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Corrosion Behavior of Copper and its Welds by Bacteria in Underground Water†

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Abstract

Microbiologically Influenced Corrosion (MIC) was suspected in the corrosion failure of heat exchanger copper piping, carrying groundwater. Laboratory simulation studies were planned to find out the mechanism of corrosion. Ground water from the same plant was transferred to the laboratory aseptically and used for the experiment. Bacteria in the test solution were activated by the addition of low concentrations of nutrients. Cu 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 solution. Culturing of bacterial strains in the ground water revealed 7 different species. Four of them were susceptible to microbicidal activity of copper. The remainder of them were incubated separately in liquid medium and exposure studies were conducted. Formation of biofilm on copper coupons was 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 protein and thereby the production of ammonia. It is concluded that the copper coupons were corroded by ammonia in the test solution. The corrosion mechanism of copper pipe in ground water is discussed with reference to available literature.

KEY WORDS: (Copper) (Microbiologically Influenced Corrosion) (*Staphylococcus* sp.) (Biofilm)

1. Introduction

Stainless steel, though is thought to be resistant to corrosion as its name implies, is not totally resistant. There are cases where through wall pitting is seen soon after installation, resulting in the dripping of liquid leading to serious industrial problems [1-4]. Most of the available literature describes the instances of corrosion process of metals occurring at welds or near them. For a long time, fabricators and others who are directly involved with the corrosion problem of welds were of the opinion that this is due to welding defects such as cracks, blow holes, undercut, lack of fusion etc. which occur at the time of welding itself. As a counter measure, the usual practice was to re-weld the affected part and reinstall. This practice continued for a long time because the knowledge of microbes that can colonize at the welded region and their deterioration processes were not known. In this connection, we have carried out some experiments on microbiologically influenced corrosion (MIC) of different materials used in drainage water piping[5]. The characteristics of MIC differ from case to case as decided by the type of microbial species involved in the process and also the nature of material in question.

Copper is a frequently used metal in the potable water supply systems, heat exchangers etc. because of its excellent corrosion resistance. When it is used for such purposes, the process of joining and welding become inevitable. Though the water in contact with the material is fresh and seems to be harmless, cases of pitting are common. In some such cases, when the free corrosion potential was measured, an increase was seen. This rise in potential in the presence of microbial species and the formation of pits has been discussed and attention drawn to their interaction [6]. It is also well known that copper is toxic in nature. So, the understanding of microbes and their influence on copper corrosion leads to the conclusion that the microbes can grow and multiply even in the presence of toxic copper ions. As of now, the relevance of microbes causing corrosion of copper is not well understood.

The present work includes some laboratory experiments on corrosion of copper exposed to groundwater, which was supposedly prone to MIC. The characteristics of microbiologically influenced corrosion of copper in groundwater are discussed with reference to

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2. Experimental

2.1 Medium for study

The groundwater used in this study was collected and transported to the laboratory from Kanto area of Japan. The chemical composition is given in Table 1. As it is shown in the table, the pH was 8.0 and Cl^- ion concentration was about 140 mg/l. These and other parameters refrect the characteristics of freshwater. Prior to the experiment, the experimental water was supplemented with 0.0025% Nutrient broth (hereafter mentioned as NB). This medium containing Difco Beef Extract and Peptone is 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

Test coupons were oxygen free high conductivity (OFHC) copper, the chemical composition of which is given in Table 2. Copper 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. Welded samples were also of the same material, OFHC copper, which was prepared as beads on plate (TIG welding technique). The width of the welded area was more than 10mm. At the time of welding, argon gas was used to reduce the formation of oxide film on the welded surface. Coupons of welded area and HAZ were machined separately as shown in Fig.1. The surface conditions of the weld metal coupons were similar to those of base metal coupons and were also polished to 1500 grit using emery paper. To remove the oxide film formed at the time of welding, coupons of HAZ also were polished to 1500 grit. Only the upper surfaces 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 ground water.

pH	$CaCO_3$ (mg/l)	Cl^- (mg/l)	SiO_2 (mg/l)	SO_4^{2-} (mg/l)	Electrical conductivity (mS/cm)
8.0	322	137	135	109	1.34

Table 2 Chemical composition of oxygen-free high conductivily Copper (OFHC Cu) (ppm).

O	H	S	As	Fe	Ni	P	Pb	Zn	Cu
1.1	0.5	3.0	0.3	0.6	0.8	<0.5	0.9	0.2	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 silicon stopper. The experimental set up is shown in Fig.2a. 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 intervals (Fig. 2b). pH measurement was done by extracting 0.15ml of experimental medium at regular intervals using micropipettes (Gilson, France). The experimental duration was 40days and the observations were made on the 14th and 40th days, respectively.

As it was not a continuous flow set up, and was kept for a long time, the bacterial activity was expected to be definitely decreased. In order to avoid this, a supply of fresh medium was given at an interval of 15 days. This was done by replacing half the quantity of the original medium with fresh medium. Similar

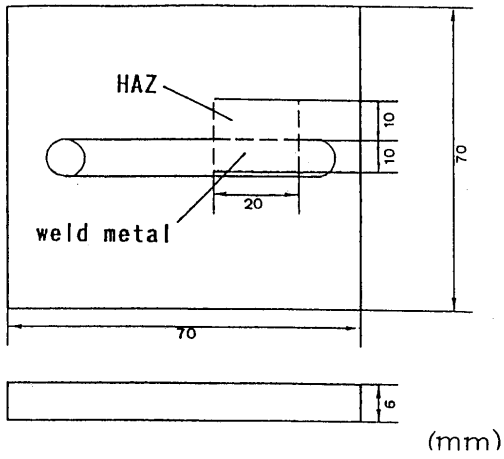

Fig.1 Appearance of oxygen free high conductivity copper weld metal and its HAZ test specimen.

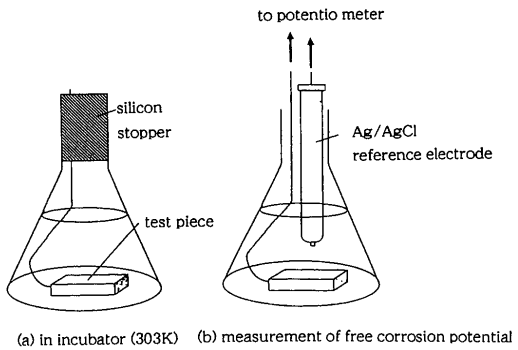

Fig.2 Appearance of exposure test.

experiments were also run using groundwater not supplemented with NB. At the prescribed intervals, the test coupons were removed from the flasks, cleaned and the corrosion status was assessed by SEM (Scanning Electron Microscope) observation.

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.

Total viable counts of the various strains present in the medium were estimated at regular intervals. Observation of bacteria on the test coupons was carried out by SEM after fixation and dehydration of the biofilm.

2.5 Evaluation of corrosion potential of various strains

Isolated and identified strains were further used to evaluate their individual corrosion potential by carrying out similar tests with single strain at a time. A loopful of inoculum was introduced into the sterile groundwater containing 0.0025%NB. Test coupons were exposed in the medium and kept in the incubator set at a temperature of 303 K for a prescribed time interval without shaking. At the end of the experiment, the corrosion of the coupons was monitored as described earlier. The detailed description of the methods is given earlier [5].

3. Results

3.1 Corrosion behavior of groundwater

3.1.1 Sterile groundwater

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.

3.1.2 Non-sterile groundwater

Test coupons kept in non-sterile groundwater supplemented with 0.0025% NB showed pitting after 40

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Test coupons kept in non-sterile groundwater supplemented with 0.0025% NB showed pitting after 40 days of exposure. The pitting occurred in OFHC copper base metal exposed in non-sterile water is shown in Fig.3.

Test coupons, after 14 days of exposure, on SEM observation showed partial discoloration (blackening) along with small pits. Bacterial cells could be seen near the discolored portion and pits. Vertical scratches seen in the photographs in Fig.3 were due to

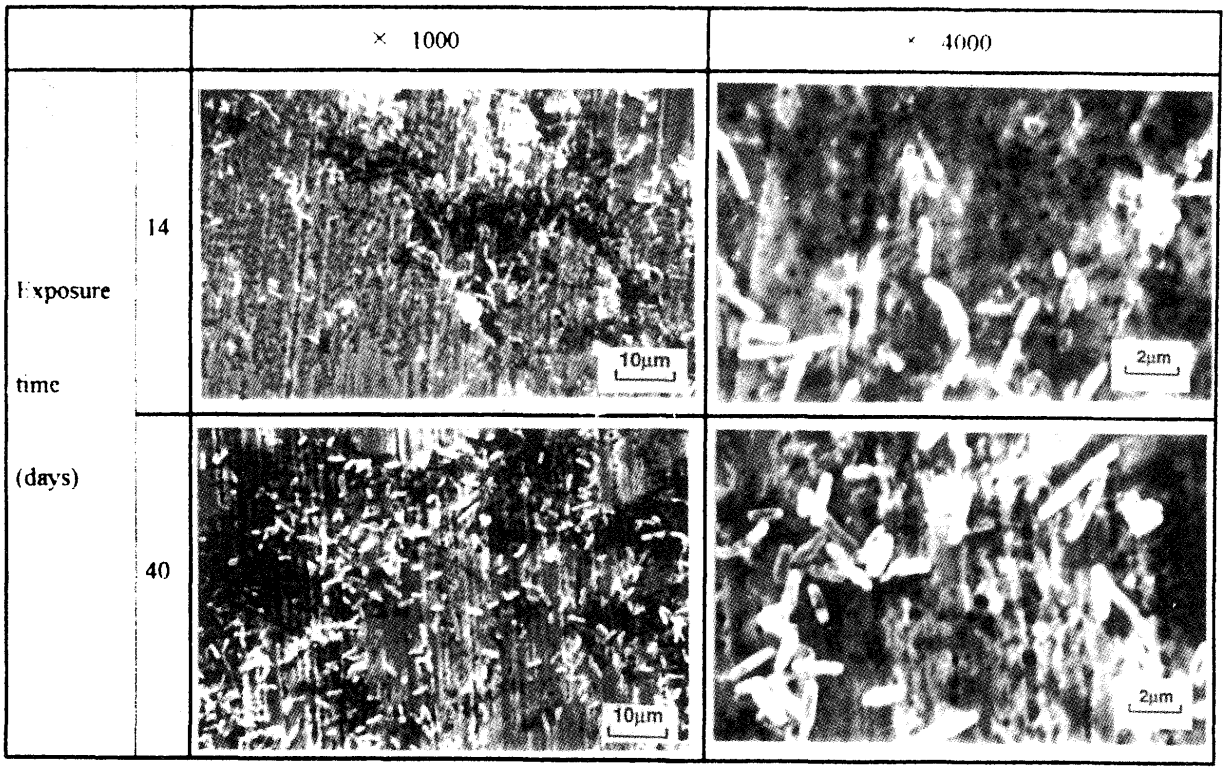


Fig.3 SEM view of corrosion pits of OFHC copper base metal surface with bacteria after the 15 and 40d exposure test(ground water 0.0025%NB).

emery paper polishing. Pits observed on test coupons after 40 days of exposure were bigger in size and larger in number. Also, it was observed that bacteria of different shapes and sizes such as cocci and rods were seen adhered to the coupons.

Results of the exposure studies of OFHC copper weld metal are given in Fig.4. Coupons exposed for 14 days showed discoloration and pitting as in the case of base metal. But, in this case, the pits were bigger. In the case of 40 days exposure, weld metal coupons showed corrosion on a larger scale. Both, OFHC copper weld metal as well as HAZ, polished by 1500 grit emery paper showed similar trends in the mode of corrosion.

Comparing the corrosion status, of base metal, weld metal and HAZ (polished coupons), the weld metal was found to be more prone to corrosion. No significant difference was seen for the corrosion status of polished and unpolished samples. Corrosion occurred in all the coupons in spite of the differences in their surface condition. Also, in the case of sterile groundwater, coupons were not corroded. Hence, the possible role of bacteria in corrosion of coupons in non-sterile groundwater is suspected.

The coupons showed no pits when the groundwater was not supplemented with NB. This observation indicates the need for active bacterial flora in the medium for the occurrence of pits.

3.2 Change in corrosion potential of the copper coupons and pH of the medium with time

It is evident that there will be a rise in free corrosion potential in the case of copper, stainless steel etc. when MIC is suspected [2, 6-9]. Therefore, in the present study also, the change in free corrosion potential and pH were measured. The variation of free corrosion potential of coupons exposed to sterile and non-sterile groundwater supplemented with 0.0025% NB is illustrated in Fig.5.

In the case of sterile groundwater, the free corrosion potential of the exposed coupons was -40mV initially, dropping down to -250 mV on the 2nd day and then remaining almost constant. The initial reading was 0 mV in the case of non-sterile groundwater. A reduction up to -280 mV was seen initially. From the 4th day onwards a rise in potential was seen which rose up to +10 mV. Then onwards, it maintained a level in between +10mV and -50 mV. The present results agree well with the results of earlier workers [2,6-9].The dotted lines in Fig.5 indicate the points for the replacement of medium.

Variation in pH is shown in Fig. 6. In the case of sterile groundwater, initially, a decreasing trend was seen, followed by a slight increase. The pH was seen fluctuating around 8.5 from the fifth day onwards. The non- sterile medium showed significant changes in pH such that by three days, it showed a decrease to about 7.1. The significant fluctuation in pH of non-sterile

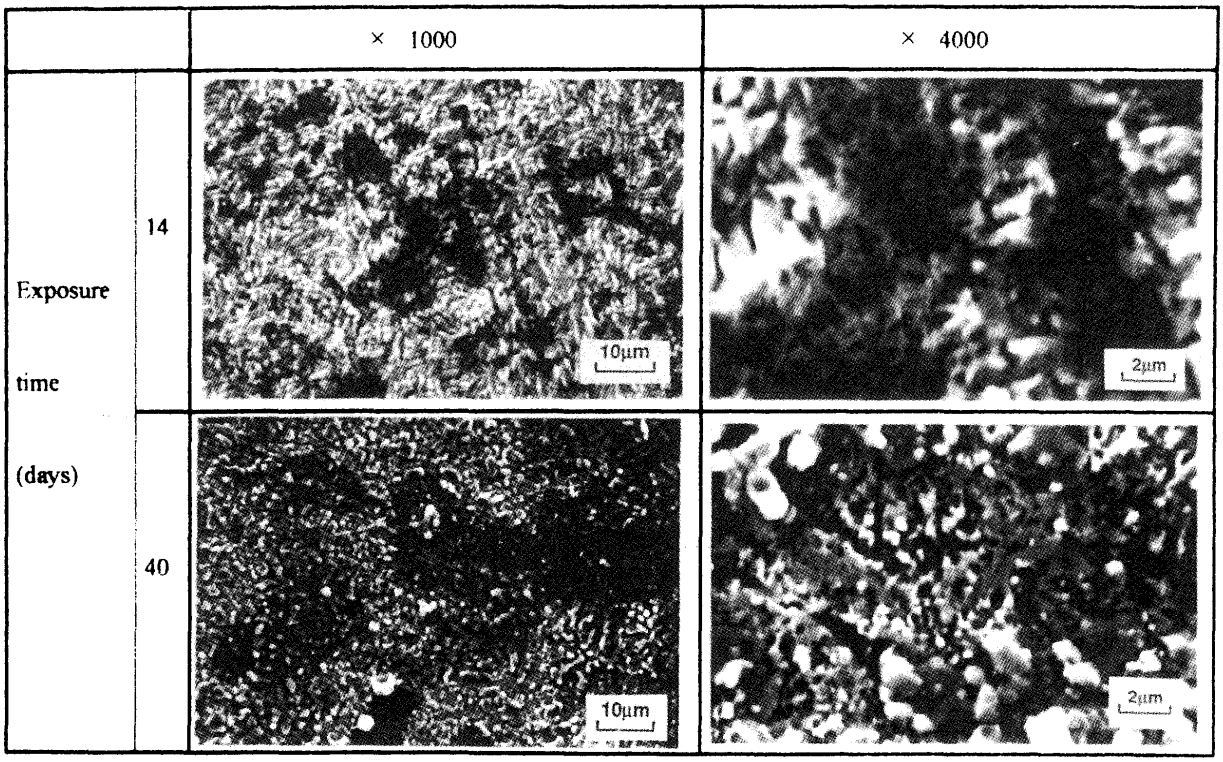


Fig.4 SEM view of corrosion pits of OFHC copper weld metal surface (as weld) after the 15 and 40d exposure test(ground water 0.0025%NB).

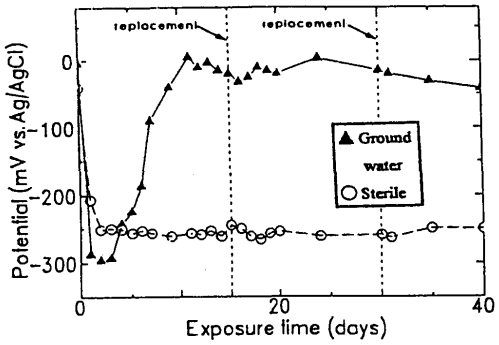


Fig.5 Variation of the free Corrosion potential in sterilized and non-sterilized ground water as a function of exposure time (0.0025%NB) (The half quantity of test solution are replaced with new one at 15 and 30d respectively).

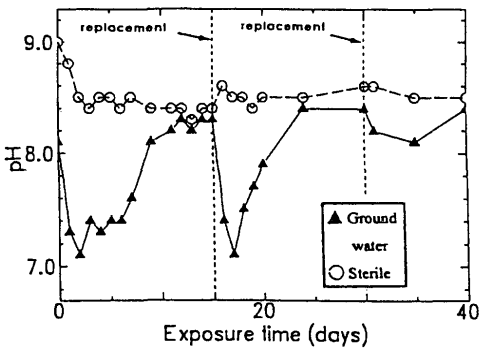


Fig.6 Variation of pH in sterilized and non-sterilized ground water as a function of exposure time (0.0025%NB).

groundwater observed in the present study can be attributed to the release of metabolites by the bacteria. This will be discussed in detail later.

3.3 Characterization of bacterial flora in groundwater

Groundwater samples were streak plated on agar medium and incubated. Colony forming units were observed on the agar surface after incubation for 5 days at 30^0 C. The shape and size of the different strains were observed by picking up a very small quantity from the colony forming units and observing through a biological microscope. The isolated strains were purified using re-streaking technique for single colony isolation. Purified strains were identified by the Institute of Fermentation, Osaka. Results of microscopic observation are given in Fig.7. Each one shown in the photograph was taken from a different colony forming unit. As seen in the figure, they were of different shapes such as cocci or rods of different sizes. Different strains are indicated by A to G. Results of identification are given in Table 3. In total, five different species were isolated.

As mentioned in the beginning, copper is toxic. Hence, the adhesion of bacteria on to copper is supposed to be less likely than for non-toxic materials[10-11]. In the present study, isolated bacterial strains were subjected to tolerance assessment to see whether they are resistant to copper ions or not. Tolerance studies were carried out as follows: Sterile groundwater supplemented with 0.25% NB was taken in conical flasks and inoculated with the respective strains of bacteria. OFHC copper coupons were introduced into the medium and bacterial counts were continuously monitored till 40 days. Control sets were also maintained without copper coupons. The results of the tolerance test are shown in Fig. 8 with bacterial number along the Y-axis and experimental time along the X-axis.

As shown in Fig. 8(a), most of the bacterial strains tested recorded up to 10^8 cells/ml or more during a period of 7 days and thereafter declined after a stationary phase. On the other hand, strains which showed only 10^6 cells/ml maximum also were there. This observation agrees with the usual growth curve of bacteria [12]. A-G in Fig.8 (a) and (b) are same as in Table 3.

In Fig.8 (b), the results of tolerance test done in the presence of copper coupons are given. The strains, B, C, F and G were dead by a period of 7 days to 14 days. This might be because they were sensitive to copper ions and were unable to thrive in the toxic medium. But, A, (*Staphylococcus* sp.), D (*Sphingomonas* sp.) and E (*Comamonas* sp.) showed their ability to grow and multiply even in the presence of toxic copper ions. Further the dotted lines in the Fig. denotes the replacement of medium. The above three strains might be harboring copper resistant traits in them. To summarize, B, C, F and G strains were not expected to be involved in the corrosion process of copper. Hence, the other strains A, D and E were used for exposure studies using consequent copper coupons and corrosion processes were studied.

3.4 Corrosion potential of A, D and E strains

Staphylococcus sp. (A), *Sphingomonas* sp. (D) and *Comamonas* sp. (E) each were inoculated into sterile groundwater containing 0.0025% NB. Exposure studies were conducted using OFHC copper coupons for 14 days. The corrosion potential of each strain was assessed by surface observation using SEM and the results are given in Fig. 9. A (I) shows the picture of coupons exposed to *Staphylococcus* sp. with adhered biofilm and an enlarged view. The biofilm seemed to be cracked at places. But, the bacterial cells were seen entangled in the biofilm. A (II) shows the surface after removal of biofilm. Pitting under the biofilm was very well seen. On the coupons exposed to *Sphingomonas* (D), bean shaped spots with a black tinge, supposed to be the bacterial attachment sites were seen. In the case of E (*Comamonas* sp.), significant changes on the surface were not seen. Though the study period was restricted to 14 days, a significant difference in

corrosion potential among the three bacterial strains tested was clearly observed. Among the three, *Staphylococcus* (A) was seen as the most potent one.

4. Discussion

From the experimental results, it can be concluded that the bacterial strains *Staphylococcus* sp. and *Sphingomonas* sp. were capable of growth and multiplication even in the presence of copper ions and also can play a role in the corrosion of copper. Another important conclusion was that the most potent strain among the three strains tested was *Staphylococcus* sp. Hence, the mechanism of corrosion of copper coupons was discussed using this strain [13]. *Staphylococcus* sp. occurs singly, in pairs or in irregular clusters. It is a facultative anaerobe i.e. the strain is capable of living in both the presence and absence of oxygen. It can be seen in soil or associated with skin and mucous membranes of animals and humans.

Two fermentative metabolic pathways are seen in this strain. 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

Table 3 Genus name of A~G bacteria.

A	:	*Staphylococcus sp.*
B, C, D	:	*Sphingomonas sp.*
E	:	*Comamonas sp.*
F	:	*Methylobacterium fujisawaensis*
G	:	Un-identified

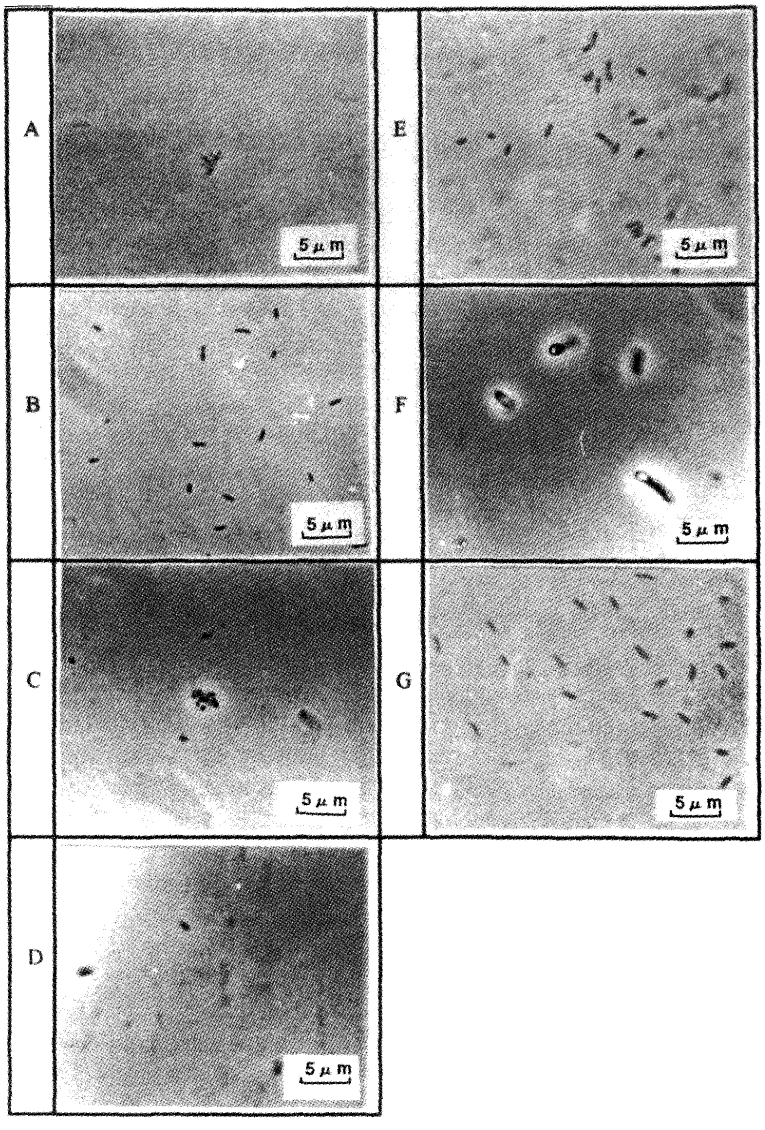
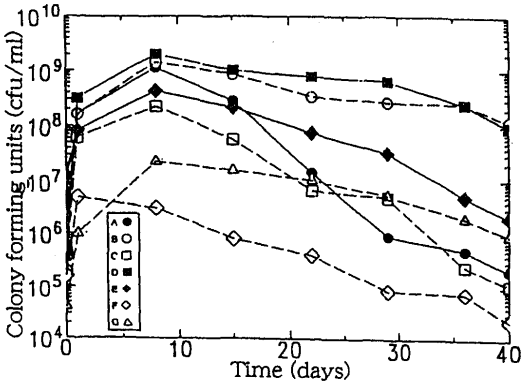
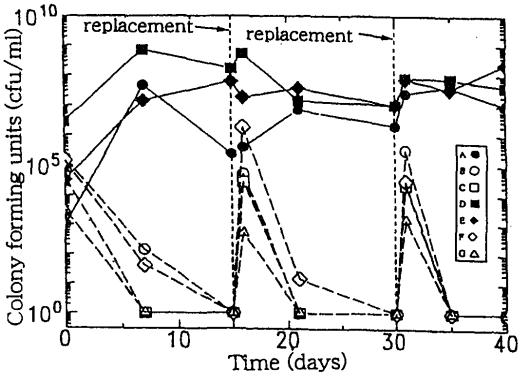


Fig.7 Biological microscope view of bacteria from the ground water.



(a) no exposure



(b) copper exposure

(The half quantities of test solutions were replaced with new one respectively after 15 and 30 days)

Fig.8 Variation of colony forming units in bacterial solution as a function of time(0.25%NB). (a)without copper (b)with copper

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, switching over to protein metabolism occurs[14].

Purified strains were inoculated to NB medium and the change in pH was recorded with time in days. This result is given in Fig. 10. During culturing, the pH showed a downward trend to 7.4 from 8.9 in two days and then showed an increase to 8.5 and stabilized in the case of *Staphylococcus* sp. (•) This particular trend of pH, fits very well for the above mentioned metabolic pathways of this strain. The initial decrease in pH can be due to the production of lactic acid as an end product of glucose fermentation. The increase afterwards can be due to the production of ammonia as a result of arginine metabolism. Corrosion occurring in copper coupons exposed to media containing *Staphylococcus* sp. might be due to the effect of lactic acid and ammonia released into the medium.

To test the above observation, simultaneous experiments were run as follows: Sterile groundwater was supplemented with different concentrations of lactic acid to prepare media of pH 3.1, 5.8 and 7.4. Exposure studies were conducted in all the three media. Likewise, 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. Coupons for both experiments were OFHC copper polished to 1500 grit. The exposure studies were carried out for 7 days at a temperature of 303K. Results of the corrosion behavior of the coupons are shown in Fig.11. Fig 11(a) shows the appearance of coupons exposed in the media containing only lactic acid and Fig11 (b), those exposed in media containing both lactic acid and ammonia. At pH 7.4, no corrosion was observed (a-I). However, at pH 5.8, the surface of the coupon showed small pits and also seems to be covered with corrosion products. At pH 3.1, the surface was comparatively rough and showed larger pits (a-II). But, the surface appearance of these coupons were dissimilar with those exposed in medium containing *Staphylococcus* sp. (Fig.3) In the latter case,

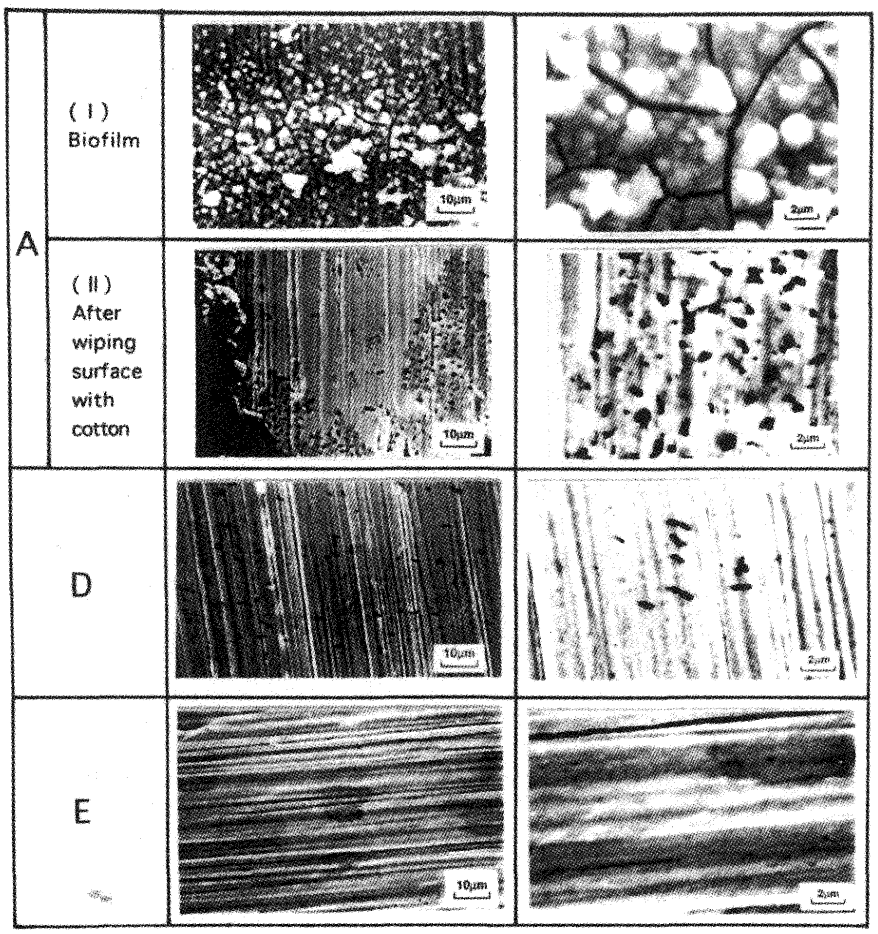


Fig.9 SEM view of surface condition on OFHC copper base metal in bacterial solution after the 14d exposure test (0.0025%NB).

i.e. with lactic acid and ammonia, at pH 8.5 no corrosion was observed (b-I). At pH 9.4, the surface showed some initiation of pitting (b-II), which became clear at pH 10.5 (b-III). At pH 11.1, an almost corroded surface was seen with larger pits (b-IV). The appearance of the coupon surface was very much similar to that of the coupon surface in the presence of *Staphylococcus* sp.

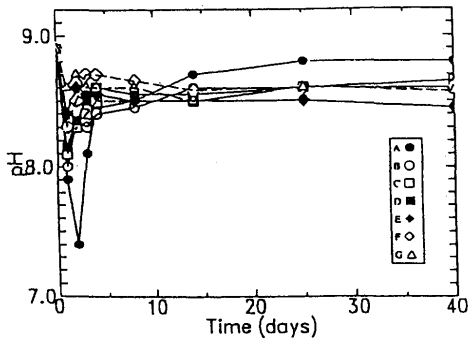


Fig.10 Variation of pH in bacterial solution as a function of exposure time (0.0025%NB).

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 copper corrosion. It can be noted that ammonium compounds are used as etching reagents for metallographic observation of copper [15].

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

Using this reasoning, a model of copper corrosion by *Staphylococcus* sp. was prepared (Fig.12). At first, *Staphylococcus* sp. adheres to the surface of OFHC copper and a biofilm is formed. This is followed by fermentation of glucose (Fig.12-I) producing lactic acid and pitting is initiated. As time passes, the

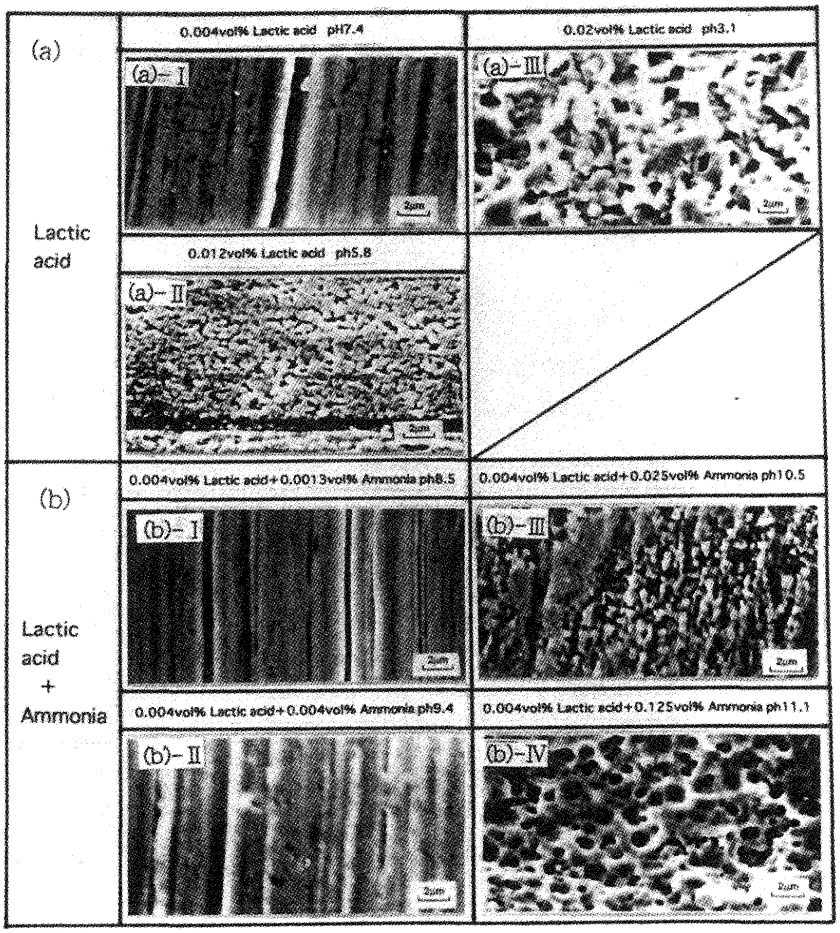


Fig.11 SEM view of corrosion pit of OFHC copper base metal surfaces in lactic acid and lactic acid + ammonia solution after 7d exposure test.

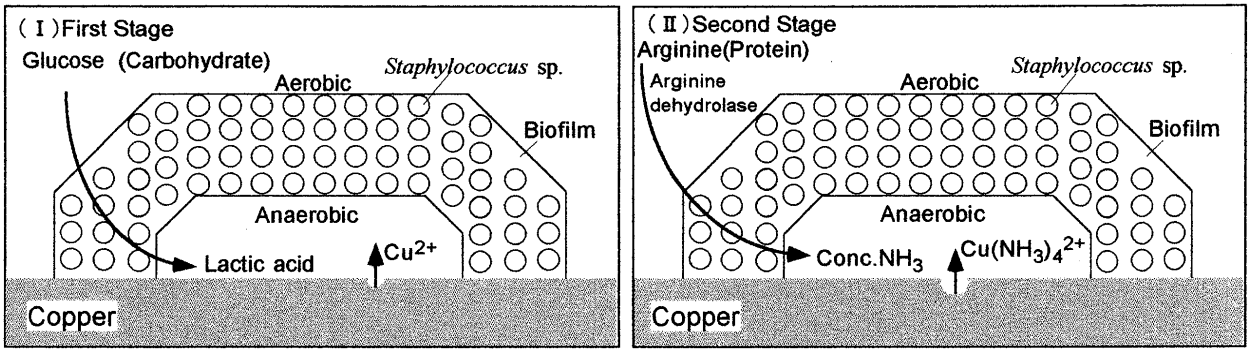


Fig.12 Schematic illustration of pitting corrosion processes in *Staphylococcus* sp. strain-A included solution.

metabolism is switched over to protein (Fig.12-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 breakdown of arginine. Copper dissolution continues with the formation of Cu $(NH3)_4^{2+}$.

5. Conclusions

Copper corrosion influenced by bacteria in groundwater was examined 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, the involvement of bacteria in corrosion of copper in ground water was suspected.

(2) Coupons exposed in groundwater lacking NB also showed no pitting. This makes clear the necessity of an active bacterial flora for the corrosion to occur.

(3) Among the seven different strains of bacteria isolated from the experimental groundwater, four were found to be susceptible to copper ion toxicity. Among the three resistant strains, *Staphylococcus* sp. was seen to be forming biofilm on OFHC copper.

(4) *Staphylococcus* sp. is a facultatively anaerobic bacterium. At first, the utilization of glucose occurs, followed by the breakdown of protein. As a result, lactic acid and ammonia are formed.

(5) Modeling experiments were done at different pH levels 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 reach a pH of about 10 was more corrosive and showed similarity to the corrosion caused by *Staphylococcus* sp. Hence, the cause for corrosion of copper in groundwater is supposed to be the metabolic by-product ammonia from *Staphylococcus* sp.

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