Microbial Corrosion of Brass in Groundwater†

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Abstract

Microbiologically Influenced Corrosion (MIC) was suspected in the corrosion failure of heat exchanger copper piping, carrying groundwater. Corrosion resistances of aluminum brass and admiralty brass in contact with groundwater were studied. Groundwater from the same plant was transferred to the laboratory aseptically and used for the experient. Bacteria in the test solution were activated by the addition of low concentrations of nutrients. Aluminum brass and admiralty brass samples were exposed to the test solution. After 2 weeks, many corrosion sites were observed on the surface in the form of pitting. On the other hand, pitting corrosion was not observed on exposure to sterile test solutions.

Results of exposure tests indicated that Staphylococcus sp. which were separated from the groundwater have corrosion activity. Formation of biofilms on aluminum brass and admiralty brass coupons were observed when they were exposed to the test solution with Staphylococcus sp. Under the biofilm, many corrosion sites in the form of pitting could be seen. Staphylococcus sp. are capable of fermentation of proteins and thereby the production of ammonia. Ammonia was detected from the biofilm by Nessler's reagent. It is concluded that aluminum brass and admiralty brass coupons were corroded by ammonia in the test solution. The corrosion mechanism of aluminum brass and admiralty brass coupons in the groundwater is discussed with available literature.

KEY WORDS: (Cu) (Corrosion) (Biocorrosion) (Underground water) (*Staphylococcus* sp.) (Ammonia)

1. Introduction

Microbial Influenced Corrosion (MIC) can be caused by bacterial adhesion, corrosive metabolic products or by direct utilization of metal ions by bacteria. MIC of steels, aluminum and many alloys other than titanium has been reported and the corrosion mechanisms have been discussed[1-5]. In the natural environment, where MIC occurs, single strains are not the rule, but there is coexistence of different microbes. So, it is assumed that MIC is affected by symbiosis of these microbes. Reports discussing MIC, the kind of microorganisms and their performance in the environment are scarce.

The present authors have reported MIC of oxygen free copper occurring in neutral groundwater, and also discussed MIC and its mechanisms with the kinds of microbes involved and their performance in the environment.

In the present study, the corrosion behavior of aluminum brass and admiralty brass which generally have higher corrosion resistance than the above mentioned copper, is discussed.

2. Examination

2.1 Medium for study

The groundwater used in this study was collected and transported to the laboratory from Kanto area of Japan. The composition is given in **Table 1**. As shown in the table, the pH was 8.0 and the Cl^- ion concentration was about 140 mg/l. These and other parameters reveal the characteristics of freshwater. Prior to the experiment, the experimental water was supplemented with 0.0025% Nutrient broth (hereafter mentioned as NB). This medium contains Difco Beef Extract and Peptone added to revive the bacterial activity. Control sets without bacteria were run simultaneously with autoclaved (393 K-0.9Ks) ground water (hereafter mentioned as sterile water) under the same conditions.

2.2 Test coupons

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Test coupons were aluminum brass and admiralty brass, the chemical composition of which is given in **Table 2**. Brass coupons used were of the size 20x10x6mm. Surface preparation was done by polishing with emery paper to 1500 grit. Test coupons (base metal coupons) prepared as above were used for exposure studies. Only the upper surface of the coupons were exposed to the experimental water as all the other sides were coated with an insulating resin. A copper wire was soldered to the lower surface of the coupon for potential measurements. Prior to exposure, the test coupons as prepared above were degreased with acetone, cleaned ultrasonically and sterilized with ethyl alcohol. Sterilization was done to avoid contamination.

Table 1 Chemical composition of groundwater.

pH	$CaCO_3$	Cl^-	SiO_2	SO_4^{2-}
8.0	322	137	135	109

Table 2 Chemical composition of aluminum brass and Admiralty brass.

	Cu	Al	Ni	As	Sn	Zn
Aluminum brass	77.5	2.0	0.3	0.04	-	Rem.
Admiralty brass	71.0	-	-	0.03	1.1	Rem.

2.3 Exposure studies

200 ml each of groundwater supplemented with 0.0025% NB was taken in conical flasks and autoclaved. 200ml each of non-sterile groundwater also were taken in sterile conical flasks for the simultaneous experimental run. Test coupons were introduced into the experimental medium horizontally, in such a way that their exposed surface (unmasked surface) faced up. To avoid contamination, the flasks were closed with silicone stoppers. The experimental set up is shown in **Fig.1a**. The flasks were kept in an incubator set at a temperature of 303K without shaking. The change in pH of the medium and free corrosion potential were measured (3.33 M KCl-Ag/AgCl reference electrodes) at regular

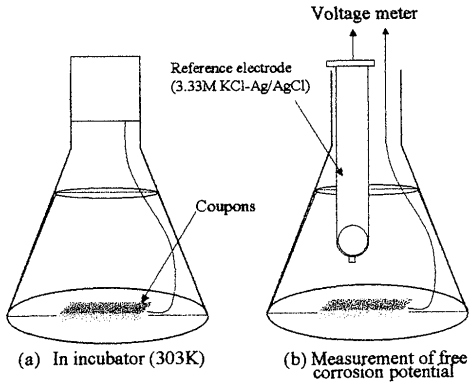

Fig.1 Experimental set up showing the details of coupon exposure.

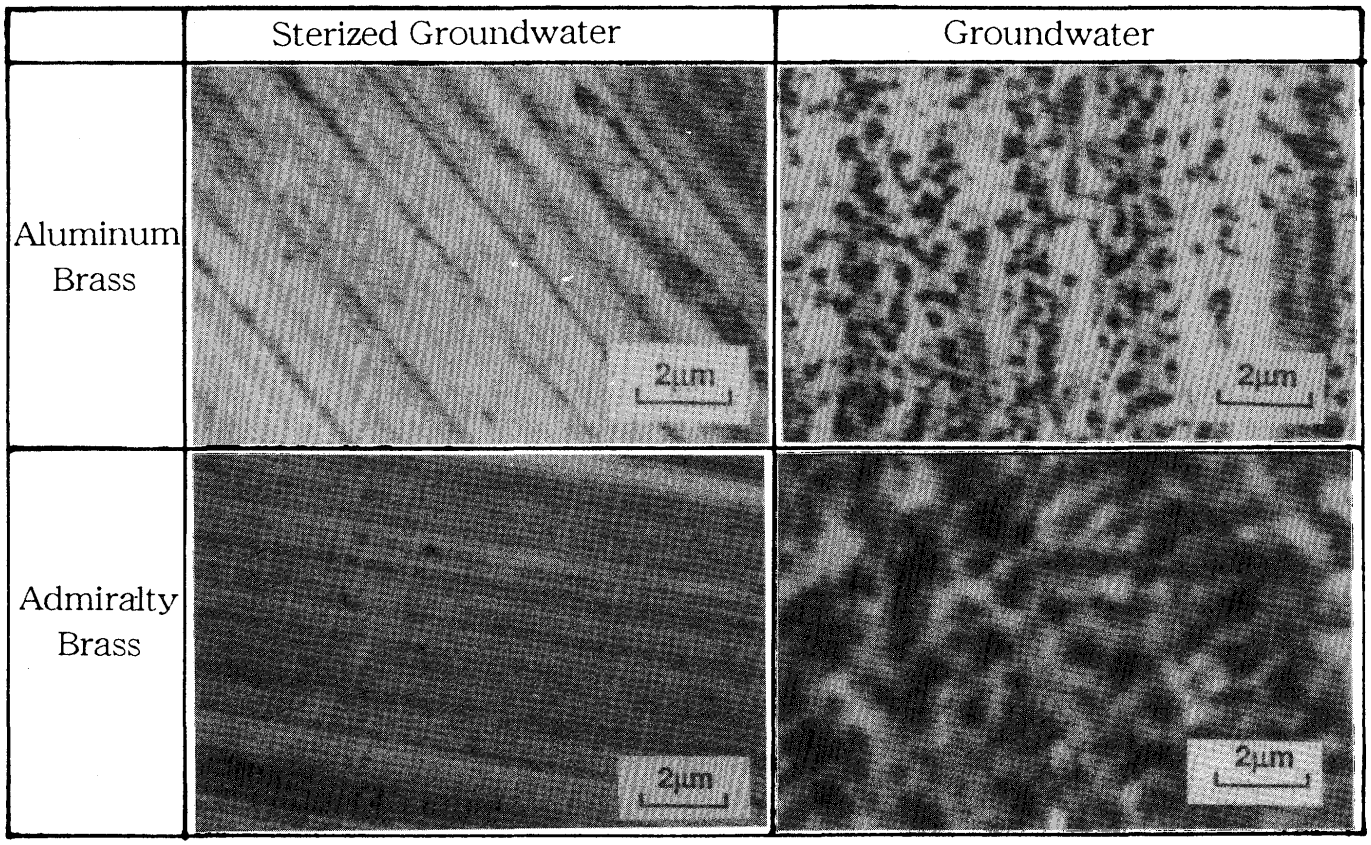

Fig.2 SEM images of Aluminum brass and Admiralty brass surfaces after the 40days exposure test.(0.0025%NB)

intervals (**Fig. 1b**). pH measurement was done by taking out 0.15ml of experimental medium at regular intervals using micropipettes. The experimental duration was 40days and the observations were made on the 14th and 40th days, respectively. Sampling for ammonia detection was done by taking out 12μl of the biofilm on the surface for the 14days exposed coupon using micropipettes. The ammonia detection was carried out by Nessler's reagent.

2.4 Isolation and identification of various strains from groundwater

A number of strains of bacteria were isolated from the experimental ground water using agar medium. Isolated strains were streaked repeatedly for purification and single strain smears were observed using a biological microscope. Identification of the strains was done in the Institute of Fermentation, Osaka.

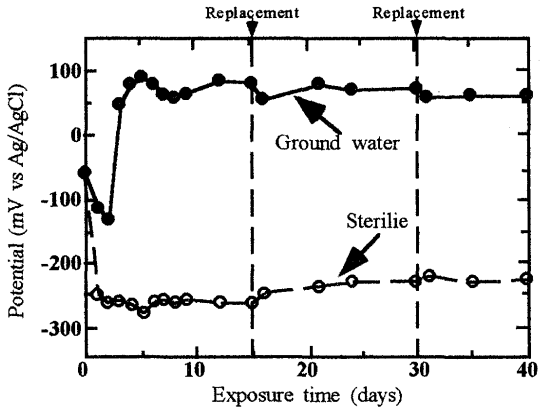

Fig.3 Variation of the free corrosion potential in Sterilized ground water as a function of exposure time for Aluminum brass.

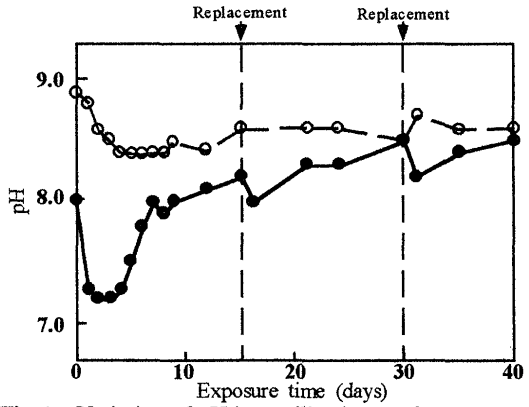

Fig.4 Variation of pH in sterilized ground water and non-sterilized ground water as a function of exposure time for Aluminum brass.

3. Results and discussion

3.1 Corrosion behavior of the brass by the groundwater

Fig.2 shows SEM images of the aluminum brass and admiralty brass surfaces after the 40days exposure to the groundwater supplemented with 0.0025% NB. Scratches seen in the photographs in this figure were due to emery paper polishing. SEM observations of the coupons showed no pitting on coupons suspended in both the sterile water supplemented with NB and without it after 40days of exposure. Test coupons kept in non-sterile groundwater supplemented with 0.0025% NB showed pitting (ranging from 0.1 to 0.5μm in size) after 40 days of exposure. Pitting on copper surfaces were reported[6]. Similarly, the aluminum brass and admiralty brass corroded.

Table 3 Characteristics of identified bacteria.

	Genus name	Tolerance to Cu	MIC*)
A	*Staphylococcus* sp.	○	★★
B	*Sphingomonas* sp.	×	–
C	〃	×	–
D	〃	○	★
E	*Comamonas* sp.	○	×
F	*Methylobacterium fujisawaensis*	×	–
G	Un-identified	×	–

*) ★★:possible, ★:only black tinge, x:impossible

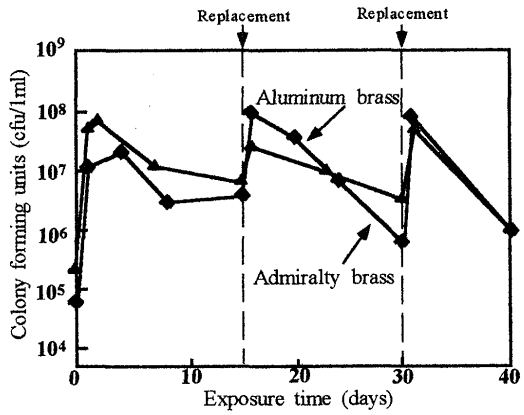

Fig.5 Variation of Colony forming units in aluminum brass and admiralty brass exposed the *Staphylococcus* sp. solutions as a function of time.

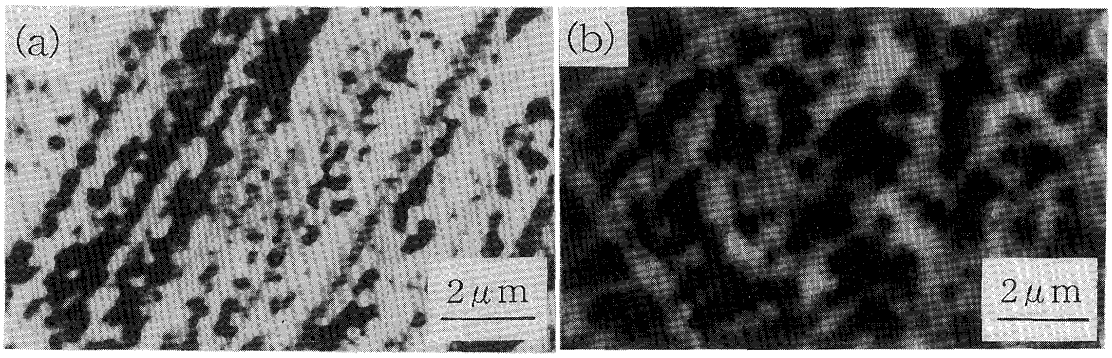

Fig.6 SEM images of surface condition on Aluminum brass(a) and Admiralty brass(b) in *Staphylococcus* sp. strain-A included solution after 40d exposure test.(0.0025%NB)

Test coupons after 14 days of exposure, showed small pits on SEM observation. The pits observed on test coupons after 40 days of exposure were bigger in size and larger in number[7].

The results indicated that microbes present in this groundwater have an ability to corrode the brasses tested. Also, it is indicated that these microbes have tolerance not only to copper but to zinc also.

3.2 Change in corrosion potential of the aluminum brass and admiralty brass coupons, and pH of the medium with time

The change in free corrosion potential and pH were measured. The variation of free corrosion potential of the aluminum brass coupons exposed to sterile and non-sterile groundwater supplemented with 0.0025% NB is given in **Fig.3**.

In the case of sterile groundwater, the free corrosion potential of the exposed coupons was -100mV initially, reduced to -250mV on the 2nd day and then remained almost constant. Initial reading was -50mV in the case of non-sterile groundwater. A reduction up to -130mV was seen initially. From the 4th day onwards a rise in potential was seen which recorded up to +50mV. Then onwards, maintained a position in between +50mV and 100mV. The dotted lines in Fig.3 indicate the replacement of the medium.

Fig.4 shows the variation in pH. In the case of sterile groundwater, initially, a decreasing trend was seen, followed by a slight increase. The pH was seen fluctuating near 8.5 from the fifth day onwards. The non-sterile medium showed significant changes in pH, for example by three days, it showed a decrease to about 7.2. The significant fluctuation in pH of non-sterile groundwater observed in the present study can be attributed to the release of metabolites by the bacteria[6]. This will be discussed in detail afterwards.

3.3 Characterization of bacterial flora in groundwater

In the previous paper[6], the bacterial flora in this groundwater were identified, and the corrosion ability of each bacterial strain was discussed. The identified bacteria and their characteristics are given in **Table3**.

Generally, it is well known that copper ion is toxic to many bacteria. But, *Staphylococcus* sp. (strain-A), *Sphingomonas* sp. (strain-D) and *Comamonas* sp. (strain-E) showed tolerance to copper ion. Especially, *Staphylococcus* sp. (strain-A) showed strong corrosion ability also.

The resistance of *Staphylococcus* sp. (strain-A) to brass was also tested. Results are shown in **Fig.5** with bacterial number along the Y-axis and experimental time along the X-axis. The maximum bacterial number recorded was up to 10^7 cells/ml. This showed that the *Staphylococcus* sp. (strain-A) has resistance not only to copper but to brasses also.

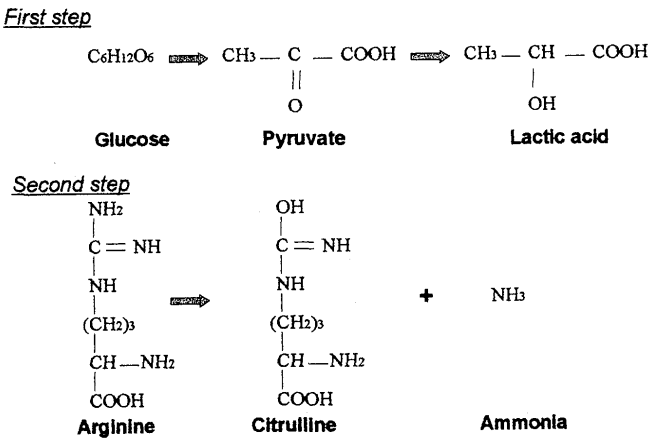

Fig.7 Metabolic pathways of *Staphylococcus* sp. in NB medium.

Staphylococcus sp. (strain-A) was inoculated into sterile groundwater containing 0.0025% NB. Exposure studies were conducted using aluminum brass and admiralty brass coupons for 40 days. The corrosion ability was examined by surface observation using on SEM and the results are given in **Fig.6**. Vertical scratches seen in the photographs in Fig.6 were due to emery paper polishing. Pits, whose morphology and size are shown in Fig.2, were observed on the test coupons. The surface of admiralty brass coupons surface showed bacterial adhesion and biofilm. It also shows that *Staphylococcus* sp. (strain-A) has corrosion ability not only to copper, but also to the brasses.

Generally, it is known that two fermentative metabolic pathways are seen in the Genus *Staphylococcus*. These pathways are shown in **Fig.7**. At first, fermentation of carbohydrate takes place. Glucose is converted into lactic acid through the Embden Meyerholf pathway. Protein metabolism takes over glucose fermentation when the glucose concentration becomes less. Thus, arginine is attacked and converted to citrulline by the help of arginine dehydrolase enzyme and the by-product is ammonia. The above two metabolic pathways are supposed to be the sources of ATP (adenosine- tri phosphate) and hence the energy sources in the case of *Staphylococcus* sp. The nutrient broth medium used in the present study contains beef extract as the main ingredient. Hence, carbohydrate and protein components necessary for the above mentioned metabolic pathways are available in it. Both the pathways never occur at the same time. Bacteria are able to choose the necessary pathway according to the availability of nutrients. Likewise, in the present case, glucose is degraded first and as and when its concentration becomes less, switches over to protein metabolism [8]. The above metabolic pathways explained the behavior of pH in Fig.4.

3.4 The discussion for corrosion mechanism

It is known that ammonia has the ability to corrode copper and copper alloy[9]. It was decided to detect the ammonia from the medium by Nessler's reagent. Traces of ammonia could be detected from the surface of the coupon, suggesting its presence in the biofilm. However, it was not detected in the medium away from the coupon surface. These results indicate that the *Staphylococcus* sp. produce ammonia, and this ammonia concentrates in the biofilm.

From the above observations, it is assumed that the

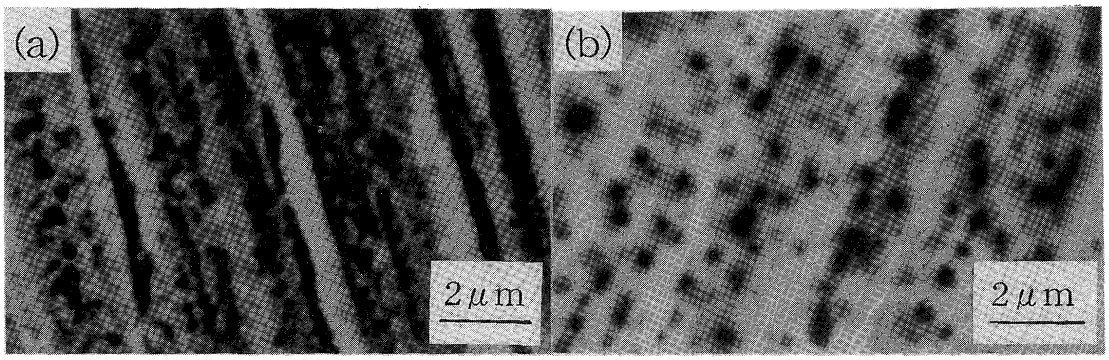

Fig.8 SEM images of surface condition on Aluminum brass(a) and Admiralty brass(b) in latic acid+ammonia solution (PH≒9.0) after 7d exposure test.

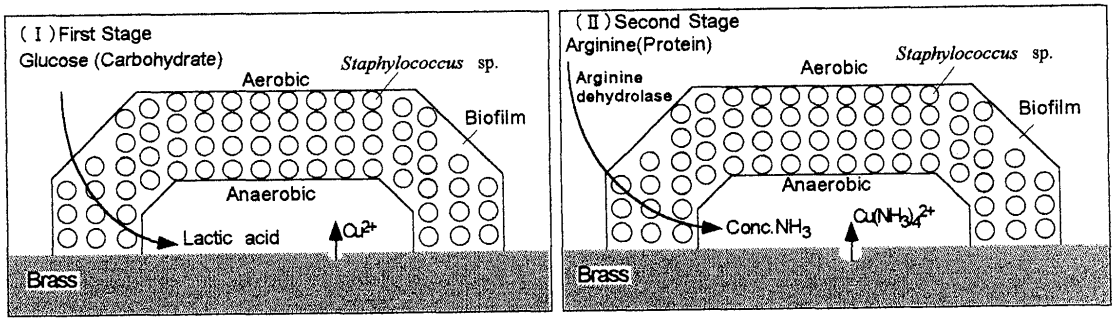

Fig.9 Schematic illustration of pitting corrosion processes in ***Staphylococcus*** sp. strain-A included solution.

corrosion occurring in the brass coupons exposed to a medium containing *Staphylococcus* sp. might be due to the effect of lactic acid and ammonia released into the medium, similar to the case of the copper[6]. As a confirmatory approach, a simultaneous experiment was run as follows: Sterile groundwater was supplemented with concentrations of lactic acid to prepare medium of pH 7.4. Groundwater of pH 8.5, 9.4, 10.5, and 11.1 were prepared by adding ammonia to the medium of pH 7.4 described above. These media also were sterilized and similar exposure studies were conducted. The exposure studies were carried out for 7 days at a temperature of 303K. Results of the corrosion behavior of the coupons in the medium of pH 9.0 are given in **Fig.8**. Pits of similar morphology as in Fig.2 and Fig.6, were observed on the test coupons.

From the above description, it can be concluded that in an environment where *Staphylococcus* sp. is actively growing, ammonia, a by-product of its protein metabolism, can be the reason for corrosion of brass coupons.

Staphylococcus sp. is known to adhere to surface of materials and form a biofilm. Once the biofilm is formed, the oxygen concentration under it is decreased. In such a condition, protein metabolism takes place and the chances of production of ammonia increase. The released ammonia might be the causative factor of brass corrosion in the present study. So, it can be concluded that wherever the biofilm was formed, the concentration of ammonia increased. At the particular points, pits were initiated and likewise, corrosion continued.

Using this conclusion, a model of copper corrosion by *Staphylococcus* sp. was prepared **Fig.9**. At first, *Staphylococcus* sp. adhere to the surface of the brass and a biofilm is formed. This is followed by the fermentation of glucose (Fig.9-I) producing lactic acid and pitting is initiated. As time passes, the metabolism is switched over to protein (Fig.9-II). By this time, the concentration of oxygen will also be very poor. Hence, protein metabolism in anaerobic condition proceeds and ammonia is released as a by-product of the breakdown of arginine. Copper dissolution continues with the formation of $Cu(NH3)_4^{2+}$.

4. Summary

The corrosion characteristics of the groundwater which caused copper corrosion were studied and the results are summarized below.

(1) Coupon exposure studies conducted in groundwater supplemented with 0.0025% NB showed pitting corrosion. When the same medium was sterilized, pitting could not be seen after the exposure studies. From these observations, similar to the case of copper, the role of bacteria in corrosion of brass in ground water was suspected.

(2) The morphology of pits, the changes of corrosion potential and the pH of the medium on coupon exposure studies showed the same trends of the case as in copper.

(3) Among the seven different strains of bacteria isolated from the experimental groundwater, *Staphylococcus* sp. was found to be resistant to copper and zinc ion toxicity. Also the *Staphylococcus* sp. was seen to be forming biofilm on aluminum brass and admiralty brass.

(4) Modeling experiments were done at different pHs using media supplemented with lactic acid and ammonia in different concentrations. From the results, it was clear that the medium supplemented with lactic acid to produce a final pH of about 9.0 was more corrosive and showed similarity to the corrosion caused by *Staphylococcus* sp. Hence, the cause for corrosion of brass in groundwater is supposed to be the metabolic by-product ammonia of *Staphylococcus* sp.

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