# Case Study on Microbiologically Influenced Corrosion of SUS316L welds†

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**Abstract**

*Microbiologically Influenced Corrosion (MIC) was suspected in the corrosion failure of a cooling system of a stainless piping welded joint, carrying marine water. Marine water, which is used for cooling water in a plant was transferred to the laboratory and used for experiments. In the first experiment, weld metal samples were exposed to the test solution for 56 days (marine water and sterilized marine water (control)). The surface condition of experimental coupons was observed using a Scanning Electron Microscope (SEM). In another experiment, the free corrosion potential of these materials was monitored for 56 days. Pitting corrosion was found in the coupons exposed to marine water. Free corrosion potential ennoblement was found to be significant compared to the controls. It was suspected that this corrosion case was MIC. In the second experiment, coupons were exposed to diluted nutrient medium containing single cultures of microbes isolated from the MIC causing marine water sample used for the first experiment. After the exposure test, surface condition of the experimental coupon was observed using SEM. Pitting corrosion was found in coupons exposed to some of the isolates. The results indicate that they contribute to the corrosive effect of the SUS316L welds.*

**KEY WORDS :** (Microbiologically Influenced Corrosion :MIC) (Microbes) (Stainless Steel) (Weld) (Pitting Corrosion)

## 1. Introduction

Corrosion and degradation of materials by the action of microorganisms have been in focus in material engineering. This phenomenon is recognized as Microbiologically Influenced Corrosion (MIC). Industries which are affected by MIC are varied such as the petrochemical industry, gas industry, nuclear power plants, semiconductor industry and also the concrete structures [1-3]. The type of corrosion in which microorganisms are implicated is also observed on various materials. More recently it has been known that MIC generates tunneling pits in high corrosion resistance material such as stainless steel (specially in welded joint) as a result of which MIC has attracted special attention in industrial technology. Moreover, there are very severe attributes to MIC in that it is faster and can occur under normal temperature and in mild environments. These are the reasons why the damage caused by MIC in some situations cannot be ignored. Although there are many research studies of MIC, including case analysis, most of them tend to focus on some special groups of microbes (iron bacteria, iron-oxidizing bacteria, sulfate reducing bacteria, etc.) and also to look at them from an electrochemical viewpoint. However, there exist a variety of microbes in the environment causing MIC and hence is apt to consider the possibilities that MIC is caused by various reactions and coexistence of these microbes. Therefore it is still more important that we investigate these phenomena from a microbiological viewpoint. We have attempted to isolate and identify the microbes in some MIC cases of stainless steel, copper and copper alloy [4-5]. During a series of investigations, we have tested the interaction of single cultures of microbes from MIC cases and material. We have confirmed from these experiments that there is a significant difference in the corrosiveness and resistance of the material with respect to different microbes. Recently, we have come across a corrosion failure case of welds in SUS316L. In this study, firstly, we examined the possibility of MIC and secondly, we examined that, if this case were MIC, what kind of microorganism was contributing to it.

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## 2. Experiment

### 2.1 Test solution

The marine water used in this study was collected and transported to the laboratory from a plant in the Kanto area of Japan. The composition is given in **Table 1**. As shown in the table, the pH was 7.8 and the $Cl^-$ ion concentration was about 19400mg/l. These and the other parameters reveal the characteristics of the test water. Prior to the experiment, the experimental water was supplemented with 0.01% Marine broth (hereafter mentioned as MB). This medium contains nutrients to revive the bacterial activity. The composition of this medium is shown in **Table 2**. Control sets without bacteria were run simultaneously with autoclaved (393K-0.9ks) marine water (hereafter mentioned as sterilized water) under the same conditions.

### 2.2 Materials

SUS316L stainless steel plate (10mm thickness) was welded by bead on plate with the TIG process (Shielding gas:100% Ar; Welding speed:0.4min/m; Welding current:80A). The chemical composition of base metal is shown in **Table 3**. Test specimen used were of the size 20 $\times 10 \times 10^t$ mm. Coupon surfaces were polished to 3 $\mu$m finish during the test with non-sterile water. Surface preparation was done by polishing with emery paper to 1000 grit in the case of test with individual strains. Test coupons prepared as above were used for exposure studies. Only the upper surface of the coupon 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 the exposure, the test coupons as prepared above were degreased with acetone, cleaned ultrasonically and sterilized with 70%v/v ethyl alcohol. Further, all specimens were dried in a sterile laminar flow air chamber under UV for 60 min. Sterilization was done to avoid contamination.

### 2.3 Exposure studies

150ml each of marine water supplemented with 0.01% MB was taken in 200ml Erlenmeyer flasks and sterilized by autoclave. 150ml each of non-sterilized marine water also were taken in sterile Erlenmeyer flasks for the simultaneous experimental run. Test coupons were introduced into the experimental medium horizontally, in such a way that their exposed surface faced up (**Fig.1(a)**). To avoid contamination, the flasks were closed with silicone stoppers. The flasks were kept in an incubator set at a temperature of 298K with shaking. The change in pH of the medium and free corrosion potential were measured (3.33MKCL-Ag/KCL reference electrodes) at regular intervals (**Fig.1(b)**). pH measurement was done by taking out 0.15ml of experimental medium at regular intervals using micropipettes. The experimental duration was 56days and the observations were made on the $14^{th}$, $28^{th}$ and $56^{th}$days, respectively. The experimental set up is shown in **Fig.2**.

As it was not a continuous flow set up, and was kept for a long time, the bacterial activity was expected to definitely decrease. In order to avoid this, a supply of fresh medium was given at intervals of 14days. This was done by replacing half the quantity of the original medium with fresh medium. At the prescribed interval, the test specimens were removed from the flasks, cleaned and the corrosion status was assessed by SEM (Scanning Electron Microscope) observation. Total viable counts of bacteria were estimated at regular intervals. Observation of bacteria on the test specimen was done by SEM after fixation and dehydration of the biofilm.

### 2.4 Isolation and identification of various strains from marine water and biofilm on specimen

Marine water sample was spread-plated on agar medium and incubated. Biofilm bacteria were removed from the surface of exposed coupon by brushing and were

Table 1 Chemical composition of marine water (mg/l)

| pH | Fe | Mn | Ca | Mg | Cl | $SO_4$ | $NH_4$ | $HCO_3$ | TOC | Alkalinity ($CaCO_3$ mg/l) | Total hardness ($CaCO_3$ mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.8 | < 0.05 | < 0.05 | 460 | 1400 | 19400 | 3020 | 0.2 | 130 | < 1 | 115 | 6910 |

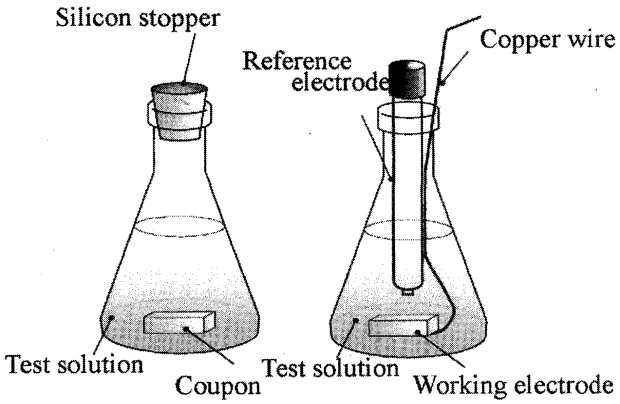
Table 2 Chemical composition of Marine broth (Difco)

| Bacto Peptone | Yeast Extract | $FeC_6H_5O_7$ | NaCl | $MgCl_2$ | $Na_2SO_4$ | $Na_2SiO_3$ | NaF |
|---|---|---|---|---|---|---|---|
| 5g | 1g | 0.1g | 19.45g | 8.8g | 3.24g | 0.004g | 0.0024g |
| $CaCl_2$ | $KClO_3$ | $NaHCO_3$ | KBr | $SrCl_2$ | $H_3BO_3$ | $Na_2PO_4$ | DistilledWater |
| 1.8g | 0.55g | 0.16g | 0.08g | 0.034g | 0.022g | 0.008g | 1L |

Table 3 Chemical composition of SUS316L used (mass%)

| C | Si | Mn | Ni | Cr | Mo | N |
|---|---|---|---|---|---|---|
| 0.016 | 0.46 | 1.49 | 13.98 | 16.4 | 2.12 | 0.026 |

spread-plated on agar medium and incubated. Colony forming units were observed on the agar surface after incubation for 3days at 298K. The shape and size of the different strains were observed and were picked up and cultivated over agar slants. The isolated strains were purified using a single colony isolation technique. Purified strains were identified by the Marine Biotechnology Institute, Co.Ltd. Kamaishi Laboratories, Iwate.




(a) Coupons for SEM observation (b) Measurement of free corrosion potential

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

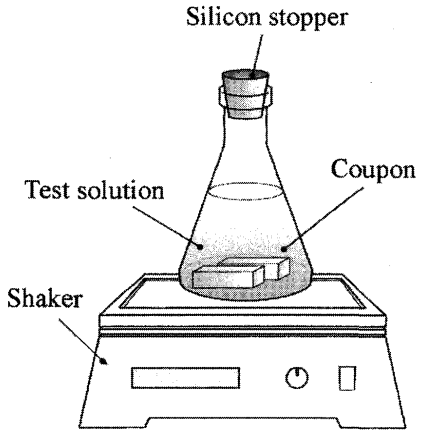



Fig.2 Apparatus for exposure experiment

**2.5 Evaluation of corrosion ability of various strains**

Isolated and identified strains were further used to evaluate their individual corrosion ability by carrying out similar tests with single strain at a time. 1 loopful of the inoculum was introduced into the sterilized marine water containing 0.01%MB. Test coupons were exposed in the medium and kept in an incubator set at a temperature of 298K for a predetermined time. At the end of the experiment, the corrosion of the coupon was monitored as described earlier.

**3.Result and discussion**

**3.1 Total viable Count in the medium**

The fluctuation in the total viable count in the experimental flasks during the period of study is given in **Fig.3**.The TVC fluctuated between$10^{6-7}$cell/ml.

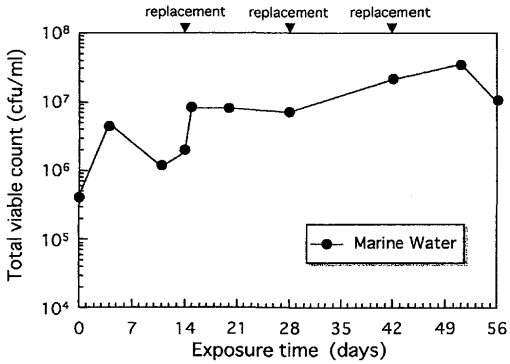
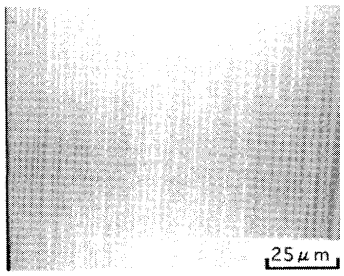


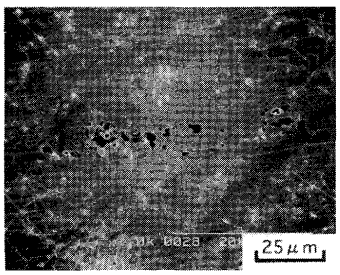

Fig.3 Variation in total viable count in the marine water as a function of time

**3.2 Corrosion behavior of the SUS316L welds by the marine water**

**Fig.4** shows SEM images of the SUS316L weld surfaces after the 56days exposure to the marine water supplemented with 0.01% MB. SEM observation of the coupon showed no pitting on coupons suspended in sterilized marine water supplemented with MB after 56 days exposure. Test specimens kept in non-sterilized marine water supplemented with 0.01% MB showed pitting after 56days of exposure. The result indicated that microbes present in this marine water have an ability to corrode the SUS316L weld tested.




(a) Sterilized marine water




(b) Marine water

Fig.4 SEM images of SUS316L weld surfaces after the 56days exposure test (0.01%MB)

### 3.3 Change in corrosion potential of the SUS316L test coupon 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 SUS316L welds coupon exposed to sterilized and non-sterilized marine water supplemented with 0.01% MB is given in **Fig.5**. In both the cases, ennoblement was seen. But in non-sterilized marine water, the width of change of ennoblement was relatively large and there was a repetition of potential increase and drop several times. **Fig.6** shows the variation in pH. In both the cases, the pH was fluctuating near 8.0 from the beginning to the end.

Therefore, it could be considered that the possibility of change in pH influencing the corrosion potential behavior is small. Hence, the participation of microorganism in non-sterilized marine water (microorganism adhesion on metal surface etc might be playing a major role). The repetition of potential increase and drop which was observed only in non-sterile marine water could be attributed to the change in the surface of the coupon due to generation of pits, microorganism adhesion or biofilm detachment etc.

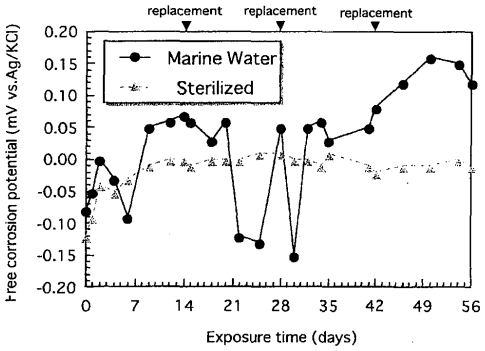



Fig.5 Variation in free corrosion potential in sterilized marine water and marine water as a function of exposure time

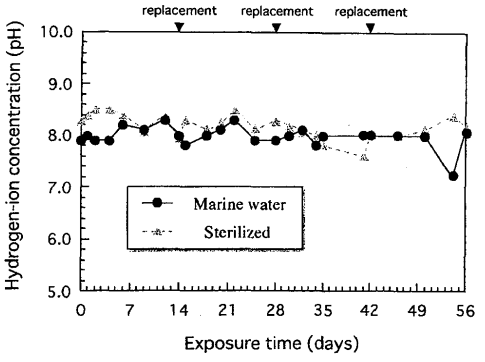



Fig.6 Variation of pH in sterilized marine water and marine water as a function of exposure time

### 3.4 Evaluation of corrosion ability of individual microorganism

A list of microorganisms isolated and identified from marine water is given in **Table 4**, and those from the surface of the test coupon is given in **Table 5**. Sulfate

Table 4 Microorganism isolated from marine water

| Strain | Genus Name |
|---|---|
| 1 | *Alteromonas* sp. |
| 2 | *Alteromonas* sp. |
| 3 | *Pseudomonas* sp. |
| 4 | *Bacillus* sp. |
| 5 | *Pseudoalteromonas* sp. |
| 6 | *Alteromonas* sp. |
| 7 | *Micrococcus* sp. |
| 8 | *Bacillus* sp. |

Table 5 Microorganism isolated from biofilm

| Strain | Genus Name |
|---|---|
| A | new genus (not identified yet) |
| B | *Alteromonas* sp. |
| C | *Cytophaga* sp. |
| D | *Cellulophaga* sp. |
| E | *Rugeria* sp. |
| F | *Muricayda* sp. |
| G | *Alteromonas* sp. |

reducing bacteria (SRB) had not been detected. The evaluation of corrosion ability of identified bacteria was carried out. Laboratory scale simulation tests were conducted using nutrient medium containing single culture of several microbes. In this report, the experimental results concerning two of the tested microorganisms: strain-4 and strain-A are discussed.

The fluctuation in the total viable count in the experimental flasks during the period of study is given in **Fig.7**. The TVC fluctuated between $10^{5-7}$cell/ml. **Fig.8** shows the SEM images of the SUS316L surface after 56days exposure. In both the cases, adhesion of microorganism and corrosion pits were observed. Strain-4 was identified as *Bacillus* sp. In our earlier report [5],it was indicated that this is one of the microorganisms that has the ability to corrode stainless steel. This corrosive ability is derived from metabolic acid production. Therefore, there seemed to be a very high possibility that this strain played a big role in this corrosion case. In the case of strain-A also, corrosion pits which could be considered as MIC were observed. However, this strain shows the likelihood of being a new genus, so the metabolic reaction of this is not known yet. Studies on the mechanism of corrosion ability of this strain are in progress.

In this experiment, we had used two different samples for microorganic isolation. Microorganism (Strain-4 and Strain-A) from both the samples showed corrosion ability. However we confirmed that there is considerable difference between strains isolated from marine water and

biofilm. This result thus indicate the need to attention of design the selection of samples for isolation of microorganism and isolation techniques.

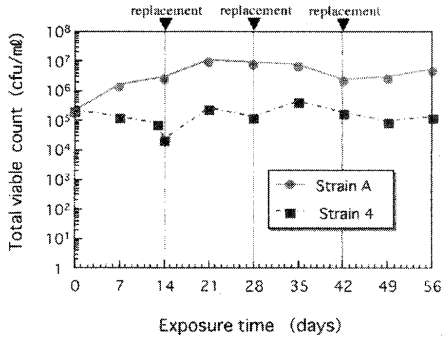
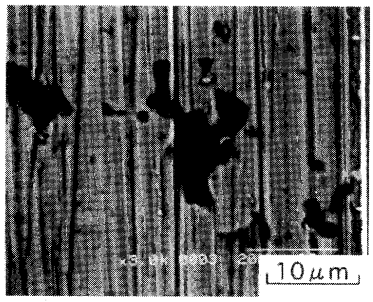
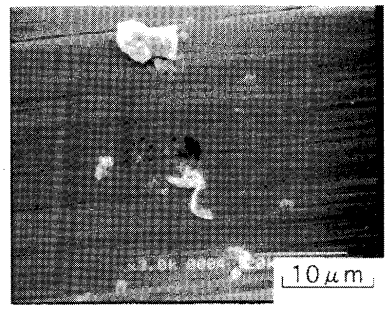



Fig.7 Variation in total viable count
in the experiment medium
added for each microorganism (Strain-A and Strain-4)




(a) Strain-4




(b) Strain-A

Fig.8 SEM images of weld coupons
after 56days of exposure to each microorganism

## 4. Conclusion

1. SUS316L welds corrosion influenced by bacteria in marine water was examined and the result are summarized below.
2. Coupon exposure studies conducted in marine water supplemented 0.01% MB showed pitting corrosion. When the same medium was sterilized, pitting could not be seen after the exposure studies. From these observations, the role of bacteria in the corrosion of SUS316L welds in marine water was suspected.
3. Fifteen bacterial strains from this case were detected. We have examined Strain-4 and strain-A and both the strains showed pitting corrosion suggesting the possibility of their interaction in MIC.

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