Survival of hydrogen sulfide oxidizing bacteria on corroded concrete surfaces of sewer systems
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ABSTRACT

The activity of hydrogen sulfide oxidizing bacteria within corroded concrete from a sewer manhole was investigated. The bacteria were exposed to hydrogen sulfide starvation for up till 18 months, upon which their hydrogen sulfide oxidizing activity was measured. It was tested whether the observed reduction in biological activity was caused by a biological lag phase or by decay of the bacteria. The results showed that the bacterial activity declined with approximately 40% pr. month during the first two months of hydrogen sulfide starvation. After 2–3 months of starvation, the activity stabilized. Even after 6 months of starvation, exposure to hydrogen sulfide for 6 hours a day on three successive days could restore the bacteriological activity to about 80% of the initial activity. After 12 months of starvation, the activity could, however, not be restored, and after 18 months the biological activity approached zero. The long-term survival aspect of concrete corroding bacteria has implications for predicting hydrogen sulfide corrosion in sewer systems subject to irregular hydrogen sulfide loadings, e.g. as they occur in temperate climates where hydrogen sulfide often is a summer-problem only.

Key words | concrete corrosion, hydrogen sulfide, oxidation, sewer networks

INTRODUCTION

Hydrogen sulfide related concrete corrosion is a widespread problem in sewer networks because it causes serious damage to sewer pipes and other sewer structures such as manholes and pumping stations, ultimately necessitating repair and replacement of the structures (Hvitved-Jacobsen 2002). The corrosion is caused by the oxidation of hydrogen sulfide to sulfuric acid on the surfaces of concrete exposed to the sewer atmosphere. The acid reacts with the alkaline components of the concrete, deteriorating the concrete, and creates a highly acidic environment. pH values below 2 have been reported on the surface of heavily corroded concrete sewer pipes (Parker 1945b; Islander et al. 1991; Mori et al. 1992). The oxidation of hydrogen sulfide on the concrete surface is mainly a biological process and the bacterium Acidithiobacillus thiooxidans (formerly Thiobacillus thiooxidans, (Kelly & Wood 2000)) is found to be the most abundant species on heavily corroded concrete surfaces (Parker 1945a; Okabe et al. 2007). This bacterium is an acidophilic chemolithotroph that oxidizes reduced inorganic sulfur components such as hydrogen sulfide, elemental sulfur, and thiosulfate as source of energy (e.g. Kelly & Wood 2000; Masau et al. 2001).

Hydrogen sulfide is formed under anaerobic conditions mainly in the inner parts of the submerged sewer biofilms. From here the hydrogen sulfide diffuses through the biofilms towards the bulk water phase. In the presence of dissolved oxygen, hydrogen sulfide is oxidized both in the biofilms and in the bulk water (Nielsen et al. 2005, 2006). This means that force mains favor the formation of hydrogen sulfide as anaerobic conditions prevail due to lack of reaeration. When the anaerobic wastewater is discharged into a partly filled gravity sewer, hydrogen sulfide can be released into the sewer atmosphere.

The release of hydrogen sulfide is strongly depended on the pH of the wastewater as only the non-dissociated species of sulfide can be released. Assuming equilibrium conditions,
about 90% of the sulfide is present on the non-dissociated form at pH 6. At pH 7 it is about 50% and at pH 8 only about 10% is found on the non-dissociated form. Hence the potential release of hydrogen sulfide is increased when pH is lowered. In Denmark, the public water supply is in many areas based on groundwater extracted from limestone aquifers. This usually results in wastewaters that are slightly alkaline and have a stable pH due to a high buffer capacity.

A recent study has demonstrated a strong correlation between drops in wastewater pH and increases in hydrogen sulfide concentration in the sewer atmosphere (Nielsen et al. 2008). The average pH of the wastewater in that study was 7.8 and at this pH, the hydrogen sulfide concentration in the sewer atmosphere was only a few percent of the equilibrium concentration, even though sulfide was present in the wastewater in significant concentrations. In that study, heavy corrosion was observed in the sewer pipes, meaning that hydrogen sulfide oxidizing bacteria should be present on the concrete surfaces. For sewer systems like the one studied by Nielsen et al. (2008), the fluctuations of the hydrogen sulfide concentration in the sewer gas phase would mean that the bacteria on the corroding concrete surfaces would experience periods with little–or even without–hydrogen sulfide available as substrate for maintenance and growth.

Fluctuations in the hydrogen sulfide concentration can also be caused by infiltration of groundwater into the sewer system, periodically diluting the substrate concentration available for the hydrogen sulfide producing biomass. Also the lowering of wastewater temperatures can cause suppression of hydrogen sulfide formation (e.g. Nielsen et al. 1998). Both of these phenomena are in Denmark associated with winter conditions.

The objective of this study was to investigate how periods of hydrogen sulfide starvation influences the activity and survival of the hydrogen sulfide oxidizing bacteria in the corrosion products from a heavily corroded sewer concrete surface.

METHODS

The activity of the hydrogen sulfide oxidizing bacteria was quantified as the rate at which hydrogen sulfide was utilized in samples of corroded concrete products. The hydrogen sulfide utilization rate was measured by suspending corrosion products sampled from a sewer manhole in oxygen saturated water, adding hydrogen sulfide and then measuring the changing concentration of hydrogen sulfide in the suspension over time. The method is described in detail in Jensen et al. (submitted).

Sampling and sample characterization

Samples of corroded concrete products were collected from a heavily corroded sewer manhole located near the town of Vårst, south of Aalborg in the northern part of Jutland (Figure 1). The manhole is located about 1 km downstream of a force main discharge. The sampled material consisted of a mixture of gypsum, sand, fine gravel, and coarse aggregates and was quite heterogeneous. Sample material was collected twice during the study: On the 13th of November 2005 and on the 30th of October 2006. A fraction of each of the two samples was used for characterization of the sampled material with respect to water content, pH and volatile solids content. The two samples were then divided into sub-samples of 12 to 14 g each. The sub-samples were contained in 50 ml centrifugation tubes (polypropylene), which were stored in an underground sewer monitoring station at temperatures similar to in situ soil temperatures.

The characterization of the sampled material with respect to pore water pH, water content and volatile solids was carried out on the day of sampling. The water content was determined as the mass loss in the determination of...
total solids. Total solids and volatile solids were determined according to the methods of APHA et al. (2005). pH in the pore water was determined by suspending approximately 5 g of sample in 20 ml of deionized water and shaking for 2 hours. The pH of the water was hereafter measured using a single pore glass pH electrode (model 238160, Hamilton Company) with a pH transmitter (model 2400, Metler Toledo). The pH was then calculated by relating the quantity of hydrogen ions to the original water content of the sample. As the site has been used for sampling on a regular basis, 14 determinations of each characterization parameter exists for this manhole.

**Experimental setup**

The experimental setup consisted of a 250 ml conic flask as reactor. The flask was sealed using a rubber stopper with a hole through which a syringe without piston was inserted as an expansion chamber. The sealed flask was placed on a magnetic stirrer to ensure mixing. Samples for hydrogen sulfide measurement were taken through the expansion chamber, using a 120 mm hypodermic needle (Figure 2).

**Experimental procedure**

A determination of the hydrogen sulfide utilization rate was carried out by weighing a sub-sample and suspending the sub-sample material in deionized water in the reactor. 5 ml of nutrient solution containing 20 g L\(^{-1}\) NH\(_4\)Cl, 100 g L\(^{-1}\) KH\(_2\)PO\(_4\), 40 g L\(^{-1}\) MgSO\(_4\)·2H\(_2\)O, 1.5 g L\(^{-1}\) CaCl\(_2\), 1 g L\(^{-1}\) FeCl\(_3\)·6H\(_2\)O, and 1 g L\(^{-1}\) MnSO\(_4\)·H\(_2\)O was added. The pH in the reactor was adjusted to 1 using 9 M sulfuric acid and aerated with atmospheric air to ensure oxygen saturation. The volume of the suspension was afterwards adjusted with deionized water until the reactor was filled and without air bobbles when the rubber stopper was put in place. A sodium sulfide stock solution was added to a hydrogen sulfide concentration in the reactor of about 4 g H\(_2\)S-S m\(^{-3}\). Samples for measurement of the hydrogen sulfide concentration were taken with regular intervals, noting the time for each sample. Sampling was continued for 6 hours. The concentration of hydrogen sulfide was measured using the spectrophotometric determination of Cline (1969). The experiments were carried out at approximately 20°C.

Within the first 6 month from sampling, determination of the hydrogen sulfide utilization rate of a sub-sample was carried out in intervals of about two weeks. Hereafter, hydrogen sulfide utilization rates were determined after 12 and 18 months, respectively. The sub-samples collected in November 2005 were used for experiments during these 18 months whereas the sub-samples collected in October 2006 were used only during the first 4 months after sampling.

**Regeneration of biological activity**

A low hydrogen sulfide utilization rate could in principle be caused by a bacterial lag phase, as the duration of one determination was in the range of some hours. It was therefore tested whether a reduced activity was caused by a biological lag phase or by decay of bacteria. For 8 of the sub-samples, the hydrogen sulfide addition (4 g H\(_2\)S-S m\(^{-3}\)) was repeated three times on three successive days, resulting in the bacteria being exposed to hydrogen sulfide for a total of up to 18 hours, hereby overcoming the effect of a possible bacterial lag-phase.

**Auto oxidation and losses**

Hydrogen sulfide is a rather reactive substance and is subject to auto-oxidation in the presence of oxygen. It furthermore tends to diffuse through a number of materials. To quantify the auto-oxidation of hydrogen sulfide in the reactor as well as a possible loss of hydrogen sulfide from the reactor by
diffusion, determinations of the hydrogen sulfide utilization rate without sample material and only with acidified water at pH 1 was carried out as quadruple determination.

Rate calculations

The hydrogen sulfide removal rate was calculated in each experiment as the slope of the linear part of the measured hydrogen sulfide concentration versus time \( \frac{dS_{\text{H}_2\text{S}}}{dt} \) and denoted \( r_{\text{total}} \), where \( S_{\text{H}_2\text{S}} \) is the concentration of hydrogen sulfide [g H\(_2\)S-S m\(^{-3}\)], and \( t \) is the time [h] (Figure 3). The rate of auto-oxidation and losses was denoted \( r_{\text{auto}} \) and the rate of corrosion product induced oxidation was denoted \( r_{\text{corr}} \). In the latter, the contribution from the biological oxidation, \( r_{\text{bio}} \), is predominant compared to the effect of catalysis by the corrosion products, i.e. \( r_{\text{corr}} \approx r_{\text{bio}} \) (Jensen et al. submitted).

In order to quantify the hydrogen sulfide removal due to microbial activity only, the rate of the auto-oxidation and losses was subtracted from the observed rate:

\[
\frac{dS_{\text{H}_2\text{S}}}{dt} = - (r_{\text{bio}} + r_{\text{auto}})
\]  

RESULTS AND DISCUSSION

Sample characterization

The median water content of the 14 samples of corrosion products collected from the sewer manhole near Vårst, Denmark, was 18% of the total solids (standard deviation: 6.8%). When sampling the corrosion products they appeared dry, indicating that the material was not saturated with water. The corrosion products were collected from the sewer manhole rather than from the actual sewer pipe, meaning that the material could have had a lower water content than the corresponding material from the sewer pipe itself, as there here would have been a steady supply of moisture from wastewater aerosols.

The median volatile solids content was 1.9% of the total solids. Due to a relatively low percentage of volatile solids, this analysis is subject to considerable uncertainty (APHA et al. 2005).

The median pH of the pore water was 0.5 (standard deviation: 0.4) and all determinations gave results below pH 1. These pH values are in accordance with the findings of Parker (1945b) who reported pH values below 2 in heavily corroded concrete.

Auto-oxidation and reactor losses

The left hand graph of Figure 3 gives an example of the rate determination of the auto-oxidation and losses from the reactor, \( r_{\text{auto}} \). The rate was 0.15 g H\(_2\)S-S m\(^{-3}\) h\(^{-1}\) with a standard deviation of 0.02 g H\(_2\)S-S m\(^{-3}\) h\(^{-1}\). This value was used in the determination of the biological hydrogen sulfide oxidation rate (Equation 1).

Development in biological activity over time

The right-hand graph of Figure 3 gives an example of the determination of the biological hydrogen sulfide utilization rate. The hydrogen sulfide concentration declines linearly till it reaches a constant level of approximately
0.3 g H₂S-S m⁻³, a level that probably reflects the presence of metal-bound sulfide. The change from the linear decline in hydrogen sulfide concentration to the constant level of hydrogen sulfide is sharp, indicating a high affinity of the biomass towards hydrogen sulfide.

Over the first half month of hydrogen sulfide starvation, the biological activity remained more or less constant, upon which it steadily declined (Figure 4). During the first two months of starvation, the biological hydrogen sulfide utilization rate declined linearly from about 28 μgH₂S-S gTS⁻¹ h⁻¹ to about 6 μgH₂S-S gTS⁻¹ h⁻¹; corresponding to a monthly decline in activity of approximately 40%. After two months of hydrogen sulfide starvation, the utilization rate became constant and stayed so for 12 months. After 18 months of hydrogen sulfide starvation, the utilization rate had dropped to a level not much higher than what could be explained by the rate of auto-oxidation and loss in the test reactor.

Regeneration of biological activity

Investigating whether the decline in hydrogen sulfide utilization rate was due to a biological lag phase or whether the involved bacteria had decayed, it became evident that even after 6 months of hydrogen sulfide starvation the major part of the initial activity could be restored within 18 hours of hydrogen sulfide exposure (Figure 5).

After 3 to 6 months of starvation and 18 hours of hydrogen sulfide exposure, the activity could be restored to about 80% of the initial activity prior to starvation. After 12 and 18 months of starvation, the activity of the bacteria could not be restored. Indicating that the bacteria survived hydrogen sulfide starvation for at least 6 months, upon which decay of the hydrogen sulfide oxidizing biomass became dominant.

The experimental evidence indicates that some weeks or months of hydrogen sulfide starvation has little effect on the activity of the hydrogen sulfide oxidizing bacteria. Even if hydrogen sulfide is absent for 6 months at a time, the activity of the hydrogen sulfide oxidizing bacteria appears to be restorable within a day or so.

A probable explanation for this prolonged survival of the hydrogen sulfide oxidizing biomass is found in the substrate utilization mechanism of the bacteria. Jensen et al. (submitted) observed that the hydrogen sulfide oxidizing biomass of corroded concrete products form intermediate products when oxidizing hydrogen sulfide. These intermediate products were suggested to mainly consist of elemental sulfur, and seemed to be oxidized at a much lower rate than hydrogen sulfide. This suggests that in hydrogen sulfide rich environments, the intermediate products would tend to accumulate in the corrosion products. This explanation is supported by the findings of e.g. Parker (1945b) and Islander et al. (1991) who report a yellowish color of concrete corrosion products, suggesting accumulation of elemental sulfur.

Practical implications

The accumulated intermediate products seem to serve as a reserve-substrate on which the sulfide oxidizing biomass can survive when hydrogen sulfide is absent for longer durations. This feature of long-term survival of the concrete corroding...
biomass has implications for the operation of sewer systems where hydrogen sulfide occurs at irregular intervals. Such conditions can be found in temperate climates where hydrogen sulfide often is a summer-problem only. Here the bacteria easily survive the winter and become active at summers first signs of hydrogen sulfide in the gas phase. Similar conditions exist when hydrogen sulfide control measures are operated inadequately. Where hydrogen sulfide is incompletely controlled, hydrogen sulfide will become available for the bacteria at irregular intervals, however sufficient to allow a continuous corrosion process to proceed—albeit at a lower rate compared to the non-controlled case.

CONCLUSION

The activity of hydrogen sulfide oxidizing bacteria from a corroded concrete sewer was investigated with respect to the effects of hydrogen sulfide starvation. During the first two months of starvation the activity of the bacteria declined at a rate of approximately 40% per month, upon which the activity stabilized for at least 12 months. After 6 months of starvation, still about 80% of the initial activity could be restored by prolonged exposure to hydrogen sulfide. After 12 months of starvation, the activity could not be restored, and after 18 months of starvation, the activity was reduced to almost zero.

The observed resistance to prolonged hydrogen sulfide starvation is of consequence when predicting the corrosion of concrete in sewers, particularly in regions of cold climate, where hydrogen sulfide is often observed only during warm weather periods. Also when predicting hydrogen sulfide oxidation in other systems where hydrogen sulfide is present only periodically, survival time of the bacteria and duration of an eventual lag phase must be taken into account.

REFERENCES