulfide	**0.2**	**mg S / hour**
Oxygen	**0.05**	**mg O_2 / hour**

As mentioned, Table 7 contains two parameters, the abiotic hydrogen sulfide removal rate and the reoxygenation rate. The abiotic sulfide rate is represented by the sum of sulfide diffusion from the wessel as hydrogen sulfide and the chemically oxidized sulfide. The reoxygenation and the diffusion of hydrogen sulfide happen through the leaks of the reactor, which mainly is the air outlet tube, shown in Figure 13. These parameters are implemented in all the measurements before they are presented.

During the experiments, 5 ml samples were taken out for protein analysis. These were frozen and analyzed at the same time with BiCinchoninic Acid (BCA) assay. The assay is a Micro BCA Protein Assay that combines the well-known reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by BCA [Pierce Protein Research Products, 2008]. The samples are acidic at the point of defrosting and the pH is adjusted to approximately 9 with sodium hydroxide, in order to make the reduction of cobber possible.

The experiments are divided into 2 different types, depending on how they are started. The first with 14 grams corroded concrete added to the reactor, as an experiment start. The second is started with 5 ml of reactor fluid from a reactor that is running, as an inoculation. This is performed in order to reach monoculture that should give a better correlation between activity and biomass concentration. The corroded concrete contains a variety of microorganisms [Okabe, 2007]. This can have influence on the ratio between hydrogen sulfide oxidation and the biomass, due to variation in the activity. Furthermore, at the end of 3 experiments, Table 8, methyl mercaptane is added to observe the microorganisms', grown on hydrogen sulfide, ability to oxidize methyl mercaptane.

The oxidation of methyl mercaptane may be done by microorganisms represented in the corroded concrete, which also contains hydrogen sulfide oxidizing microorganisms. The sodium methyl mercaptane solution contains 0.5% methyl mercaptane. The methyl mercaptane is also available for the microorganisms upon injection due to pH change.

The experiments are not performed under the same conditions, as just described and the same analysis have not been performed in all experiments. In Table 8, the different parameters of each experiment are shown.

Table 8. Measurements and start conditions of each experiment.

Experiment	A	B	C	D
Corroded concrete as start	X	X		
Inoculation as start			X	X
Hydrogen sulfide measurement	X	X	X	X
Oxygen measurement	X	X	X	X
Protein measurement		X	X	X
Methyl mercaptane addition		X	X	X

Table 8 shows that experiment A and B is started with corroded concrete and experiment C and D is started by inoculation from a running reactor. The inoculation in experiment C is made from B and the inoculation in experiment D is made from C. This procedure should isolate the hydrogen sulfide oxidizing culture. The bacterial diversity has been determined in experiment A and C, in order to support this theory. The determination was performed by a PCR with nonspecific 16S ribosomal primers, followed by a cloning and sequencing. These sequencings was analyzed by phylogeny and compared with available public database.

Calculations

There are different calculations involved in the data analysis. The hydrogen sulfide uptake rate (SUR) is calculated as:

$$\frac{\mathbf{dS_{H_2S}}}{\mathbf{dt}} = \mathbf{SUR} \qquad (10)$$

where S_{H_2S} is the sulfide concentration [g S m^{-3}] dt is time [hours] and SUR is the hydrogen sulfide uptake rate [g S m^{-3} h^{-1}].

The increase in hydrogen sulfide uptake is assumed to be caused by an increase in hydrogen sulfide oxidizing organisms, which enables the description of SUR as a function of biomass:

$$\frac{\mathbf{dX_{SOB}}}{\mathbf{dt}} \cdot \frac{\mathbf{1}}{\mathbf{Y_{H_2S}}} = \mathbf{SUR} \qquad (11)$$

where X_{SOB} is the biomass concentration [g m^{-3}] and Y_{H_2S} is the yield constant of biomass with respect to hydrogen sulfide [g biomass (g S)$^{-1}$]

The oxygen uptake rate (OUR) is calculated as:

$$\frac{\mathbf{dS_O}}{\mathbf{dt}} = \mathbf{OUR} \qquad (12)$$

where S_O is the oxygen concentration [g O_2 m^{-3}] OUR is the oxygen uptake rate [g O_2 m^{-3} h^{-1}]

When the uptake rates are compared, the stoichiometric of hydrogen sulfide oxidation is discovered as the ratio between oxygen and hydrogen sulfide:

$$\mathbf{ratio}\ \frac{\mathbf{O_2}}{\mathbf{S}} = \frac{\int \frac{\mathbf{dS_O}}{\mathbf{dt}}}{\int \frac{\mathbf{dS_{H_2S}}}{\mathbf{dt}}} \qquad (13)$$

The ratio is used to determine the variation in the reaction stoichiometry throughout the experiment.

Results and Discussion

The experiments with hydrogen sulfide oxidation are, as mentioned in the method, divided into two different experiments with either corroded concrete or inoculation as experiment start. The experiments differ in activity, depending on these start conditions. The oxygen concentration throughout an experiment started with corroded concrete is shown in principal in Figure 14.

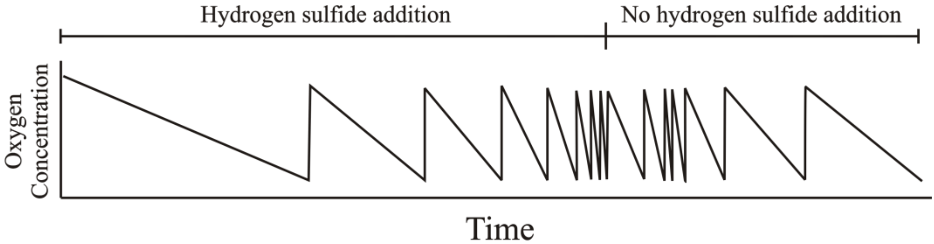

Figure 14. The oxygen curve describes the oxygen uptake through an experiment with corroded concrete as start condition in principal.

Figure 14 indicates a low activity at first, but a steady increase in oxygen uptake until the addition of disodium sulfide stops, after which the oxygen uptake suddenly decreases. The oxygen uptake increases again after the termination of sodium sulfide addition. When all sulfur is oxidized into sulfate, the activity decreases to a much lower level.

The other start condition is when the experiment is started by inoculation and this result in an oxygen uptake tendency, which is indicated in principal by Figure 15.

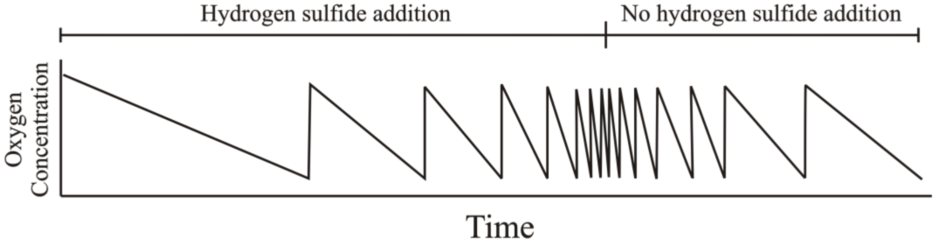

Figure 15. The oxygen curve describes the oxygen uptake through an experiment with inoculation as start condition in principal.

Figure 15 indicates low oxygen uptake in the beginning, which increases until the addition of sodium sulfide stops. The oxygen uptake decreases after termination of sodium sulfide addition.

The tendency of the hydrogen sulfide concentration is similar to the tendency oxygen. The hydrogen sulfide concentration is zero at the time of hydrogen sulfide addition due to the aeration of the reactor, which causes a striping of the hydrogen sulfide. It is assumed that the hydrogen sulfide uptake continues, during the period of aeration, with the rate determined before the aeration. This means that the hydrogen sulfide uptake rate is unaffected by stripping hydrogen sulfide.

The two different experiments are both executed twice, which resulted in four hydrogen sulfide removal rate curves and four oxygen uptake rate curves that all are shown in Figure 16.

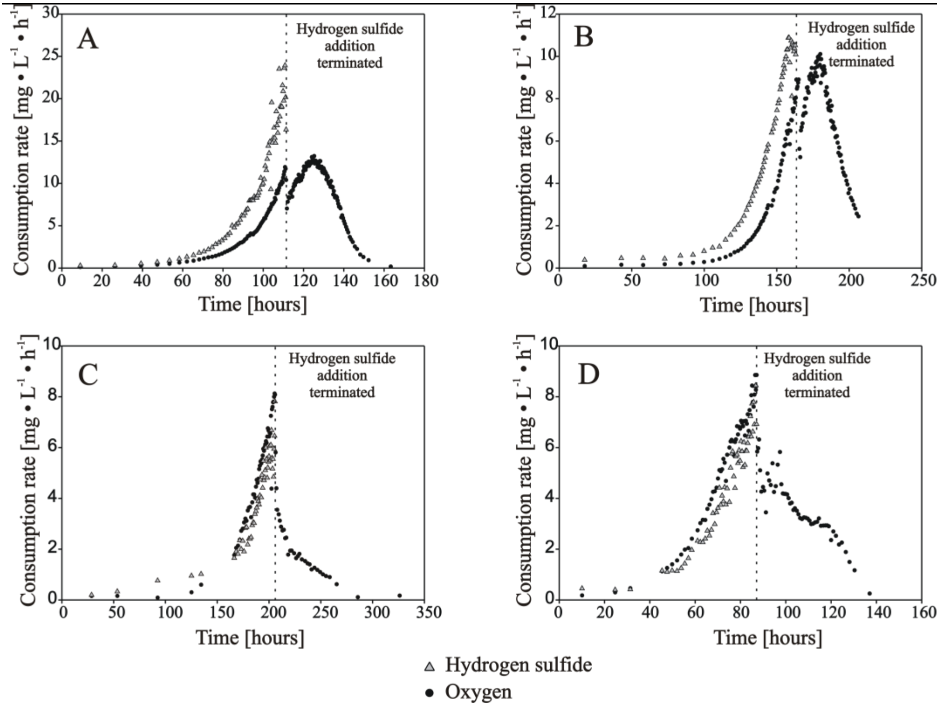

Figure 16. The 4 hydrogen sulfide removal rate curves and 4 oxygen uptake rate curves that are corresponding. Furthermore, they are paired, where A and B are started with corroded concrete, and the curves of C and D are started with inoculation.

The hydrogen sulfide removal rate has a tendency to be increasing exponentially during the disodium sulfide addition in all experiments. The tendency of oxygen uptake differs between the two different experiments with either corroded concrete or inoculation as experiment start. The experiments with corroded concrete as start has an exponential tendency in oxygen uptake during the hydrogen sulfide addition, and at the end of hydrogen sulfide addition, there is a sudden drop in oxygen uptake. A build-up phase in oxygen uptake follows the sudden drop, which could be caused by a partly oxidation of the injected sulfide, to elemental sulfur, and then a further oxidation to sulfate, as the reaction patterns of Table 6 indicate [Jensen *et. al.*, in preparation]. In the experiment with inoculation as experiment start, the oxygen uptake has an exponential tendency during the hydrogen sulfide addition phase. This is similar to the tendency in the experiment with corroded concrete as an experiment start. However, there is a difference because the hydrogen sulfide uptake rate is lower than the oxygen uptake rate in experiment C and D, which is the opposite of experiment A and B. Furthermore, the following phase has a different tendency, where the oxygen uptake decreases to zero instead of a build-up phase. The difference of the uptake rate indicates different ratios between hydrogen sulfide and oxygen in the different experiments. The ratio between consumed oxygen and oxidized hydrogen sulfide is shown in Figure 17.

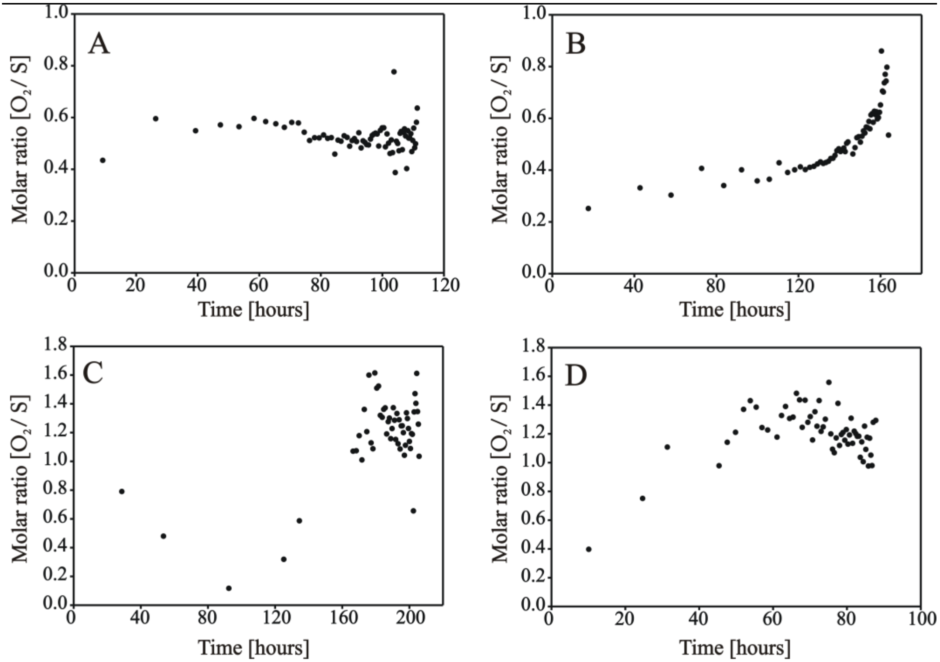

Figure 17. Ratio between oxygen and hydrogen sulfide uptake rates, which are corresponding with the plots in Figure 16.

The oxygen-hydrogen sulfide ratios show that different amounts of oxygen are used to oxidize hydrogen sulfide. The A and B experiments have a ratio between 0.5 and 0.9. Experiment A has a constant ratio and the ratio in experiment B is increasing throughout the experiment. The C and D experiments have a ratio around 1.4 with variations. Both starting low and building up to a stable level, although the D experiment has a slight tendency to decrease in the end. The average values of the ratio between oxygen and hydrogen sulfide are shown in Table 9.

Table 9. The average oxidation values of hydrogen sulfide, with reoxygenation, hydrogen sulfide stripping and chemical oxidation taken into account.

Oxidation plot	A	B	C	D
Reaction stoichiometry during hydrogen sulfide addition [O_2/S]	0.55	0.60	1.40	1.41
Total reaction stoichiometry [O_2/S]	1.50	1.90	2.18	2.58

The total oxidation ratio in Table 9 shows different tendencies that vary from the theoretical total oxidation, which would result in a ratio of 2 O_2/S. Experiment A and B have a tendency to be partly oxidized, which may be caused by the production of non-biodegradable elemental sulfur**.** The total oxidation ratio of C and D have a tendency to be fully oxidized and to use more oxygen to oxidize the added hydrogen sulfide than actually possible, which may be caused by inaccuracy of the hydrogen sulfide electrode that was especially unstable during experiment D.

Protein concentration

The protein concentration measurements, which were performed during 3 of the experiments, are shown together with the hydrogen sulfide and oxygen uptake rates in Figure 18.

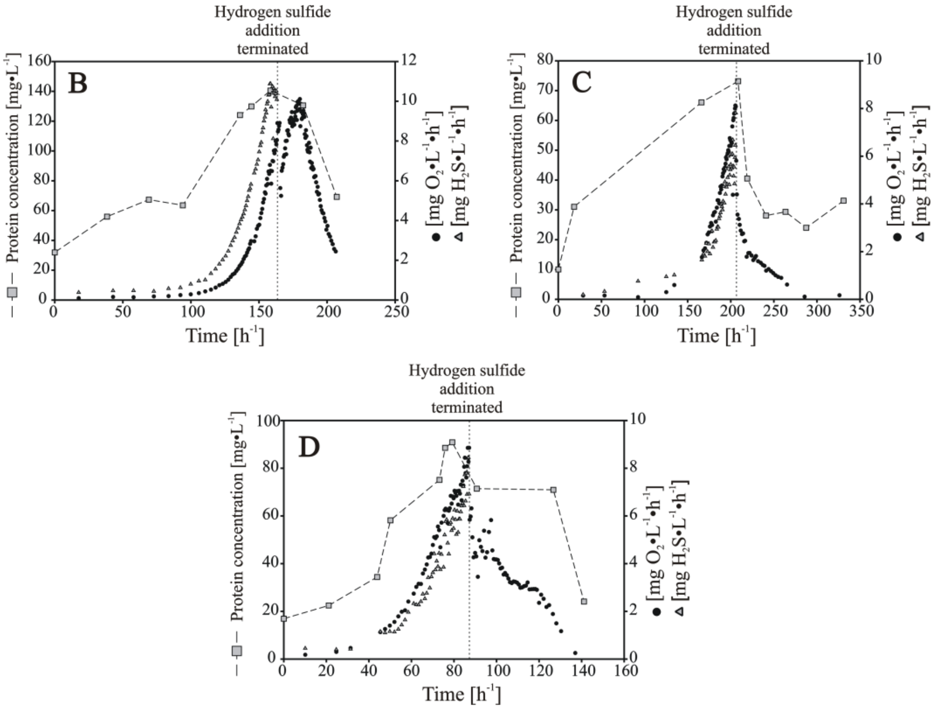

Figure 18. Protein concentration measurements shown together with the oxygen and hydrogen sulfide uptake rates during the experiments

The measurements of oxygen and hydrogen sulfide uptake rates and protein concentration all have an increasing tendency, thus an exponential increasing rate is calculated for each of the measurements. These fitted tendencies are referred to as the growth rate and the rates are displayed in Table 10. The growth characteristics, indicated by the protein measurements, are that the hydrogen sulfide oxidizing microorganisms grow during the hydrogen sulfide addition.

Table 10. Growth rate described by oxygen uptake, hydrogen sulfide oxidation and protein concentration.

Rate	O_2 [d^{-1}]	H_2S [d^{-1}]	Protein [d^{-1}]
A	1.5	1.5	
B	1.4	1.1	0.6
C	0.8	0.9	0.5
D	1.0	1.3	1.2

The growth rates of Table 10 indicate that the protein concentration does not follow the others entirely. This might be caused by the variation of microorganisms, thus a difference in the activity. Earlier studies of biomass concentration and activity in sewage have been addressed by this problem [Vollertsen *et. al.*, 2000]. The complications should be less significant in a monoculture, thus the inoculation. The experiments of Figure 18 are steps of inoculation, and Figure 19 shows the development in ratio between the oxygen growth rate and the protein growth rate, and hydrogen sulfide growth rate and the protein growth rate.

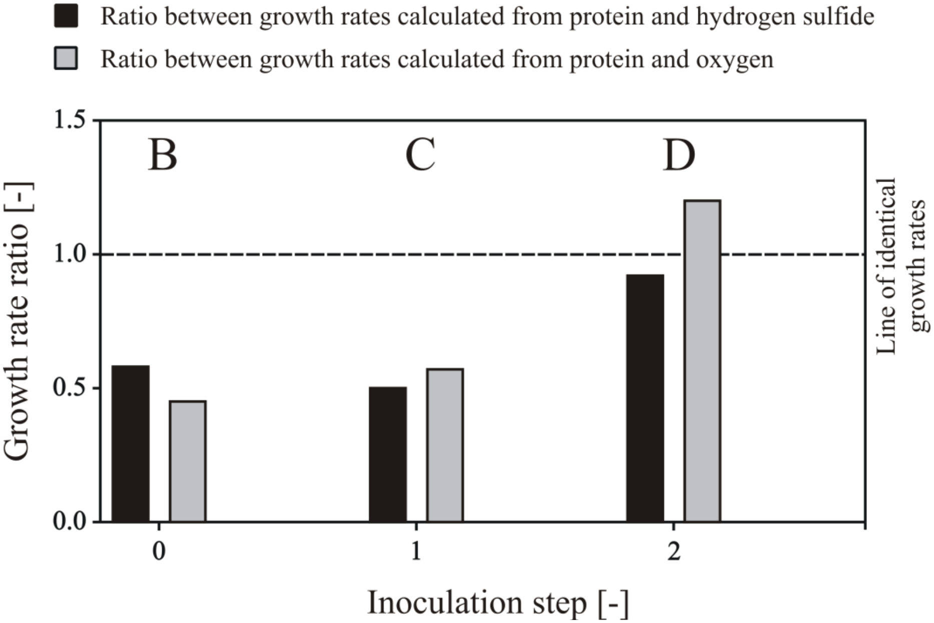

Figure 19. The ratio between protein and oxygen growth rates, and protein and hydrogen sulfide growth rates, through the inoculation steps.

The growth rate ratios of Figure 19 indicate an increasing proportionality between growth rates, calculated from protein measurements and both the hydrogen sulfide and oxygen uptake rates, when the inoculation is performed to isolates the active microorganisms. Although the first inoculation indicates a drop in ratio, regarding hydrogen sulfide, Figure 19. This drop could be caused by the measurements of protein concentration being inadequate in the growth stage of experiment C, Figure 18. The tendency in protein concentration of experiment D, Figure 18, which is the second inoculation, is very similar to the tendency of the oxygen and hydrogen sulfide uptake rates. This is further supported by growth rate ratios in Figure 19, which shows that the growth rate calculated from the protein concentrations is higher than the one calculated from the oxygen uptake rate. The growth rate ratio between protein and hydrogen sulfide at the second inoculation shows that they are very similar. When observing Figure 18, with the protein concentration measurements, a fast decrease in protein concentration is observed, shortly after the termination of hydrogen sulfide addition. This could be caused by a high decay rate of the microorganisms due to the extreme conditions of their environment. The decreasing protein concentration could furthermore be caused by a reorganization of the intercellular protein as a preparation to a hibernation mode, due to the lack of hydrogen sulfide. In order to describe such parameters, a further investigation, with more advanced molecular microbiology, is needed.

The used of more advanced molecular microbiology has begun. The bacterial diversity, at the point of hydrogen sulfide addition termination in experiment A and C, has been determined. The sample from experiment A had a broad diversity of bacteria and the sample from experiment C showed two bacteria; Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans, which are closely related. The bacterial diversity of the samples is shown in Figure 20 in a phylogenetic tree. The phylogenetic tree consists of the bacteria found in the 2 samples, the bacteria found by Okabe *et. al.* (2007) and the bacteria found by Vincke *et. al.* (2001), who both have performed similar studies.

The bacterial diversity of the phylogenetic tree in Figure 20, shows that the reactor experiment A, started with corroded concrete, has a higher diversity of organisms than reactor experiment C that has been started by inoculation. Furthermore the general bacterial diversity is similar to the one of Okabe *et. al.* (2007) and Vincke *et. al.* (2001).

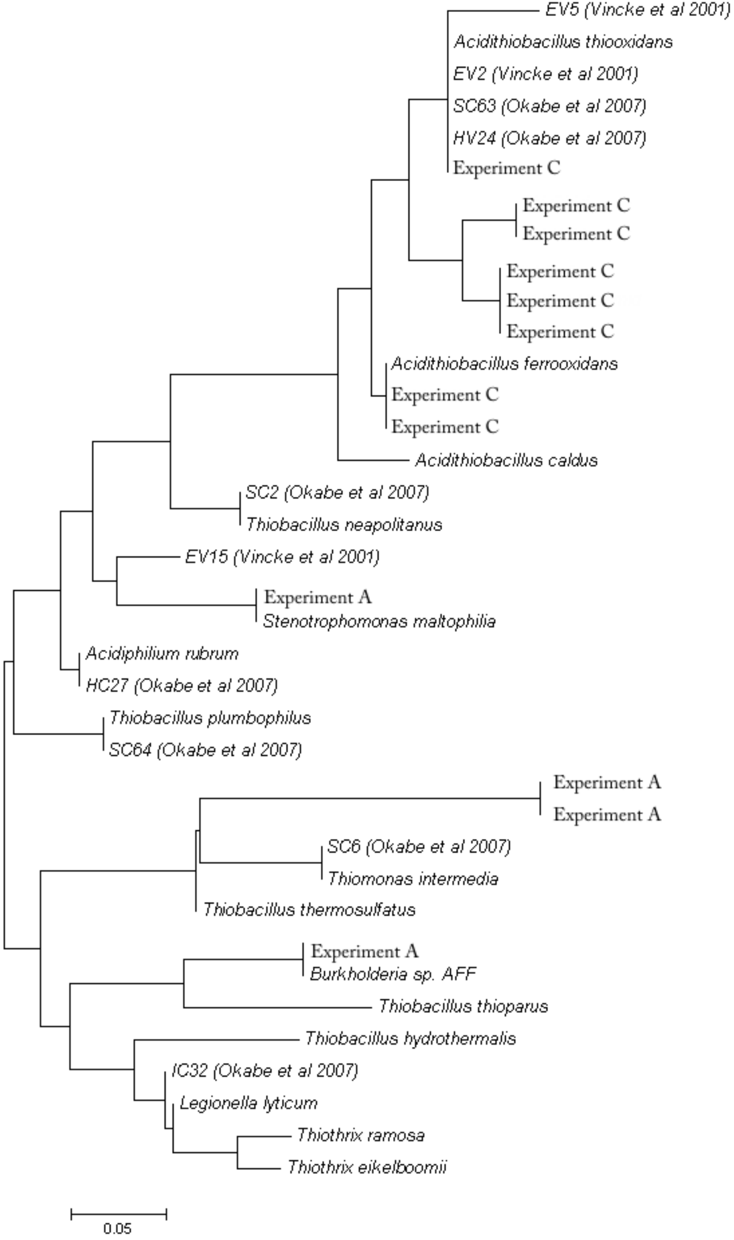

Figure 20. Phylogenetic tree of the bacterial diversity in experiment A and C, supported by the diversity found by Okabe *et. al.* (2007) and Vincke *et. al.* (2001) in similar experiments.

The phylogenetic tree shows that there are large genetic differences of the microorganisms in the sample from experiment A, compared to the sample from experiment C. This is illustrated by length of the lines of the phylogenetic tree. Two bacteria far from each other have less in common genetically than two bacteria close two each other. The bacteria from experiment A has a diversity similar to the one determined by Okabe *et. al.* (2007). This indicates a general bacterial diversity in corroded concrete. The close related bacterial diversity of experiment C is also found to be active in the oxidation of hydrogen sulfide by Vincke *et. al.* (2001).

The results of experiment with biomass dependency of the hydrogen sulfide uptake rate combined with the results of the bacterial diversity determination indicates that the biomass concentration can be use to describe the hydrogen sulfide uptake rate when working with a genetically close related culture of microorganisms. Furthermore it indicates that the genetic diversity of microorganisms in corroded concrete results in a sum of different uptake kinetics that can not be described by the biomass concentration.

Methyl mercaptane

The microorganisms were exposed to methyl mercaptane at the end of 3 growth experiments of hydrogen sulfide oxidizing microorganisms. This was done in experiment B, C and D, Table 8. The microorganisms instantly began to oxidize methyl mercaptane in experiment B, which is shown in Figure 21 as a tendency of the oxygen concentration.

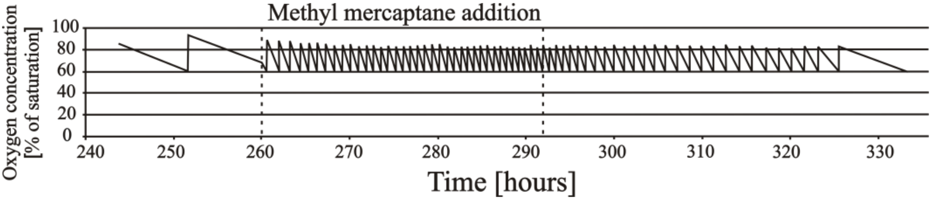

Figure 21. The oxygen concentration, during the methyl mercaptane oxidation, in experiment B.

An increase of the oxygen uptake was only present in experiment B and nothing was detected in experiments C and D. The methyl mercaptane addition and uptake of oxygen in experiment B is shown in Figure 22, as a tendency of the oxygen uptake rate.

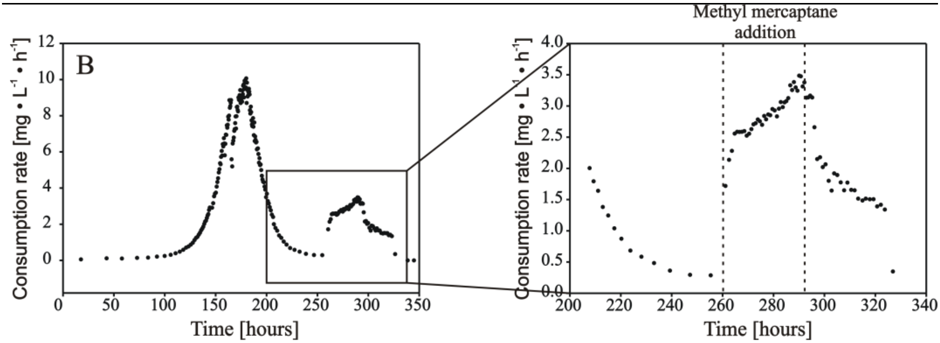

Figure 22. The total oxygen uptake rate of the experiment including methyl mercaptane oxidation with methyl mercaptane oxidation being from 260 to 350 hours, which is cut from the total oxidation rate.

Figure 22 shows the total oxygen uptake rate of experiment B. The methyl mercaptane addition begins at 260 hours. In order to view the methyl mercaptane uptake, a zoom cut is made as shown in Figure 22.

When observing the oxidation curve from experiment B, the methyl mercaptane uptake seems to happen with an instantaneous change in activity followed by an increasing activity with a tendency of growth. Furthermore, methyl mercaptane was added to experiment C and D without any real effect, although experiment C had an insignificant rise in oxygen uptake of 0.05 mg $O_2 \cdot L^{-1} \cdot h^{-1}$. The tendency that inoculation eliminated methyl mercaptane oxidation indicates that the varying culture of microorganisms in corroded concrete has different energy sources and that the, at pH 1, highly active hydrogen sulfide oxidizing microorganisms do not oxidize methyl mercaptane. The fact that two experiments resulted in no methyl mercaptane reinforces the tendency of the methyl mercaptane being biologically degraded in experiment B. Furthermore, it supports the fact that the microbial diversity was higher in experiment B than in experiment C and D.

Conclusion

The study of hydrogen sulfide uptake rate shows that the development in protein concentration is non-proportional with the hydrogen sulfide uptake rate in corroded concrete, due to the variation in microorganisms. This shows that it is not possible to use the development in biomass concentration in corroded concrete to describe the hydrogen sulfide uptake rate. The bacterial diversity of reactor approached a monoculture of Acidithio-bacillus through inoculation. This resulted in an increasing proportionality between protein concentration and hydrogen sulfide uptake rate. The results indicate that the hydrogen sulfide uptake rate is proportional with the biomass concentration of the individual microorganisms, but not with the mixed culture.

The experiments with methyl mercaptane indicate that the microorganisms in corroded concrete are able to oxidize and grow on methyl mercaptane**.** Furthermore, it is indicated that the Acidithiobacillus, isolated through inoculation, are unable to consume methyl mercaptane.

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Vollertsen J., Jahn A., Nielsen J. L., Hvitved-Jacobsen T. and Nielsen P. H., 2000: Comparison of methods fpr determination of microbial biomass in wastewater, Environmental Engineering Laboratory, Aalborg University, Denmark. Water Research Vol. 35, No. 7, pp. 1649–1658. Elsevier Science Ltd., Great Britain

This Master thesis is produced at Aalborg University and consists of 2 articles, in the area of hydrogen sulfide oxidation and sewer corrosion, describing:

- The relation between actual and potential corrosion was investigated and described as a corrosion reduction factor.
- A study on the fate of partly oxidized hydrogen sulfide that is immobilized in corroded concrete was performed.
- An investigation of the influence of atmosphere velocity on the hydrogen sulfide removal rate in sewer systems was performed.
- Hydrogen sulfide oxidation in sewer systems was investigated in a bench scale bioreactor
- A study on the kinetics of methyl mercaptane oxidation in sewer systems was performed