# II-473 Microbial Characteristics of Corroded Concrete Sewer Pipe

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## 1. INTRODUCTION

The corrosion of concrete sewer pipes is a serious problem in sewage works. Anaerobic conditions in sewage support microorganisms that convert sulfate and organic sulfur compounds to hydrogen sulfide ($H_2S$). $H_2S$ volatilizes into the sewer atmosphere and redissolves into condensate on the surface of sewer pipes. The community of sulfate-producing microorganisms, dominated by species of the genus *Thiobacillus*, oxidizes the dissolved sulfide into corrosive sulfate, resulting in the corrosion of concrete sewer pipes. The participation of different species of *Thiobacillus* in the corrosion process has been shown to depend upon the respective optimum conditions of pH and nutrient for their growth. The final community of microorganisms was reported to be dominated by an acidophilic bacterium, *Thiobacillus thiooxidans.* However, there is little evidence which shows effects of other characteristics of final microflora on the corrosion process of sewer pipes.

The present paper describes the characteristics of microflora on severely corroded sewer pipe, and the roles of microorganisms in the corrosion process.

## 2. EXPERIMENTS

**Sampling:** The severely corroded concrete materials were sampled at the water level in sewer pipes connecting to sewage treatment plant in Ohmuta city, Fukuoka.

**Microbial analysis:** One g-wet of the corroded material was placed in 9 ml of distilled water and homogenized by ultrasonic disruption for 1 min at 40 W. After serial dilutions with distilled water, the suspensions were spread on the following solid media; T 6.5, TO 6.5, T 2.5, TO 2.5, F 4. The chemical composition of the media is shown in Table 1. After incubating the plates at 30 °C for 2 - 3 weeks, 60 colonies from each medium were randomly selected, and characterized.

Table 1 Composition of media

| Compositions | Media | | | | |
|---|---|---|---|---|---|
| | T 6.5 | TO 6.5 | T 2.5 | TO 2.5 | F4 |
| Basal salt | Sol. A1) | Sol. A | Sol. A | Sol. A | Sol. B2) |
| Nutrient: $Na_2SO_4 \cdot 5H_2O$ (g/l) | 8.0 | 8.0 | 8.0 | 8.0 | / |
| Yeast extract | / | 2.0 | / | 2.0 | / |
| $FeSO_4 \cdot 7H_2O$ | / | / | / | / | 44.0 |
| Gelling agent | Agar | Agar | Colloidal silica | Colloidal silica | Colloidal silica |
| pH | 6.5 | 6.5 | 2.5 | 2.5 | 4.0 |

1), $NH_4Cl$ 0.5, $K_2HPO_4$ 4.0, $KH_2PO_4$ 4.0, $MgSO_4 \cdot 7H_2O$ 0.8, EDTA 0.5, $ZnSO_4 \cdot 7H_2O$ 0.22, $CaCl_2$ 0.05, $MnCl_2 \cdot 4H_2O$ 0.05, $FeSO_4 \cdot 7H_2O$ 0.05, $(NH_4)_6Mo_7O_{24}$ 0.01, $CuSO_4 \cdot 5H_2O$ 0.02, $CoCl_2 \cdot 6H_2O$ 0.02 (g/l).
2), $(NH_4)_2SO_4$ 3.0, $K_2HPO_4$ 0.5, KCl 0.1, $MgSO_4 \cdot 7H_2O$ 0.5, $Ca(NO_3)_2$ 0.01 (g/l).

**Characterization of isolates:** Two representative microorganisms were isolated, and identified. Their abilities of $H_2S$ oxidation were investigated, and the metabolic products were identified. $H_2S$ and anionic sulfur compounds were analyzed by using gas chromatography and high performance liquid chromatography(HPLC), respectively.

## 3. RESULTS AND DISCUSSION

**Microflora on corroded concrete sewer pipe:** The corroded sample was very soft and acidic with pH 1.9. When the suspended solution of the sample was inoculated on 5 kinds of media, only 2 kinds of microorganisms appeared; One was a whitish yellow bacterial colony, and the other was a dark green fungus. The bacterial colonies appeared only acid solid media (pH 2.5). The fungi were detected on neutral solid media (pH 6.5) as well as on acidic ones (pH 2.5). All of 120 bacterial colonies, which were randomly selected from T 2.5 and TO 2.5 media, showed similar characteristics, were identified as *Thiobacillus thiooxidans*.

The dark green fungi that appeared on the isolation media showed similar morphological characteristics, even though the media used for isolation varied in pH and nutrients. Although their optimum pH for growth was found to be in neutral conditions, they showed tolerance against acid. Through taxomonical study, the fungus was identified as the new species of the genus *Sporormia*, and named as *Sporormia concretivora*. It was observed that no other heterotroph except the fungus was found on the most severely corroded sewer pipe, even if other heterotrophs could have been detected on TO 2.5 and/or TO 6.5 media due to the presence of the organic substance. The results showed that the most severely corroded sewer pipe at the water level was inhabited by only two kinds of microorganisms, *Thiobacillus thiooxidans* and *Sporormia concretivora*. The simplification of the microflora may be caused by low pH and a single energy source of sulfide.

**Roles of *T. thiooxidans* and *S. concretivora* in corrosion process:** $H_2S$ oxidation by *T. thiooxidans*, isolated from corroded concrete sewer pipe, is shown in Fig. 1. A slight decrease of $H_2S$ concentration was observed in the cell-free basal salt solution. However, a remarkable decrease of $H_2S$ was found in the test tubes containing viable cells, suggesting $H_2S$ was removed by biological activity. The oxidation product of $H_2S$ was identified as sulfate.

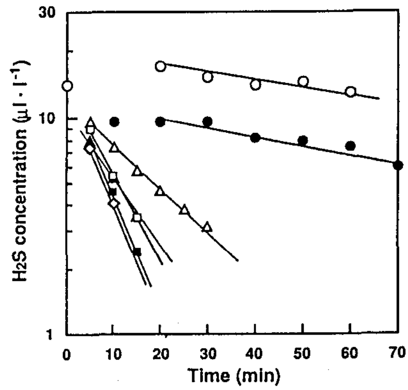

Fig. 1 Oxidation of $H_2S$ by *T. thiooxidans*
Cellular protein concentration ($mg \cdot l^{-1}$): ○, 0(control); ●, 0.39; △, 0.78; ▲, 1.17; □, 1.56; ■, 3.12; ◇, 4.68.

*S. concretivora* was able to degrade $H_2S$. The viable cells show the degradation of $H_2S$, but no degradation of $H_2S$ was found by the autoclaved cells. This fact indicates that the degradation of $H_2S$ by *S. concretivora* was biological reaction. *S. concretivora* was found to oxidize $H_2S$ to thiosulfate via sulfite as shown as Fig. 2.

It is new finding that the $H_2S$-oxidizing fungus, *S. concretivora*, coexisted with *T. thiooxidans* on the severely corroded sewer pipe. Thiosulfate, which was produced from $H_2S$ by this fungus, can be oxidized to corrosive sulfate by *T. thiooxidans*. *T. thiooxidans* excretes as much as 20 % of the $CO_2$ it fixes as organic substances. It excretes pyruvic acid and oxalacetic acid, which are self-inhibitory at $2 \times 10^{-5}$ to $7 \times 10^{-5}$ M. Therefore, continued vigorous growth of *T. thiooxidans* requires a mutualistic relationship with acidophilic or acid-resistant heterotrophs that can degrade the organics excreted. Considering these facts, the mutualistic relationship between *T. thiooxidans* and *S. concretivora* on the concrete sewer pipe can be existed (Fig. 3). It is possible that the close association between two microorganisms accelerates the corrosion of concrete sewer pipes.

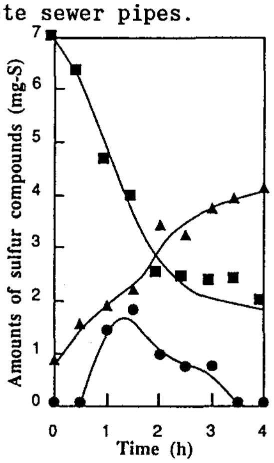

Fig. 2 Oxidation of sulfide by *S. concretivora*
■, sulfide; ▲, thiosulfate; ●, sulfite

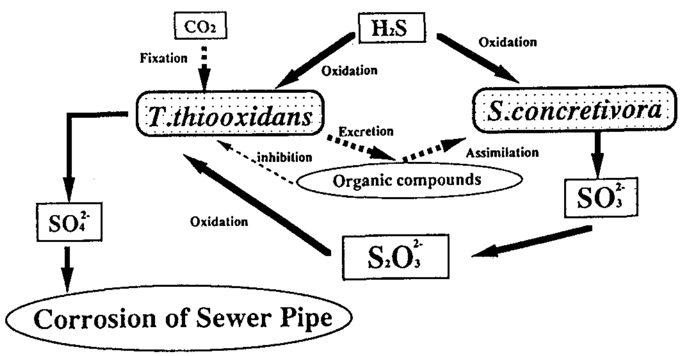

Fig. 3 Hypothetical relationship between *T. thiooxidans* and *S. concretivora* on concrete sewer pipe