APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1987, p. 1645–1648 Vol. 53, No. 7
0099-2240/87/071645-04$02.00/0

Importance of Hydrogen Sulfide, Thiosulfate, and Methylmercaptan for Growth of Thiobacilli during Simulation of Concrete Corrosion

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Received 11 February 1987/Accepted 9 April 1987

Biogenic sulfuric acid corrosion of concrete surfaces caused by thiobacilli was reproduced in simulation experiments. At 9 months after inoculation with thiobacilli, concrete blocks were severely corroded. The sulfur compounds hydrogen sulfide, thiosulfate, and methylmercaptan were tested for their corrosive action. With hydrogen sulfide, severe corrosion was noted. The flora was dominated by *Thiobacillus thiooxidans*. Thiosulfate led to medium corrosion and a dominance of *Thiobacillus neapolitanus* and *Thiobacillus intermedius*. Methylmercaptan resulted in negligible corrosion. A flora of heterotrophs and fungi grew on the blocks. This result implies that methylmercaptan cannot be degraded by thiobacilli.

Due to growing public concern about environmental pollution, the construction of sewage trunks has been enforced in the last decades. The construction of trunks in an open pit is often impossible. By constructing new waste water trunks underground, sewage pipes are pressured by shield machines. For this procedure, concrete is the most suitable material.

Concrete in its main structure is composed of calcium oxides, hydroxides, and carbonates. These compounds can react with acids: $Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4 + 2H_2O$. The reaction product is gypsum. The calcium loss diminishes the resistance of the building concerning statics. In the last decade an increasing number of reports have been published describing microbial attack on and degradation of concrete. Most of the reports deal with biogenic sulfuric acid corrosion of concrete (1, 4; EWPCA International State-of-the-Art Seminar on Corrosion in Sewage Plants, 1982, Hamburg, Federal Republic of Germany [FRG]). It is caused by the activity of sulfur-oxidizing bacteria—thiobacilli. My group was involved in a research program to elucidate the mechanisms of extensive sulfuric acid corrosion in newly built sewage pipelines in Hamburg, FRG. The results of the study have been published in part (7). To summarize the work, it was demonstrated that the corrosion was caused by thiobacilli. The degree of corrosion was dependent on the number of *Thiobacillus thiooxidans* on the concrete surface. This type of corrosion was termed biogenic sulfuric acid corrosion. It could be reproduced in an H_2S simulation chamber. With these results we were able to make proposals to the city of Hamburg about the type of concrete suitable for sewage pipelines.

As several reports have been published on methylmercaptan as a substrate for thiobacilli, the relevant substrates in sewage pipelines—hydrogen sulfide, thiosulfate, and methylmercaptan—were examined in simulation experiments (9, 10).

In this communication I report the effect of these sulfur compounds on the growth of thiobacilli on concrete blocks and the resulting degree of corrosion.

MATERIALS AND METHODS

Pure cultures of *Thiobacillus intermedius*, *Thiobacillus novellus*, *Thiobacillus neapolitanus*, and *T. thiooxidans* were grown on thiosulfate (10 g/liter) or sulfur (10 g/liter) for *T. thiooxidans* and stored at 4°C for 3 weeks. Carboys (10 liter) were inoculated with active cells and cultivated at 28°C for 10 days. The cells were harvested, washed, and concentrated. After this procedure the concentrates of the four species were mixed. The mixture contained a total of 10^{13} cells for each inoculation cycle. It was sprayed as an aerosol on the surface of the concrete test blocks. In a breeding chamber made of stainless steel (Weiss-Technik, Giessen, FRG) the test blocks were standing on end with 10 cm immersed in water (7). The surface of the test blocks was sawed to yield cubes of 1.8 by 1.8 by 2 cm for sampling and monitoring purposes (Fig. 1). The experiments were run at 30°C and a relative humidity of more than 95%. Five experiments lasting from 9 to 12 months have been completed. The experiments were made with comparable test blocks. The blocks were not sterilized in order to prevent changes of the crystal structure. Random samples were found to be virtually free of microorganisms. Aseptic techniques were not used to inoculate or incubate the test blocks due to the construction of the apparatus.

In two experiments H_2S gas at a concentration of 15 ± 3 mg/m^3 was used as the source of sulfur and concomitantly of biogenic sulfuric acid. The gas concentration was adjusted by a strictly controlled circuit consisting of the H_2S module and the gas chromatograph (Fig. 1). H_2S gas pulses were given to replenish the breeding chamber. For one experiment, sodium thiosulfate (10 g/liter), solubilized in mineral salts solution, was sprayed in intervals of 5 min of spraying per h as an aerosol on the test blocks. In two experiments the gas methylmercaptan at concentrations of 22 ± 4 and 2 ± 1 mg/m^3 was used. The above-mentioned concentrations were chosen to avoid substrate limitation of bacterial growth. Chemical monitoring showed an abundance of sulfur or thiosulfate, respectively, on the test blocks.

During the experiments with hydrogen sulfide, SO_2 gas at a concentration of 2 mg/m^3 was detected in the atmosphere of the breeding chamber (W. A. Koenig, unpublished results). This SO_2 gas was demonstrated in the spent air of aerated cultures of *T. thiooxidans* growing with sulfur.

The counts of thiobacilli, heterotrophic bacteria, and fungi as well as the pH of the surface water and loss of substance were measured as follows. The pH of the surface water on the test blocks was measured with pH indicator sticks (Merck, Darmstadt, FRG). The sticks were embedded in sheets of filter paper to ensure good contact with the concrete surface. The error of the sticks amounts to ±0.2 pH

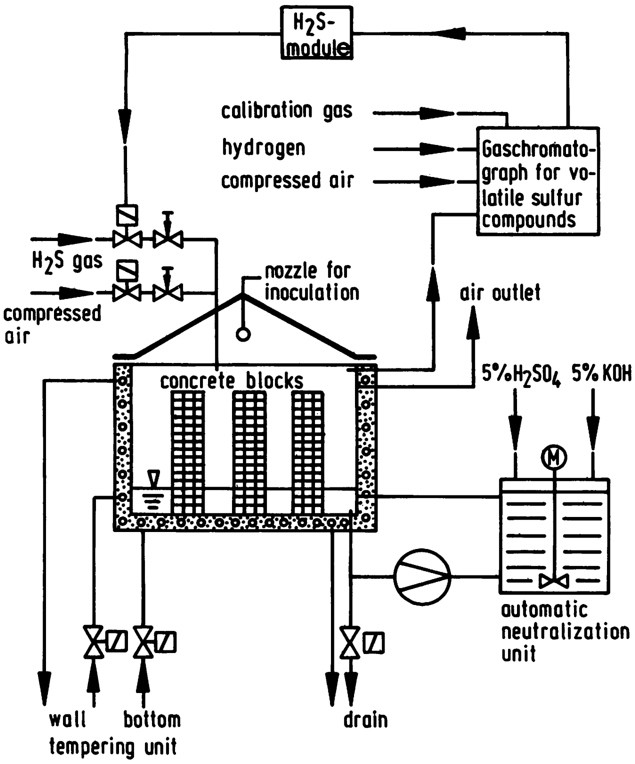

FIG. 1. Diagram of the H_2S-breeding chamber for simulation of biogenic sulfuric acid corrosion.

units. Electrodes were not used because the diaphragm often became plugged.

Loss of substance was determined by (i) weighing the cubes of the test blocks, (ii) shaking the cubes in bottles filled with washing solution for 2 h, and (iii) weighing the remaining cubes and the residues. The values are given as percentage of the intact cube.

With washing solution, serial dilutions were made to determine the biological parameters of the biogenic corrosion.

Cell counts of thiobacilli were determined by a five-tube most-probable-number technique with selective nutrient solutions (4) for *T. neapolitanus* (10 g of thiosulfate per liter,

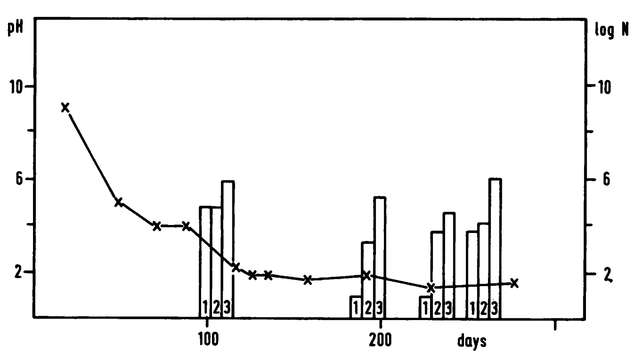

FIG. 2. Profiles of cell counts and pH on a test block with good resistance to biogenic sulfuric acid attack (Portland-type cement). Bars 1, 2, and 3, cell counts of *T. intermedius*, *T. neapolitanus*, and *T. thiooxidans*, respectively, given as logarithmized values (log N). ×, pH (±0.2 pH units). Standard deviations of the cell counts did not exceed one order of magnitude (not indicated).

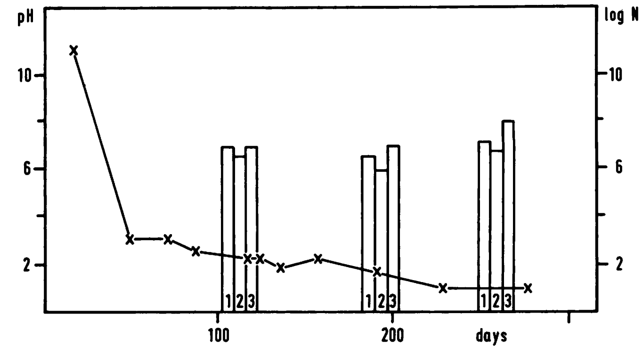

FIG. 3. Profiles of cell counts and pH on a test block with low resistance to biogenic sulfuric acid attack (blast furnace-type cement). Bars 1, 2, and 3, cell counts of *T. intermedius*, *T. neapolitanus*, and *T. thiooxidans*, respectively, given as logarithmized values (log N). ×, pH (±0.2 pH units). Standard deviations of the cell counts did not exceed one order of magnitude (not indicated).

pH 6.5), *T. intermedius* and *T. novellus* (5 g of thiosulfate with 0.02 g of biotin per liter, pH 6.5), and *T. thiooxidans* (10 g of sulfur per liter, pH 4.5).

The cell counts of heterotrophic bacteria were determined by Koch's plating technique with DEV-Agar (Merck, FRG), which contains gelatin, meat extract, and peptone.

Fungi were counted by the same technique on Sabouraud maltose agar (Merck, FRG) containing 4% maltose, meat, casein, and peptone.

RESULTS

In two simulation experiments, H_2S was used as a nutrient for thiobacilli. By chemical autooxidation reactions, H_2S is decomposed to sulfur (5). Thiobacilli oxidize sulfur to sulfuric acid for metabolic energy. As is demonstrated in Fig. 2 and 3, differences could be established for the various concrete types. Figure 2 presents, together with the pH values, the cell counts of thiobacilli on a concrete block with good resistance to microbial attack. Figure 3 shows a concrete block with low resistance. The values for the two test blocks indicate that the degree of corrosion, measured as loss of substance, was dependent on the cell number of *T. thiooxidans* on the concrete surface. This result was also true for the other concrete blocks. In Table 1 the mean values for the two experiments with hydrogen sulfide are presented. The blocks differed in corrosion, loss of substance, and cell counts. According to the results, the test blocks were classified in three groups—severely corroded, moderately corroded, and slightly corroded. On severely

TABLE 1. Importance of sulfur compounds to growth of *T. thiooxidans* and the corrosion of concrete in simulation experiments[a]

Sulfur compound	Mean pH ± SD	Mean loss of substance (%)	Mean no. of cells/cm²		
			T. intermedius	*T. neapolitanus*	*T. thiooxidans*
H_2S	1.5 ± 0.5	3.3	10^6	10^6	10^7
$S_2O_3^{2-}$	2.5 ± 0.5	1.8	10^7	10^7	10^6
CH_3SH	8.5 ± 0.5	0	$<10^1$	$<10^1$	$<10^1$

[a] H_2S and CH_3SH were added as a gas, and $S_2O_3^{2-}$ was added as a sodium salt in mineral salts solution. The duration of a single experiment was 9 months.

corroded test blocks with a loss of substance of 5.8%, the count of *T. thiooxidans* amounted to 8×10^7 cells per cm^2. On test blocks with slight corrosion, a loss of substance of 0.7% correlated with cell counts of *T. thiooxidans* of $6 \times 10^6/cm^2$.

One experiment was run with sodium thiosulfate. This sulfur compound has been detected in sewage pipelines (2). After 9 months of simulation, the test blocks were corroded (Table 1). The flora on the concrete was dominated by *T. neapolitanus*, *T. intermedius*, and *T. novellus*. The cell counts of *T. thiooxidans* were 1/10 power lower than in previous experiments.

Another two experiments were run with methylmercaptan, which is a compound of the atmosphere found in sewage pipelines (3, 6). Due to reports about degradation of this compound, methylmercaptan was deemed to be a possible nutrient for thiobacilli and hence a source for biogenic sulfuric acid (9, 10).

In contrast to the previous experiments at a methylmercaptan concentration of 22 mg/m^3, growth of thiobacilli and hence corrosion of the test blocks could not be measured. Although inoculation of the test blocks was repeated, the cell counts of thiobacilli fell below the detection limit (Table 1). Only heterotrophic bacteria and fungi were present. To prove that the growth of the thiobacilli was not inhibited by this concentration of methylmercaptan and also to exclude any poisonous effects, another experiment with a reduced concentration of 2 mg/m^3 was made. During this experiment the pH of the surface water again did not change. This fact as well as the cell counts of the thiobacilli, which decreased below the detection limit of 10 cells per cm^2, indicated that the thiobacilli could not grow with methylmercaptan. In both experiments, neither sulfuric acid nor sulfur was detectable on the concrete test blocks (W. A. Koenig, unpublished results).

These simulation experiments were accompanied by long field experiments. Identical test blocks were incubated in an aggressive sewer atmosphere. The atmosphere contained volatile organic and inorganic sulfur compounds at a total concentration of 2 to 10 mg/m^3. The temperature amounted to an annual median value of 15.5°C, and the relative humidity was more than 90%. After 6 years of incubation, the first signs of corrosion were measurable. Calculations indicate that the natural corrosion process takes eight times longer than the simulated process.

DISCUSSION

Since 1945 (5) it has been well known that thiobacilli can cause rapid degradation of concrete in sewage works. Hydrogen sulfide was believed to be the nutrient and sulfur source. Studies of the importance of other sulfur compounds for biogenic sulfuric acid corrosion of concrete, particularly in relation to hydrogen sulfide, have not been performed. My results provide data which allow for the first time a differentiation of the three main classes of sulfur compounds, namely hydrogen sulfide, thiosulfate, and methylmercaptan, in relation to their importance as nutrients and sources of biogenic sulfuric acid. In addition the data allow predictions about the composition of the thiobacilli flora if the main substrate is known.

To summarize the results of the five simulation experiments, it became obvious that with the nutrient sulfur (source H_2S), *T. thiooxidans* dominated the flora on the test blocks. Under these circumstances the concrete blocks were severely corroded. Dominance of *T. thiooxidans* was demonstrated at field sites that also showed strong corrosion (4). When the nutrient thiosulfate was used, the flora was dominated by *T. neapolitanus*, *T. intermedius*, and *T. novellus*. With thiosulfate a medium, corrosion became evident. In both instances the substrates were supplied in abundance. Methylmercaptan and other organic sulfides such as dimethylsulfide, dimethyldisulfide, etc., can be excluded as nutrients for thiobacilli and hence sources of sulfuric acid. This result, obtained in two experiments with methylmercaptan, is in contrast to reports describing the growth of thiobacilli with these sulfur compounds (9, 10). However, it may be possible that methylmercaptan and other volatile organic sulfur compounds can be degraded aerobically by heterotrophic bacteria and fungi. For example, *Hyphomicrobium* sp. may be able to decompose methylmercaptan (11). The end products include hydrogen sulfide, which in turn would be oxidized by thiobacilli to sulfuric acid. Similar conditions may have caused the thiobacilli to be able to oxidize and grow with methylmercaptan (9, 10).

The degradation of organic volatile sulfur compounds under anaerobic conditions is well established (12). Presumably, methanogenic bacteria degrade methionine in lake sediments. The end products are methylmercaptan and hydrogen sulfide. During the simulation of aerobic sulfur oxidation, these processes are totally inhibited and thus can be ignored. Hence, organic sulfides may not be of direct importance as a substrate for thiobacilli and the biogenic sulfuric acid corrosion of concrete.

The data also show that under the optimized simulation conditions, thiobacilli cause a degree of corrosion within 1 year which equals at least 8 years of incubation at natural sites.

ACKNOWLEDGMENTS

This work was supported by grants from the Bundesministerium für Forschung und Technologie and the Freie und Hansestadt, Hamburg, FRG.

In particular I am grateful to E. Bock of the Universität Hamburg, FRG.

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