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Sulfur cycle of microbial corrosion on carbon steel in soil model

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ABSTRACT

This study examined the effects of *Desulfovibrio desulfricans* and bacteria consortia on the rate of carbon steel corrosion in soil model. Microbial corrosion was measured using the corroded mean depth after 56 days incubation under aerobic and anaerobic conditions. The effects of water content and dissolved oxygen in soil on the corrosion rate were also analyzed. Results showed that aerobic conditions increased corrosion rate. Moreover, sole *Desulfovibrio desulfricans* treatment ceased the corrosion as a protective ferrous sulfide film formed on the carbon steel. While the heterogeneous biofilm of the bacterial consortia formed uneven oxygen concentration which accelerated the corrosion.

Keywords - **Bacteria , Carbon steel, Corrosion, SRB, *Desulfovibrio desulfricans***

I. Introduction

Corrosion is an ever-present degradation mechanism in wetted components and systems. There are many forms of corrosion in metals, that include; pitting, stress corrosion, general corrosion, galvanic corrosion, and others [1], in which microbiologist have recognized and are constantly addressing. When a system first encounters microbial corrosion (MC) such event usually occurs during the system initial exposure to an aqueous environment, such as during hydrotest, wet lay-up, or moist soil. The presence of certain bacteria in an environment will lead to the production of microbial corrosions. This mode of corrosion can be accelerated by microbial organisms, either because they manufacture aggressive species, such as protons or sulphide ions, or because they catalyze the electrochemical reactions themselves [2]. MC induction also requires the presence of nutrients and water to ensure the survival and growth of microorganisms. Thus, the ability of microorganisms to sense and rapidly response to harsh environmental changes is vital for their survival and MC capabilities [3]. Microbes can grow in fluids with pH values ranging from -1 to 10, where -1 is the most acidic concentration, and derive energy from organic or inorganic materials. Moreover, microbes can survive temperatures which range from -4 to 210^{o}F (-20 to 99^{o}C) [4]. The majority of the active organisms involved in corrosion are bacteria, about 1-5 micrometers long, which either oxidize or reduce sulphur compounds as some part of their life process. Although many culturable bacterial types with known corrosion effects have been identified [5], in both aquatic and terrestrial environments the primary corrosion-causing bacteria are the sulfate-reducing iron-oxidizing bacteria. *Desulfovibrio desulfricans* is examined in this report as a source of Sulfate reducers, which is found to exist in all soil and water types, as well as lives symbiotically with facultative anaerobic bacteria [6]. Sulfate-reducing bacteria (SRB) are a group of anaerobic diverse organisms in which have varied morphological and nutritional characteristics. They utilize organic matter to produce sulfide by either reducing or oxidizing sulfate compounds [7] , as a source of energy. Therefore, sulphate (SO_4^{2}–) can be reduced to sulphide (S^{2}–) by SRB leading to the generation of hydrogen sulfide as a metabolic bi-product. Both physical and chemical processes transfer Hydrogen sulfide (H2S) across the air and water boundaries to environments where chemoautotrophic bacteria oxidize the sulfide to sulfuric acid [8]. The corrosion process will hence occur by the reaction of the biogenic sulfuric acid with the metallic surfaces [9]. In order to evaluate the significance of microbial corrosions, a look at the economical perspective on such effects is essential. In 2001, the cost of microbial-influenced corrosion on oil and gas industries accounted for about $2 billion annually [10]. Microbial induced corrosion in the US economy has reached $350 billion annually as of 2010 [11]. Mild steel in used widely in piping systems, storage tanks, cooling towers and aquatic structures and is the most readily corroded metals [12]. Since bacteria form colonies beneath which corrosion can occur, prevention of colonization is one of the potential ways to prevent MC. This report will examine the effects of *Desulfovibrio desulfricans* and bacteria consortia on the rate of carbon steel corrosion in soil model with respect to dissolved oxygen and water content distribution in such a system.

II. Materials and Methods

2.1 Organisms

The sulfate reducing bacterial stain *Desulfovibrio desulfricans* DSMZ 642 was used as the model *SRB* in this study. Activated sludge from a municipal wastewater plant was used as model *Bacteria consortia*.

2.2 Culture medium

Modified Baar`s Medium (MBM), containing (in g/L) anhydrous sodium citrate 5.0, 50% sodium lactate 4.9, yeast extract 1.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 2.0, $CaSO_4$ 1.0, and K_2HPO_4 0.5, was used as culture medium. The pH, sulfate concentration and total organic carbon concentration (TOC) of MBM are 7.0, 1500 mg/L, and 2500 mg/L, respectively. SRB and activated sludge were pre-incubated in MBM at 37°C and 25°C, respectively, for seven days. Then, one-tenth of these pre-incubated culture broths were inoculated into test culture medium. The inoculated SRB size was $4x10^5$ colony forming unit (CFU) CFU/mL *D. desulfricans* and $3x10^7$ CFU/mL facultative anaerobic bacteria. The inoculated activated sludge size was 7500mg/L mixed liquor suspended solid (MLSS). Sterile MBM was prepared by autoclaving the medium at 120□ C for 20min.

2.3 Carbon steel coupons preparation

Rectangular carbon steel coupons (20x10mm and 0.35mm thick) were cut from a sheet stock. The composition of the carbon steel coupons was (in wt %) 99.71 Fe, 0.03 C, 0.01 Si, 0.19 Mn, 0.013 P, 0.017 S, 0.0017 N, and 0.026Al. The surface was wet-polished with 800-grid polishing paper. The polished coupons were cleaned ultrasonically in acetone for 15min, weighed, air-dried, and stored in a desecrator.

2.4 Corrosion Measurements

The corroded mean depth (CMD) was used as an indicator of the extent of corrosion. Corrosion products were selectively removed from tested coupons by incubating at 60°C for 60 min in a 10wt% HCl solution with 0.3vol% Ibit, a polycationic amine derivative that protects metal steel surface from HCl. Then the treated coupons were weighed. Mass loss of the carbon steel coupon was divided by the specific gravity of iron ($7.86g/cm^3$) and by the area (cm^2) of the carbon steel coupon, and CMD was derived.

2.5 Model soil preparation

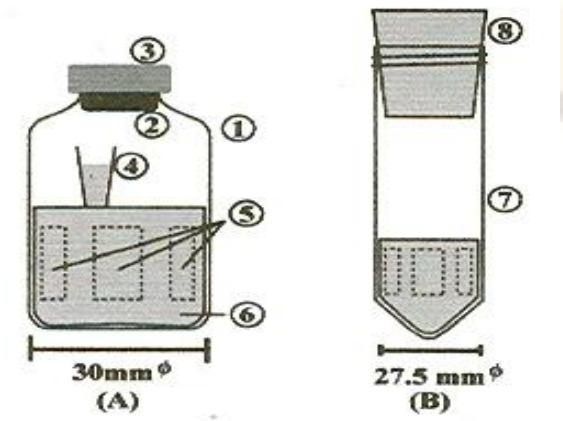

Figure 1. Experimental apparatuses for preparation of artificial model soil. (A) Anaerobic conditions: (1) 30mL vial bottle; (2) butyl rubber plug; (3) aluminum cap; (4) Na_2S solution in 500µL microtube; (5) carbon steel coupons; (6) culture medium in SiO_2 soil. (B) Aerobic conditions: (7) 50mL centrifuge tube; (8) silicone plug.

To create artificial model soil, 30mL vials (Nichiden Rika Garasu, Hyogo) containing 25g silicon dioxide (SiO_2) sand, a 500µL microtube, and three carbon steel coupons were autoclaved, sealed with a butyl rubber plug and an aluminum cap, and replaced with N_2 gas (N_2 >99.9995%) (Figure, 1A). To maintain the anaerobic conditions throughout the experiment, 400µL of 27.2g/L Na_2S solution was poured into the microtube. Then, 7.5mL of the degassed culture medium was inoculated. To maintain aerobic conditions, a 50mL centrifuge tube containing 25g SiO_2 and three carbon steel coupons was autoclaved. Followed by the inoculation with 7.5mL of the culture medium (Fig, 1B).

2.6 Measurement of water content on corrosion

To investigate the effects of water content on corrosion, 4.5 or 1.5mL culture medium was inoculated. Water content (WC) was defined as the ratio of water volume to void of the model soil. Since the void of 25g SiO_2 7.5mL, addition of 7.5, 4.5 and 1.5mL of culture medium resulted in 100, 60 and 20% WC, respectively. To keep the water constant in the test tube, sterile water was added and the tube was centrifuged (1500G, 3min) every week. After incubation for 0, 7, 14, 28, and 56 d, saline (NaCl 8.0 g/L, KCl 0.2 g/L) was added to the model soil. The model soil was sonicated for 20 sec, and mixed with a spatula.

2.7 Measurement of Total Organic Carbon and Sulfate Concentrations

Sulfate concentration and TOC concentration in the culture was measured by sulfur analyzer Antek (9000 Series) and a TOC analyzer, respectively. The pH of the culture tenfold-diluted solution was measured by a pH meter. Corrosion products were selectively removed from the corroded carbon steel coupons as described above. The roughness of the coupon was analyzed by a laser 3D profile microscope (VK-8500).

2.8 Bacterial count

To count the aerobes in the culture, saline was added to the model soil and the supernatant was spread-plated on Minimized Luria-Bertani (MLB) agar plate, containing (in g/L) polypeptone 1.0, yeast extract 0.5, NaCl 10.0, and agar 15, in triplicate. After incubation for 7 d at 28°C, the observed colonies were counted as aerobes. For the counting of anaerobes and SRB in the experimental culture, the supernatant was added dropwise to the plates and the MBM agar medium with 173 (in g/L) $Fe(NH_4)_2(SO_4)_2$ was poured. The plates were incubated in sealed jars with an oxygen absorbing and carbon dioxide generating agent. After incubation for 14 d at 37°C, the observed black

colonies were counted as SRB and others were counted as anaerobes. The concentration of bacteria was expressed as (CFU) per mL.

III. Results

3.1 Analysis of corroded carbon steel

The CMDs of carbon steel coupons under anaerobic and aerobic conditions were compared after 56 days incubation period. In *Bacterial consortia*, the CMD under aerobic conditions reached 34.1 µm, which was 28 times higher than anaerobic conditions with CMD measurement of only 1.2 µm. However, the CMD for SRB-inoculation or sterile conditions reached 5.42 or 5.11 µm respectively, in which was 8.9 or 8.8 times higher than the anaerobic conditions (Fig. 2A, Table 1). There weren't any significant differences between *SRB* and sterile control conditions, yet in *Bacterial consortia* a change was noted with 2.1 and 6.7 times increase in anaerobic and aerobic treatments respectively, when compared with sterile CMD values. These observations indicated that existence of oxygen causes carbon steel corrosion in model soil.

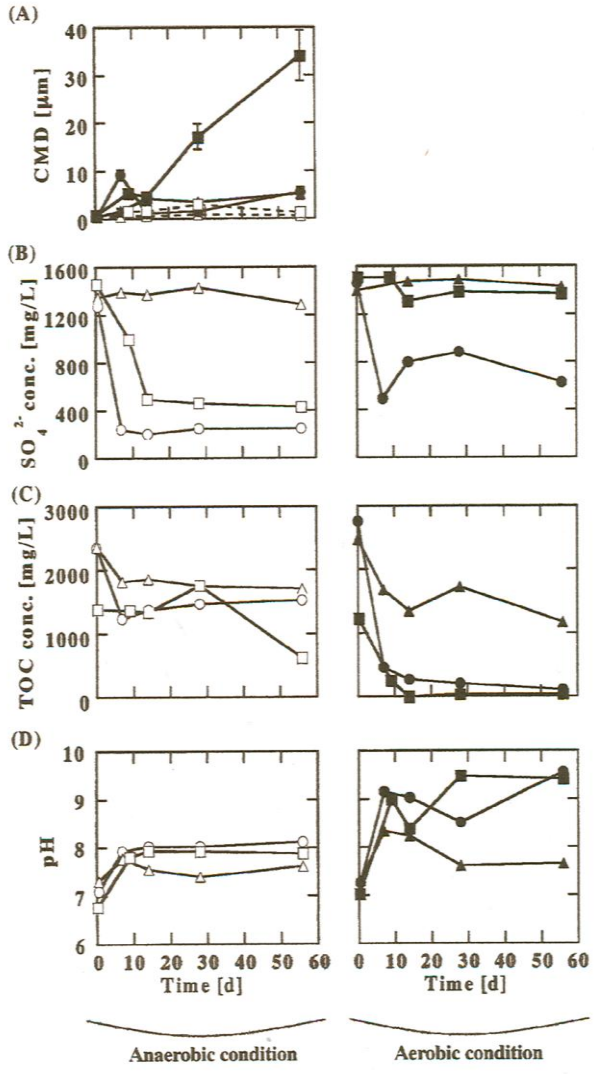

Figure 2. Time Course of CMD (A), sulfate concentration (B), TOC concentration (C) , and pH (D) under anaerobic (○, □, △) and aerobic (●, ■, ▲) conditions. The test was conducted in SRB culture (○, ●), bacterial consortia (□, ■) and in sterile conditions as a control (△, ▲).

Table 1. Effect of dissolved Oxygen Existence and Bacteria on Endpoint CMD (µm) after 56 days Incubation

	SRB	*Bacterial consortia*	Sterile
Anaerobic	0.61	1.2	0.58
Aerobic	5.42	34.1	5.11

Upon inoculation of *SRB* under aerobic conditions, little corrosion on the steel coupons was observed during the 14-28 days incubation period. In the model soil analyzed, a gap separating the soil into upper and lower parts was observed just after incubation for 7 days. This gap seemed to influence corrosion acceleration, as the coupons were corroded unexpectedly at day 7. The whole surface of coupon immersed into the *SRB* culture was covered with homogeneous black corrosion products that were easily wiped off. When the corroded coupons were incubated with HCl, hydrogen sulfide (H_2S) gas was produced, indicating that FeS was formed. In addition, local corrosion under black tubercles was observed under aerobic conditions. These observations showed that the sole existence of *SRB* didn`t accelerate carbon steel corrosion, while that of *Bacterial consortia* did indeed accelerate the corrosion process.

3.2 Influence of pH, Sulfate and TOC concentrations on bacterial growth over time

Sulfate concentration for the *SRB* inoculated in both anaerobic and aerobic conditions stopped decreasing at day 7 suggesting that the sulfate-reducing activity of *SRB* was high until this day as it began to decrease afterwards. While, the sulfate concentration for *Bacterial consortia* stopped decreasing at day 14 (Fig. 2B). Incubation of carbon steel coupons with *SRB* or *Bacterial consortia* inoculae lead to the depletion of TOC concentration at day 7; furthermore, heterotrophic bacteria were inactive during the 14-56 day incubation period (Fig. 2C) and the concentration of aerobes decreased as well (Fig. 3A). Under anaerobic conditions, the TOC was not consumed completely, because oxygen as electron acceptor was limited. TOC concentration decreased even under sterile conditions. In another experiment with sterile conditions that did not contain carbon steel coupons, the TOC concentration however did not decrease (data not shown). This indicates that the

corrosion products adsorbed the organic carbon. Under anaerobic conditions, *SRB* concentration was decreased and became negligible during the 28-56 day incubation period (Fig. 3C). This is due to the fact that insufficient sulfate and hydrogen sulfide that filled the tested bottle inhibited the growth of *SRB*. Under aerobic conditions, the presence of *SRB* suggested the formation of anaerobic regions in the soil.

When examining the pH concentration under anaerobic conditions, it increased to 7.9 after incubation for 7 days and remained constant for 50 days. On the other hand, the pH under aerobic conditions was slightly higher as it increased to 9 after similar incubation time and remained constant for 50 days as well (Fig. 2D). In SRB culture incubated under both anaerobic and aerobic conditions, there was no detection of *SRB* during the 28-56 day incubation period (Fig. 3C). In contrast, *Bacterial consortia* cell concentrations reached 10^8 CFU/mL under aerobic conditions, while *SRB* reached just over 10^5 CFU/mL, after incubation for 28 days. As for the sulfate concentration, it remained around 1400mg/L throughout the experiment. The increase of *SRB* at 7 day incubation time indicated that *SRB* was not dormant and sulfate-reducing activity occurred during this time. This observation suggested that sulfur-oxidizing bacteria (SOB) generated sulfate and lived symbiotically with *SRB*, in addition to the oxidation of sulfide within the dissolved oxygen.

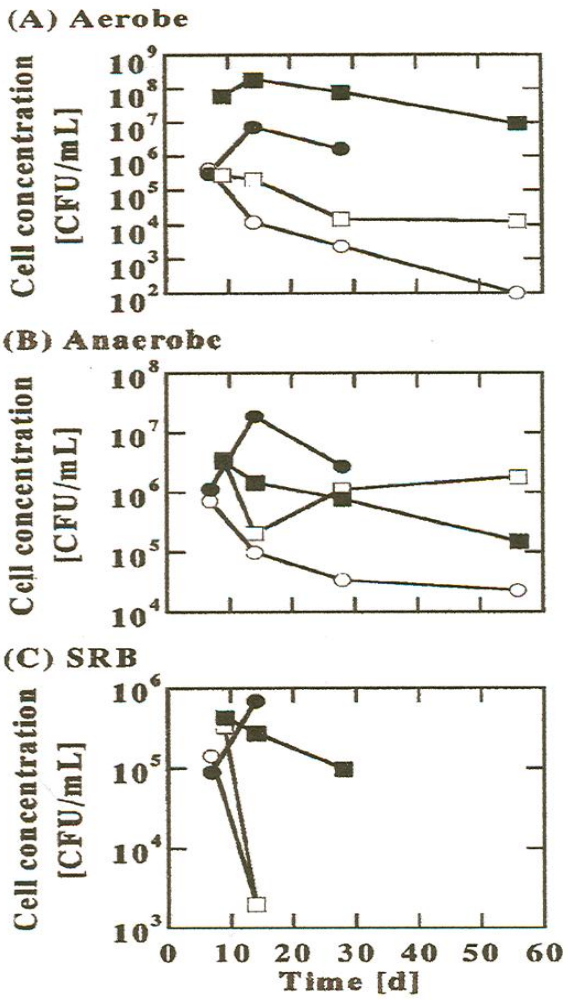

Figure 3. Time course on the concentration of aerobes (A), anaerobes (B), and SRB (C). Incubation was carried out under anaerobic (○, □) and aerobic conditions (●, ■). ○, ● : In SRB culture, □, ■ : In bacterial consortia. Cell concentrations were measured over a 60 day (d) period.

3.3 Characteristic of *SRB* colonies

For the counting of *SRB* in the culture of *Bacterial consortia*, the culture broth was inoculated into MBM agar plate under anaerobic condition. After incubation for 7 days, a black colony was observed in the plate. Following this period, the entire plate became black due to H_2S produced from the *SRB* colony. Clear zones surrounding the bacterial colonies in the FeS on the MBM agar plates were observed (Fig. 4).

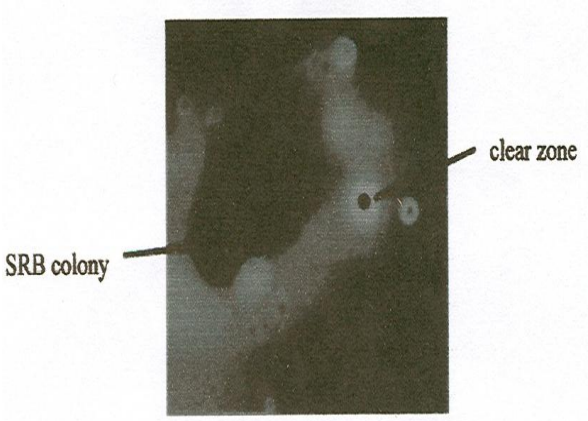

Figure 4. Clear zones surrounding the bacterial colonies in the FeS on the MBM agar plates incubated under anaerobic conditions.

3.4 Effects of Water Content on carbon steel coupons roughness

In case of 60% WC, the boundary line between water phase and air phase was observed at the center of the carbon steel coupons. Yet, for 20% WC, the boundary line was not observed on the coupons. Examination of the eroded region on the corroded coupon in terms of 60% WC, it was limited to the lower half (Fig. 5C). On the other hand, in the case of 20% WC, the eroded region was observed over the whole area on the coupons. Shiny metal surfaces remained after incubation for 56 days (Fig. 5D). In comparison with 100% WC, the eroded region was observed over the entire coupon, and developed pitting was observed (Fig. 5B). The CMD under sterile conditions with 60% WC reached 22.5µm and was 4.4 times higher than 100% WC (5.11µm) after incubation for 56 days (Fig. 6B, Table 2). CMD was directly proportional to the incubation time (R^2= 0.993). CMD under sterile conditions with 20% WC reached 30.0µm and was 5.9 times more than 100% WC after incubation for 56 days. The CMD was directly proportional to the incubation time (R^2 = 0.999).

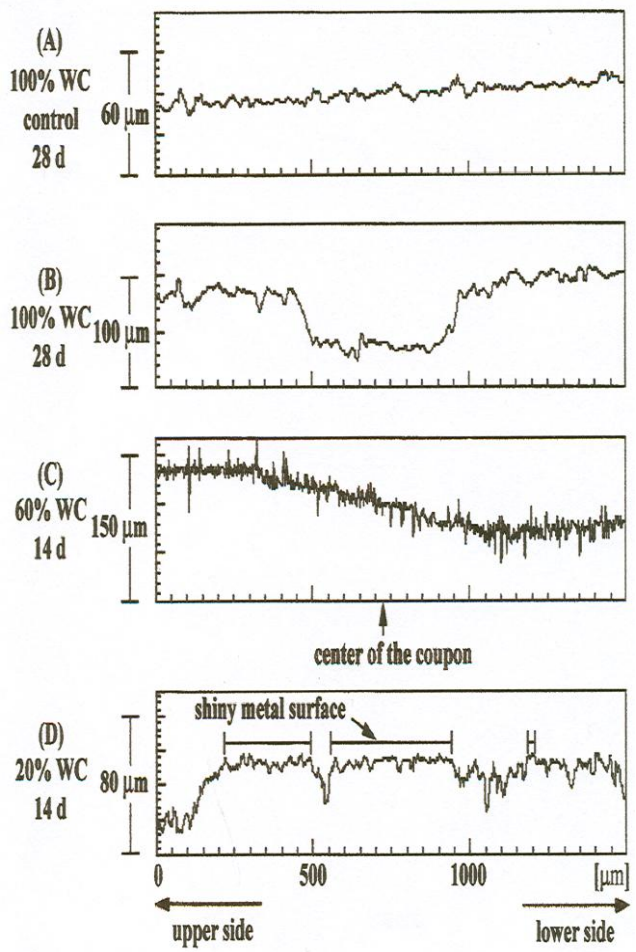

Figure 5. Surface roughness analysis of corroded carbon steel coupons immersed in bacterial consortia under 100, 60 and 20% WC conditions. Corrosion products were selectively removed and the roughness of the metal surface was analyzed. The control coupon was immersed in sterile conditions. Under 60% WC condition, the lower half of coupon was eluted. Under 20% WC condition, shiny metal surfaces were observed.

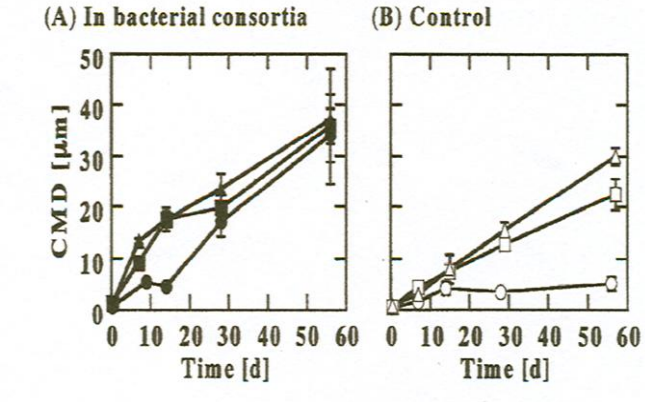

Figure 6. Time course of CMD in bacterial consortia (○, □ , △) and sterile conditions (●, ■ , ▲) with 100% WC (● , ○), 60% WC (■ , □), and 20% WC (▲ , △). Cell concentrations were measured over a 60 day (d) period.

Table 2. Effect of Water Content on Endpoint CMD (μm) after 56 days Incubation

	Bacterial consortia	**Sterile**
100% WC	34.1	5.11
60% WC	35.9	22.5
20% WC	37.1	30.0

Bacterial consortia accelerated the corrosion of the carbon steel coupons under three WC conditions. CMDs in the cases of 100, 60 and 20% WC reached 34.1, 35.9 and 37.1μm, respectively after incubation for 56 days (Fig. 6A, Table 2). In the case of 100% WC, CMD was slightly increased until the 14th incubation day and the corrosion rate was accelerated after that. No distinct difference in CMD among the three WC conditions was observed nor was there any detection of SRB after incubation for 56 days. The concentrations of aerobes and anaerobes decreased under incubation with all three WC conditions (Fig. 7).

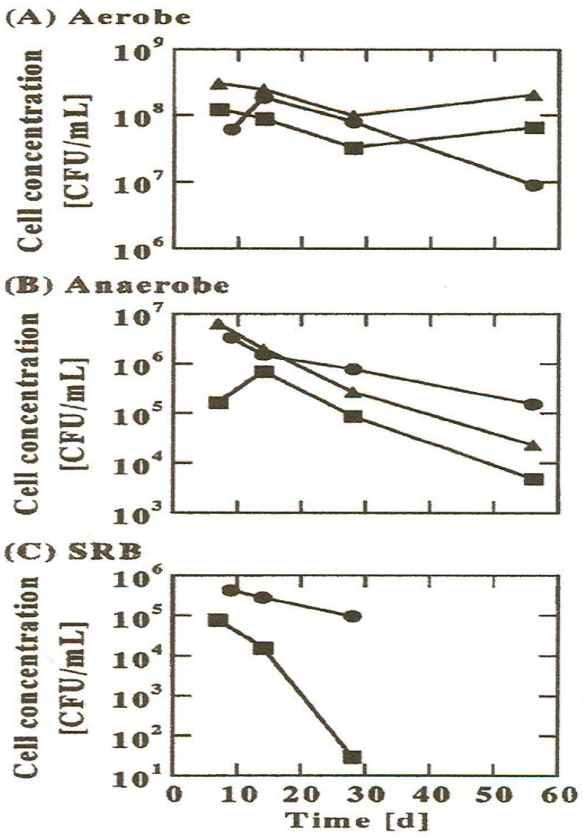

Figure 7. Time course of concentration of aerobes (A), anaerobes (B), and SRB (C). Incubation was carried out under 100% WC (●), 60% WC (■), and 20% WC (▲). Cell concentrations were measured over a 60 day (d) period.

IV. Discussion

Aerobic conditions accelerated the corrosion of carbon steel in model soil. *SRB* inoculation inhibited the corrosion under aerobic conditions at day 14 and 28. The whole surfaces of the corroded steel coupons were covered with homogeneous sulfide (FeS). Ma et al. reported that corrosion of 99.99% pure iron immersed in solution was inhibited by a protective layer of FeS under such conditions as less than 0.04 mmol dm^{-3} H_2S concentration, a pH value of 3-5 and an immersion time longer than 2 h [13]. When the entire coupon surface was covered with homogeneous ferrous sulfide, corrosion of carbon steel was inhibited. Under anaerobic or aerobic condition, sulfate concentration for the *SRB* inoculated condition stopped decreasing at day 7 because of either the product (H_2S) inhibition or lactate depletion, respectively. The observation under anaerobic condition indicated that hydrogen sulfide didn`t accelerate corrosion of carbon steel. Corrosion acceleration by *SRB* activity such as the cathodic depolarization was stopped at day 7.

Bacterial consortia accelerated the corrosion of carbon steel. However, in the soil contained water, little convection occurred. Therefore, segregation of bacterial habitat and uneven distribution of dissolved oxygen in the culture were considered to be formed in the soil. It is indicated that the heterogeneous structure led to the formation of a heterogeneous biofilm with corrosion products on the carbon steel coupons. The heterogeneous biofilm that resulted in the uneven distribution of dissolved oxygen on the metal surface and the formation of oxygen concentration cells accelerated microbial corrosion. Therefore, the heterogeneity caused acceleration in model soil. Dubiel et al. reported that the corrosion of carbon steel in the culture inoculated with both SOB and SRB was accelerated compared to that in the culture inoculated with either SOB or SRB [14]. In our experiments, *SRB* colonies and FeS were observed on MBM agar plates inoculated with *Bacterial consortia* culture. The entire plate became black due to H_2S produced by the *SRB* colony. Clear zones surrounding the bacterial colonies in the FeS were observed. On the carbon steel coupons immersed into *Bacterial consortia*, the protective FeS film formed by *SRB* was broken by the bacteria observed in the clear zone. The bacteria were considered to be SOB and the mechanism of corrosion was proposed in Fig. 8. That is being, (1) SRB generate H_2S from sulfate, (2) carbon steel

coupons are protected by FeS, (3) SOB break the protective FeS film and sulfate is generated.

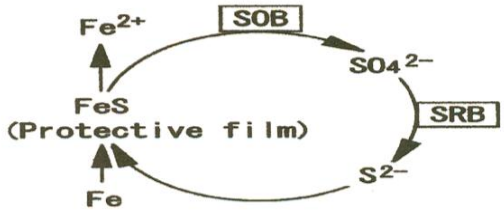

Figure 8. Sulfur cycle with SRB and SOB accelerating carbon steel corrosion.

The corrosion rate of carbon steel coupons increased in the order of 20, 60, and 100% WC. In the case of 60% WC, boundary line between the water phase and the air phase was observed at the center of carbon steel coupons. Above the water phase, the soil had adhering water. In the case of 20% WC, corrosion that begun from adhering water extended to cover the entire carbon steel coupons. In the case of 60% WC, the corrosion was observed not on the upper half of coupon but on the lower half. The difference in dissolved oxygen concentration between the parts under adhering water and the metal surface on the upper half of the coupon was less than that between the lower half and the upper half of the coupons. Therefore, the oxygen concentration cell reaction was limited between the lower half and the upper half of the coupon. The uneven distribution of water and air spaces on the carbon steel coupons accelerated the corrosion.

In *Bacteria consortia*, the corrosion of carbon steel coupons under 60 and 100% WC conditions was accelerated to almost same level as the corrosion observed under the 20% WC condition. Therefore, the heterogeneity resulting from bacteria led to an uneven distribution of dissolved oxygen on the carbon steel coupon, which is equal to the distribution under the 20% WC condition, and that was the major mechanism of microbial corrosion acceleration.

V. Conclusion

Carbon steel coupons were immersed into artificial model soil consisting of silica sand, microbes, and medium. Incubation was carried out under anaerobic and aerobic conditions, and aerobic conditions were found to accelerate corrosion of carbon steel. Carbon steel coupons were immersed into artificial model soils with 100, 60 and 20% WC. The existence of air space and the uneven distribution of medium on the carbon steel coupons accelerated corrosion. Sole SRB ceased the corrosion of carbon steel with protective ferrous sulfide film. Uneven distribution of dissolved oxygen on the carbon steel, resulting from the heterogeneity of bacterial consortia habitat, accelerated the corrosion of carbon steel.

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