CONCRETE DETERIORATION CAUSED BY AEROBIC BACTERIA

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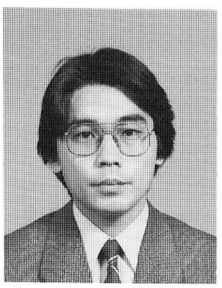
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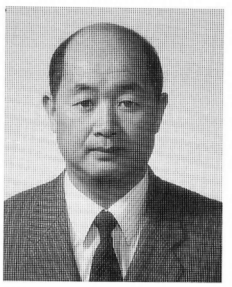
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ABSTRACT

A certain underground concrete structure had been severely damaged, and the cause was believed to have been metabolites produced by microorganisms. In this study, several types of bacteria in the soil around the structure were isolated and cultured under aerobic conditions. Mortar simulation tests using the bacteria were then carried out and the composition of the metabolites produced by the bacteria analyzed. As a result, calcium ion was dissolved out from mortar soaked in the culture medium bred with the bacteria, and it was found that organic acids and carbonic acid were metabolized by the bacteria. It is already known that concrete can be severely damaged by anaerobic bacteria, but this study suggests that organic acids and carbonic acid metabolized by aerobic bacteria can also cause concrete to deteriorate.

Keywords: deterioration, aerobic bacteria, metabolites, hydrogen sulfide, organic acid, carbon dioxide

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1. INTRODUCTION

The chemical deterioration of concrete has been considered to be caused mainly by acids, alkalis, or salts. And recently, some investigations on biological deterioration of concrete structure in sewers have been reported in Japan. Biologically caused deterioration of concrete in sewage and waste water treatment plant has been known for a long time in Europe and America [1,2], and is becoming an important issue as public sewerage spreads. The deterioration mechanism is as follows.

When organic matter in sewage is decomposed by microorganisms and oxygen is consumed, the sewage becomes anaerobic and sulfate-reducing bacteria become highly active. As a result, sulfate in the sewage is reduced by the sulfate-reducing bacteria and a large amount of hydrogen sulfide is produced. This hydrogen sulfide is released from the liquid phase into the gas phase, and dissolves into condensation on the wall. In the gas phase, aerobic sulfur-oxidizing bacteria, of which *Thiobacillus thiooxidans* is typical, oxidize hydrogen sulfide to form sulfuric acid, which attacks concrete [3-5].

The authors have clarified that deterioration of one underground concrete structure, which is different from a sewerage, could be caused by microorganisms as a result of investigations on the structure [6,7]. In this study, after microorganisms were isolated around the deteriorated concrete structure and was cultured, simulation tests of mortar deterioration using microorganisms (hydrogen sulfide-producing bacteria and sulfur-oxidizing bacteria), which would be concerned in the concrete deterioration, were carried out and the compositions of the metabolites of the microorganisms analyzed.

2. EXPERIMENTAL METHOD

2.1 Bacteria

(a) Hydrogen sulfide-producing bacteria

Two types of bacteria (Bacteria 1 and Bacteria 2), which were collected from soil around the underground structure and were isolated under aerobic condition with an ordinary agar medium (10 g of nutrient broth, 10 g of peptone, 10 g of glucose, and 20 g of agar in 1000 ml of distilled water), were used. To identify these bacteria, observation by gram staining, observation of shape by transmission electron microscope, hydrogen sulfide testing, nitrate reductase testing, methyl red reaction testing, Vogers-Proskauer reaction testing, indol testing, measurement of G-C content of DNA, and identification of quinones were carried out [8].

(b) Sulfur-oxidizing bacteria

One type of bacteria (Bacteria 3), which was collected from water around the underground structure and was isolated under aerobic condition with medium for *Thiobacillus* (2 g of K_2HPO_4, 0.4 g of NH_4Cl, 0.4 g of Na_2CO_3, 0.2 g of $MgSO_4$, 5 ml of vitamin mixture, 2

ml of trace metal solution, 3.95 g of $Na_2S_2O_3$, and 20 g of agar in 1000 ml of distilled water), was used. To identify this bacteria, observation by gram staining, observation of shape by transmission electron microscope, growth test on inorganic medium (2 g of K_2HPO_4, 6.2 g of $Na_2S_2O_3 \cdot 5H_2O$, 2 g of KH_2PO_4, 0.4 g of NH_4Cl , 0.4 g of Na_2CO_3, 0.2 g of $MgSO_4 \cdot 7H_2O$, 5 ml of vitamin mixture, 3 ml of trace metal solution, and 20 g of agar in 1000 ml of distilled water), measurement of the optimum pH and the optimum temperature for growth, sulfur culture which was tested for knowledge of potential for oxidizing sulfur compound with liquid medium (2 g of K_2HPO_4, 2 g of KH_2PO_4, 0.4 g of NH_4Cl, 0.4 g of Na_2CO_3, 0.2 g of $MgSO_4 \cdot 7H_2O$, 60 g of sulfur, 5 ml of vitamin mixture and 2 ml of trace metal solution in 1000 ml of distilled water), growth test against oxygen, denitrification test, measurement of G-C content of DNA and identification of quinone were carried out.

2.2 Simulation Test Using Mortar

(a) Mortar specimen

Mortar was prepared with a water-cement ratio of 0.5 and a sand-cement ratio of 1.0, using ordinary Portland cement and Japanese standard sand (Toyoura sand). Mortar prisms (40 x 40 x 160 mm) were cast and cured in water at 20°C until the age of 28 days. After that, the prisms were cut into the size of 40 x 40 x 20 mm and used in the experiments.

(b) Culture medium

In this experiment, ordinary liquid medium (10 g of nutrient broth, 10 g of peptone, 10 g of glucose in 1000 ml of distilled water) was used as a culture medium.

(c) Culture method

A mortar specimen in a culture bottle was immersed in 300 ml of ordinary liquid medium and the bottle sterilized for 20 minutes at 120°C and 1.5 atm. Bacteria were then inoculated upon the medium (photograph 1). A medium containing no bacteria was also prepared as a control.

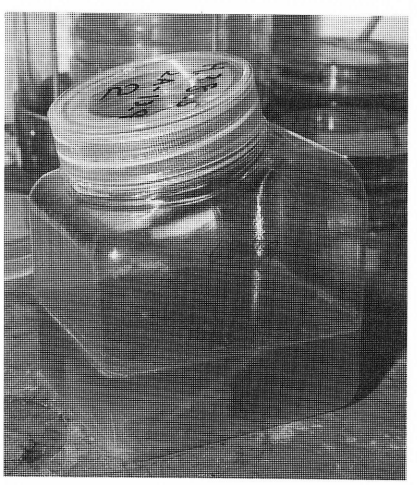

Photo 1 Picture of Culture

Single culture and mixed culture were carried out as shown in figure 1. Two species of hydrogen sulfide-producing bacteria (Bacteria 1 and Bacteria 2) and one species of sulfur-oxidizing bacteria (Bacteria 3) were used. The sulfur-oxidizing bacteria was inoculated in 9 days after culture. All the bacteria used in this tests were in the logarithmic growth phase.

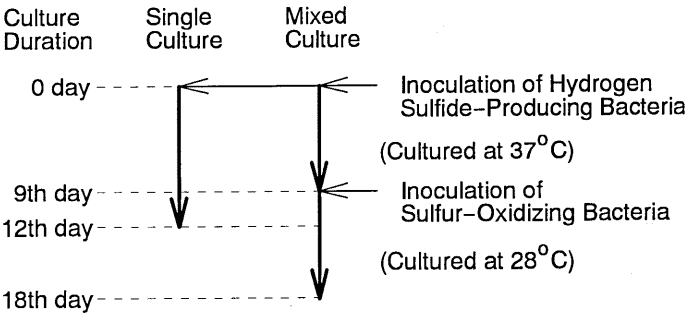

Fig.1 Method of Culture

Long-term culturing of 10 weeks were also carried out on the same way for the analysis of mortar specimen. In this culture, sulfur-oxidizing bacteria were inoculated 14 days after culture.

(d) Analysis method

Eight milliliter of the sample solution was extracted from the medium every third day on a sterile bench. After the solution was filtered with a 0.45 μ m filter, the pH value, calcium ion concentration, sulfate ion concentration, and dissolved hydrogen sulfide concentration of the solution were measured with a pH meter, flame emission spectrophotometer, and ion chromatoanalyzer and with methylene blue method, respectively. The growth of bacteria was determined from the absorbance (wavelength 660 nm) of the solution before filtration.

After 10 weeks of culturing, a mortar specimen was taken out of medium. Samples were extracted from the depth of 0 to 5 mm (S) and 5 to 10 mm (I) with a drill and were ground with agate mortar before being analyzed with powder X-ray diffraction and TG-DTA.

2.3 Analysis of Compositions of Metabolites

Since biological deterioration of concrete was connected with the metabolites of microorganisms, the following analysis was carried out to determine the changes in composition and concentration in culture medium as the microorganisms grew.

After hydrogen sulfide-producing bacteria was inoculated in ordinary liquid medium (100 ml) in a culture bottle and was cultured at 37°C, growth curve was drawn by measuring absorbance of solution with absorptiometry. Based on the curve, before inoculation of bacteria, on the logarithmic growth phase, in which bacteria were active, and on the stationary growth phase, in which bacteria stopped growing, culture medium was extracted and compositions of the medium investigated. Organic acid, inorganic anions, and inorganic cations in the medium were determined by gas chromatoanalyzer, ion chromatoanalyzer, and flame emission spectrophotometer, respectively.

3. IDENTIFICATION OF BACTERIA

3.1 Hydrogen Sulfide-Producing Bacteria

The results of identification tests of hydrogen sulfide-producing bacteria (Bacteria 1 and Bacteria 2) are shown in table 1.
Bacteria 1 could not be identified because the bacteria stopped propagating during identification tests.
From the results shown in table 1, Bacteria 2 was thought to belong to *Flavobacterium* sp. or *Xanthomonas* sp. Since some of *Xanthomonas* sp. produced hydrogen sulfide from peptone, Bacteria 2 was identified as *Xanthomonas* sp. *Xanthomonas* sp. is known as a bacterium found in plants.

3.2 Sulfur-Oxidizing Bacteria

The results of identification tests of sulfur-oxidizing bacteria (Bacteria 3) are shown in table 2. From the results, Bacteria 3 was identified as *Thiobacillus intermedius*.

4. SIMULATION TEST OF MORTAR DETERIORATION

4.1 Results of Analysis of Culture Medium

The changes in calcium ion concentration in the medium are shown in figure 2. The concentration in vertical coordinate represents the percentage of dissolved calcium content to calcium content in the mortar. From the figure, it was found that the calcium ion concentration in the medium inoculated with bacteria increased in

Table 1 Result of Identification of Hydrogen Sulfide-Producing Bacteria (Bacteria 1 and Bacteria 2)

No.	Gram Staining	Shape of Cell	Size of Cell	Hydrogen Sulfide Test	Reduction of Nitrate 1 day	3 days	5 days
Bacteria 1	Negative	Rod		Positive	+	+	+
Bacteria 2	Negative	Rod	0.8-1.5×2-3 μm	Positive	-	-	-

No.	V-P Reaction	Methyl Red Test	Indol Test	G-C Content of DNA	Quinones
Bacteria 1	Positive	Positive	Negative		
Bacteria 2	Positive	Negative	Negative	57.5%	Menaquinone

Table 2 Result of Identification of Sulfur-Oxidizing Bacteria (Bacteria 3)

Gram Staining	Shape of Cell	Size of Cell	Flagella	Chemoautotrophy	Optimum pH for Growth
Negative	Rod	1.5-1.8×0.6 μm	Polar	+	8.0

Optimum Temperature for Growth	Sulfur Culture	Oxygen	Denitrification	G-C Content of DNA	Quinones
30°C	+	Aerobic	-	68.8%	Uviquinone Q-8

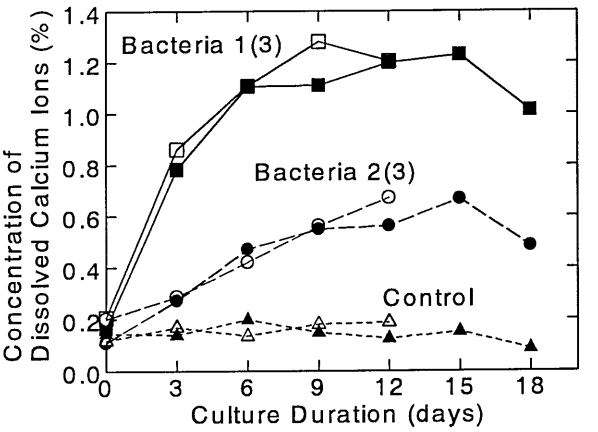

Fig.2 Calcium Ion Concentration in Culture Medium (White: Single Culture; Black: Mixed Culture)

the early stage of culture, while there was no change in calcium ion concentration in the medium containing no bacteria. Few calcium ions were detected in the medium containing bacteria and no mortar specimen. Therefore it is considered that the calcium ions in the medium was not metabolite of bacteria itself but it was dissolved out of the mortar by the effects of the introduced bacteria. The calcium ion concentration in the medium increased up to the ages of 9 or 12 days for the single culture, and up to 15 days for the mixed culture. Then calcium ion concentrations started to decrease in both cases. This might be because the bacteria ingested calcium ions as a nutrient or because the calcium ions reacted with the carbon dioxide produced by the respiration of the bacteria and calcium carbonate was produced and precipitated.

Growth curve of microorganism presents as absorbance of the medium measured by absorptiometry as shown in figure 3. The quantity of microorganism increased up to the culture age of 3 or 6 days and then plateaued. Considering this tendency with the results of

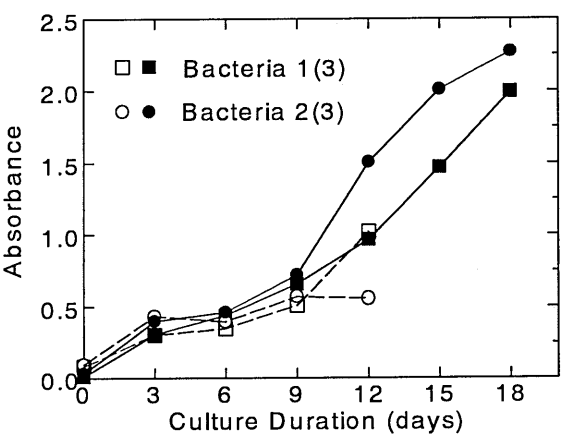

Fig.3 · Growth Curves of Microorganisms (White: Single Culture; Black: Mixed Culture)

calcium ion concentration, the growth of bacteria caused lack of nutrient in the medium and calcium ions in the medium might be ingested as nutrient by the bacteria.

The changes in the pH value of the culture medium are shown in figure 4. The pH value shows the one which was measured with respect to the culture medium diluted with distilled water up to 100 times. Compared with the control samples, the pH value in the medium inoculated with bacteria decreased in the early stage of culture duration and increased after that. In the preparatory examination, when the pH value of the culture medium containing bacteria and no mortar specimen was measured, the value which was first adjusted to 7.0 was decreased to 5.0 and 5.5. Consequently the decrease in pH would result from organic acid and carbonic acid excreted by bacteria. It is thought that the acid reacted with calcium hydroxide in the mortar and the pH value increased. As a result of experiments on the relationship between compositions and concentrations in the culture medium and bacterial activity (Bacteria 2), it was found that concentrations of acetic acid and propionic acid increased and the concentration of phosphoric acid decreased as bacteria grew, while the concentrations of sulfate and nitrate ions increased a little during the logarithmic growth phase and decreased during stationary growth phase as shown in figure 5. From this result, it was thought that organic acids metabolized by bacteria influenced the decrease of pH in the culture medium and accelerated dissolution of calcium ions out of the mortar.

The changes of dissolved hydrogen sulfide concentration in the medium are shown in figure 6. The peak of the concentration was found in 3 or 6 days after culture. In the mixed culture, another peak of the concentration was found after sulfur-oxidizing bacteria was inoculated. The second peak in the mixed culture is thought to be caused by hydrogen sulfide produced by hydrogen sulfide-producing bacteria, which consumed sulfuric acid produced by sulfur-oxidizing bacteria inoculated.

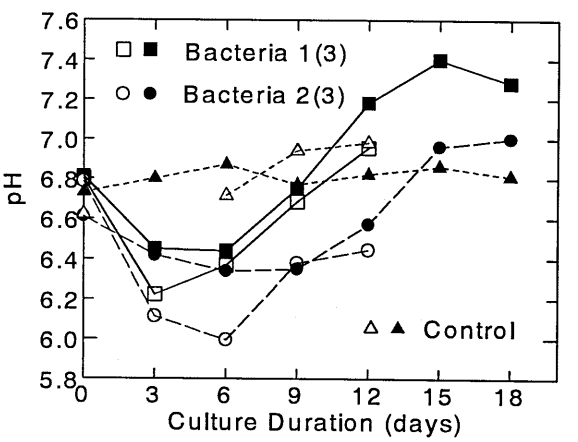

Fig.4 Value of pH in Culture Medium(White : Single Culture; Black : Mixed Culture)

The changes in sulfate ion concentration in the culture medium are shown in figure 7. In the mixed culture, the increase of sulfate ion concentration cannot be seen after inoculation of sulfur-

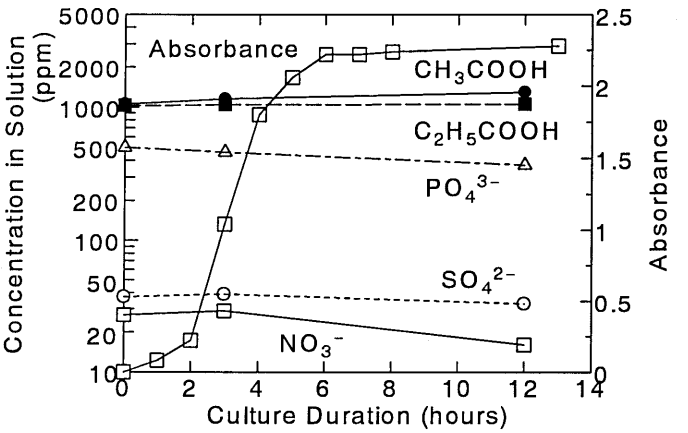

Fig.5 Growth Curve of Bacteria and Concentration of Compositions in Culture Medium

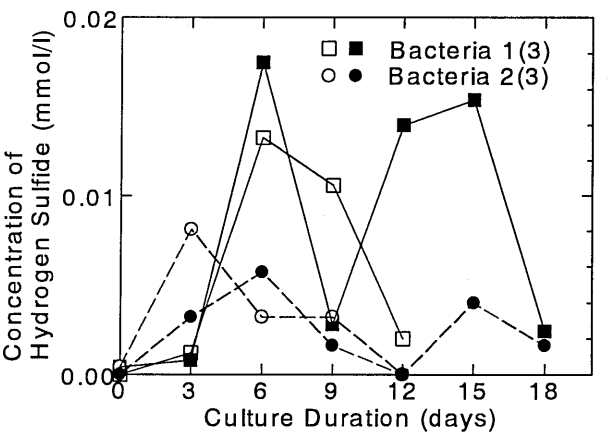

Fig.6 Hydrogen Sulfide Concentration in Culture Medium (White: Single Culture; Black: Mixed Culture)

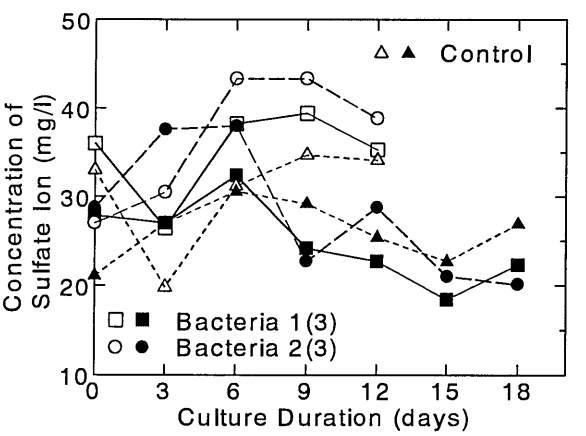

Fig.7 Sulfate Ion Concentration in Culture Medium (White: Single Culture; Black: Mixed Culture)

oxidizing bacteria. This is because the bacteria used in this study did not have high enough activity to produce sulfuric acid as sulfur-oxidizing bacteria (*Thiobacillus thiooxidans*), which caused biological deterioration of concrete in sewage, had.

4.2 Results of Analysis of Mortar Specimen

The calcium content of the surface area (S: 0-5 mm) and internal area (I: 5-10 mm) of mortars was obtained from the TG-DTA results, and the calcium carbonate content was converted to the equivalent calcium hydroxide content as shown in figures 8 and 9. From the figures, the calcium hydroxide content decreased and the calcium carbonate content increased in mortars soaked in the culture medium inoculated with bacteria. This was because carbon dioxide

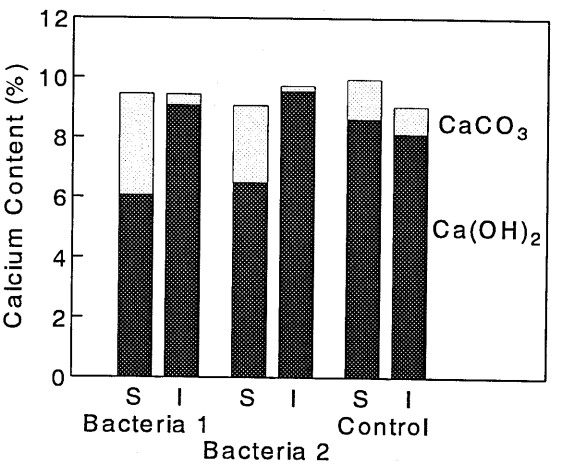

Fig.8 Calcium Content in Mortar in Single Culture (S: Surface Area of Mortar; I: Internal Area of Mortar. Calcium carbonate content was converted into equivalent calcium hydroxide content.)

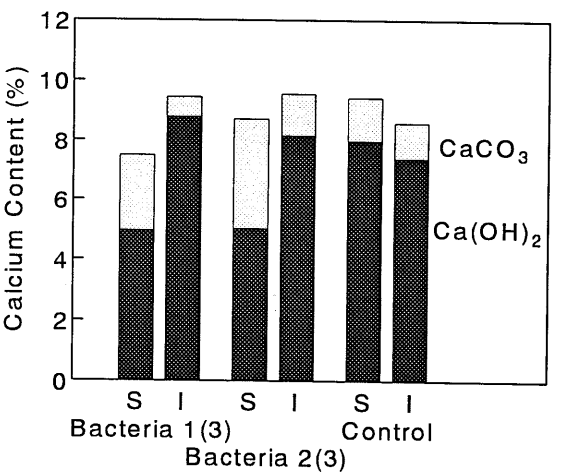

Fig.9 Calcium Content in Mortar in Mixed Culture (S: Surface Area of Mortar; I: Internal Area of Mortar. Calcium carbonate content was converted into equivalent calcium hydroxide content.)

produced by bacterial respiration caused carbonation of the mortar. In a mixed culture, the total calcium content of the mortar soaked in the culture medium with bacteria was smaller than that in the control specimen. This decrease in calcium content would be due to dissolution of calcium ions by organic acid and/or carbonate acid, which were metabolized by the bacteria.

As a result of powder X-ray diffraction analysis, neither ettringite nor gypsum was identified in the mortar. Since enough nutrient for bacteria to grow could not be supplied and the production of sulfuric acid by sulfur-oxidizing bacteria was low, so little ettringite and gypsum was produced in the mortar that those products could not be detected by powder X-ray diffraction analysis.

5. RELATIONSHIP BETWEEN CONCRETE DETERIORATION AND SIMULATION TEST

The underground structure in which biological deterioration occurred was constructed by shield tunnelling. The concrete was soft enough to collapse by hand and was damaged especially around the joints of concrete segments, where leakage of water was found, and the gutter of pavement, where the water had stagnated. Therefore it was thought that underground water was involved in the deterioration of concrete. As this deterioration was found only on the surface inside the structure, it was thought corrosive substance was produced during penetration of underground water into concrete.

It is known that hydrogen sulfide was often encountered during excavation work in this district. And it was clarified that soil and underground water contained many anions and cations as a result of analysis and soil was permeated with sea water [9]. Furthermore, soil had comparatively much organic substance and acid consumption of underground water was very high since microorganisms produced carbon dioxide by decomposition of organic substances.

Consequently, external factors with respect to concrete deterioration by bacteria are shown in figure 10. Organic

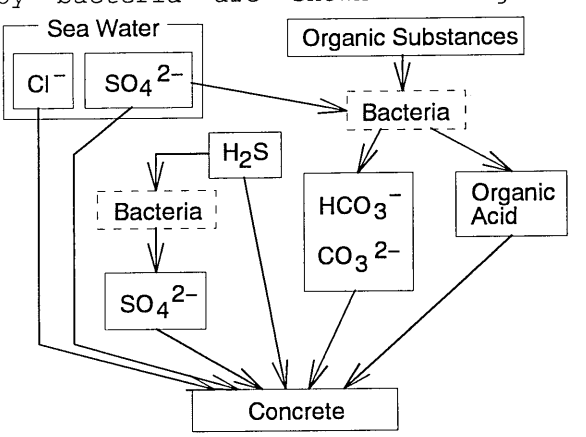

Fig.10 External Factors Concerned with Concrete Deterioration by Bacteria

substance contained in the soil was ingested as nutrient by bacteria and both carbonic acid and organic acid were excreted. Chloride ions were supplied from sea water, and the oxidizing or reducing action of bacteria produces sulfuric acid or hydrogen sulfide.. These factors would affect concrete.

Sulfate ions and chloride ions permeated into the pore solution in the concrete reacted with cement hydrate and produced monosulfate, ettringite, or Friedel's salt.

$$3CaO \cdot Al_2O_3 + 3Ca(OH)_2 + 3SO_4^{2-} + 6H^+ + 26H_2O \rightarrow 3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$$

$$3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O + 2(3CaO \cdot Al_2O_3) + 4H_2O \rightarrow 3(3CaO \cdot Al_2O_3 \cdot CaSO_4 \cdot 12H_2O)$$

$$3CaO \cdot Al_2O_3 \cdot CaSO_4 \cdot 12H_2O + 2Cl^- \rightarrow 3CaO \cdot Al_2O_3 \cdot CaCl_2 \cdot 10H_2O + SO_4^{2-} + 2H_2O$$

The complex salts and cement hydrate were decomposed by carbonic acid and/or bicarbonic acid produced by the bacteria. By repetition of product and decomposition, the concrete structure became porous and weak.

$$Ca(OH)_2 + CO_3^{2-} + 2H^+ \rightarrow CaCO_3 + 2H_2O$$

$$3CaO \cdot 2SiO_2 \cdot 3H_2O + 3CO_3^{2-} + 6H^+ \rightarrow 3CaCO_3 + 2SiO_2 + 6H_2O$$

$$3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O + 3CO_3^{2-} + 6H^+ \rightarrow 3CaCO_3 + 2Al(OH)_3 + 3Ca^{2+} + 3SO_4^{2-} + 32H_2O$$

$$3CaO \cdot Al_2O_3 \cdot CaSO_4 \cdot 12H_2O + 3CO_3^{2-} + 6H^+ \rightarrow 3CaCO_3 + 2Al(OH)_3 + Ca^{2+} + SO_4^{2-} + 12H_2O$$

$$3CaO \cdot Al_2O_3 \cdot CaCl_2 \cdot 10H_2O + 3CO_3^{2-} + 6H^+ \rightarrow 3CaCO_3 + 2Al(OH)_3 + Ca^{2+} + 2Cl^- + 10H_2O$$

It is thought that these consecutive reactions shown in figure 11 break down the cement structure.

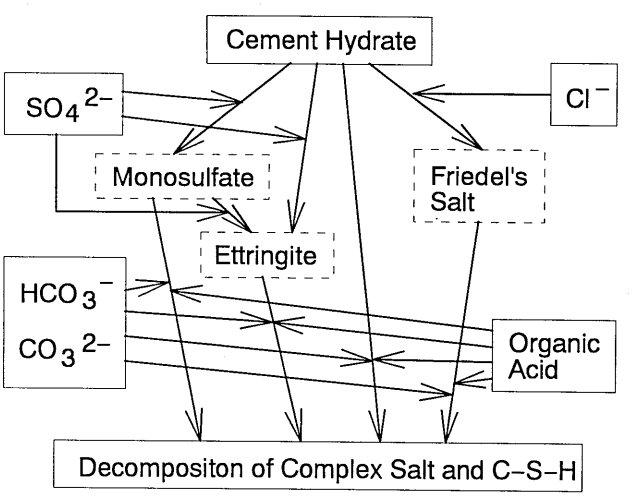

Fig.11 Internal Factors Concerned with Concrete Deterioration by Bacteria

Since calcium carbonate such as calcite and vaterite was much detected as a result of analyzing the concrete in the structure, one of the reasons for deterioration is thought to be extraordinary carbonation.

In this study, it was found that concrete was deteriorated by metabolites resulting from microorganism respiration when microbiological activity was high as a result of mortar simulation tests using hydrogen sulfide-producing bacteria and sulfur-oxidizing bacteria which was isolated under aerobic condition. It is said that about 100 million microorganisms inhabit a gram of fertile soil. Since the soil around the underground structure contained much organic substance, many types of microorganism were isolated [10]. Although it is not possible to totally simulate effects of microorganism on concrete by experiments using only one or two species of bacteria, some of the effects were clarified in this study.

6. CONCLUSIONS

As a result of this study, calcium ions were dissolved out from mortar soaked in the culture medium inoculated with hydrogen-producing bacteria and sulfur-oxidizing bacteria, which were isolated under aerobic condition, and it was found that the main compositions of metabolites of bacteria used in this study were acetic acid, propionic acid, and carbonic acid. Although severe deterioration of concrete, which was caused by anaerobic bacteria, could not be simulated as seen in sewage, it was found that concrete deterioration could be caused by metabolites of aerobic bacteria if the structure was constructed in a place having high microbiological activity.

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