LABORATORY STUDIES ON MIC OF AISI TYPE 304 STAINLESS STEEL USING BACTERIA ISOLATED FROM A WASTEWATER TREATMENT SYSTEM

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ABSTRACT

Microbiologically Influenced Corrosion (MIC) is one of the most deleterious effects of metal microbe interactions. When a fresh metal surface comes in contact with a non-sterile fluid, biofilm formation is ensued. This might result in the initiation of corrosion. The sites and materials where MIC is implicated are versatile. Industries such as shipping, power generation, chemical etc are reported to be affected. The rapid and unexpected failure of AISI type 304 stainless steel was investigated in the laboratory by simulation studies for a period of 4 months. Slime and water samples from the failure site were screened for corrosion causing bacteria. Both aerobic and anaerobic flora were enumerated and identified using PCR techniques. *Pseudomonas* sp. and *Bacillus* sp. were the most common aerobic bacteria isolated from the water and slime samples, whilst sulfate reducing bacteria (SRB) were the major anaerobic bacteria. The aerobic bacteria were used for the corrosion experiments in the laboratory. Coupon exposure studies were conducted using a very dilute (0.1%V/V) nutrient broth medium. The coupons after retrieval were observed under a Scanning Electron Microscope (SEM) for the presence of MIC pits. Compared to sterile controls, metal coupons exposed to *Pseudomonas* sp and *Bacillus* sp. showed the initiation of severe pitting corrosion. However, amongst these two strains, *Psudomonas* sp. caused pits in a very short span of 14 days. Towards the end of the experiment, severe pitting was observed in both the cases. The detailed observation of pits showed they vary both in number and shapes. Whilst the coupons exposed to *Bacillus* sp. showed widely spread scales like pits, those exposed to *Pseudomonas* sp. showed smaller and circular pits, which had grown in number and size by the end of the experiment. From these results it is inferred that the rapid and unexpected failure of 304 SS might be due to MIC. *Pseudomonas* sp. could be considered as the major responsible bacteria that could initiate pits in the metallic structures. As the appearance of pits was different in both the tested strains, it was thought that the mechanisms of pit formation are different. Experiments on these lines are being continued.

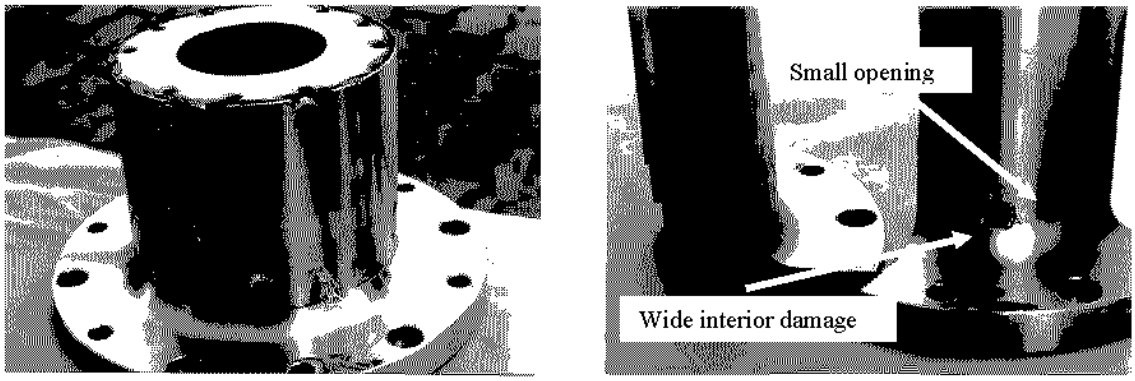
KEYWORDS
Bacteria; Biofilms; pitting; Microbiologically Influenced Corrosion; Stainless steel

1. Introduction

Microbiologicaly influenced corrosion (MIC) is one of the most deleterious effects of metal microbe interactions. Unexpected and rapid failure cases occur in otherwise resistant materials even in mild environment (Walsh, 1999). MIC occurs often at or near welds (Borenstein 1991). Materials affected by MIC include stainless steels among many other structural materials (Borenstein 1991). This phenomenon is reported in different industries such as power, chemical, shipping, oil and gas and potable water supply lines. Localized corrosion in the form of pitting in stainless steels is a common failure cause due to the influence of microbes present in the surroundings. When a fresh metal surface is exposed to a non-sterile medium, a conditioning film is formed followed by biofilm. The first ones to attach over the surface is bacteria, followed by unicellular algae, fungi etc. (Borenstein, 1994). Biofilms are never uniform and they form patchy non uniform areas with or without biofilm. A change in the chemistry of the surface follows and consequently aggressive micro niches results. Depending on the nature of metabolism and the community interaction, the characteristic reactions makes the surface corrode. Though the focus at the electrochemical aspects of MIC is in limelight in the recent past (Dexter et al, 1989; Little et al, 19990; Mansfeld and Little 1990; Videla 2001), emphasis on the aftermath of biofilm formation on it is lacking attention. We had attempted to investigate on a corrosion failure case from a point of view of presence of bacteria and film formation.

2. Background

An unexpected and rapid failure case occurred in a waste water treatment plant. The post combusted ash is collected in a reservoir; rainwater sweeps through it and the seepage water is collected and purified before it is let to the river water system. Leaking in the disc used to stir the wastewater as it is being purified made the shaft not rotating and consequently stopped running. The failure was observed in less than six months period which was unexplainable from the material side as the structural material was AISI type 304 stainless steel. On examination, severe pitting was seen at and near the welds completely covered with slime. The appearance of corrosion sites on the failed samples and the surroundings represented typical MIC features. Pits were typical in having tiny openings outside which on internal observation led to wide and severe damage (Fig.1). Anticipating the possibility of microbiologically influenced corrosion, laboratory simulation studies were carried out using bacterial isolates from the water and slime samples brought from the corroded site. Results were compared with that obtained in the case of sterile medium to elucidate the effect of microorganisms present in the environment.



A. Corroded component showing pitting and crevices B. Enlarged view showing pits

Fig. 1 Photograph of the failure sample

3. Materials & Methods

Enumeration, isolation and identification of bacteria: Slime and water samples from the corrosion site were used for isolation of native flora. Both the samples were diluted to appropriate dilutions and enumeration was done following standard plate count method. Nutrient agar medium was used for isolation of aerobic heterotrophic bacteria. Total viable count in water is expressed as colony forming units (cfu)/ml and that in slime sample as cfu/g wet wt. Most common colonies were picked up from the highest dilution standard plate. They were purified using single colony isolation technique and were identified using PCR techniques. Presence of Sulfate reducing bacteria (SRB) was investigated using postgate medium.

Material used: AISI type 304 SS base metal coupons were used for the present study. Experimental coupons were of the size 25mmX10mm. A surface finish of 1000 grit was attained by polishing with emery paper. The coupons were then degreased with acetone and stored in dessicator until use.

Laboratory simulation studies: Coupon exposure studies were carried out using the identified most common bacterial strains. A very dilute nutrient broth (0.1%)medium (Difco make; Peptone-5mg/L; Yeast extract- 3mg/L; prepared in distilled water of Cl- concentration <0.1 ppm) was used as the experimental medium. The most common strains were *Bacillus* sp. isolated from slime and *Pseudomonas* sp. isolated from water samples. These selected strains were inoculated separately into sterile medium. Experimental coupons as prepared above were sterilized using 70% ethanol and dried under UV in a sterile chamber. Coupons were introduced aseptically to the inoculated medium. A set of control i.e. sterile medium without inoculation was also maintained for comparison. Same numbers of sterile coupons were introduced into the control set also. Maximum exposure period was 120 days. Coupons were retrieved at pre determined intervals (7^{th} day, 14^{th} day, 21^{st} day, 28^{th} day, 60^{th} day and 120^{th} day). Half the volume of the experimental medium was replaced every week by the same amount of fresh sterile medium in order to keep the live cells at normal concentration. The presence of live cells in adequate concentration was constantly monitored by intermittent sampling and plate counting.

Observation: Coupons after exposure to different intervals of time were observed for pitting corrosion using Scanning Electron Microscopy (SEM). Results were compared with the control coupon surfaces.

4. Results and Discussion

Enumeration, isolation and identification of bacteria: Figure 2 gives the total viable bacterial count of different samples. Two different slime samples and water sample were used for enumeration of bacteria. Both the slime samples showed more bacterial number compared to water sample. Aerobic and anaerobic bacteria were isolated. The results are given in Table 1. Water sample did not show the presence of anaerobic bacteria whereas the slime samples harbored sulfate reducing bacteria (SRB). However, the quantitative estimation of SRB was not done during this study. The most common bacterial strains present in the slime samples were *Bacillus* sp. as per the PCR identification techniques. *Pseudomonas* sp. were the dominant bacterial strain in the case of water samples.

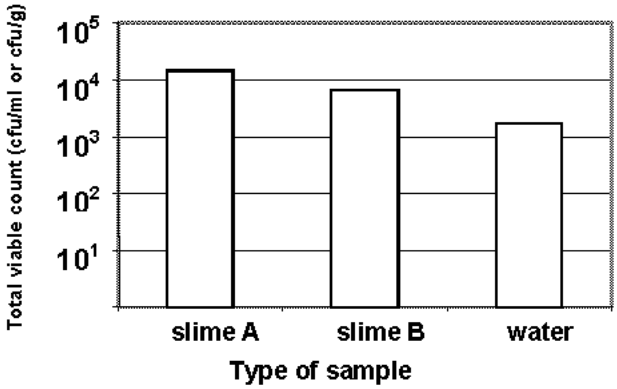


Figure 2. Total viable count of different samples

Table.1 Type of bacteria present in different samples

Type of sample	Slime A	Slime B	Water
Bacteria present	***Bacillus* sp.** **Sulfate reducing bacteria (SRB)**	***Bacillus* sp.** **Sulfate reducing bacteria (SRB)**	***Pseudomonas* sp.**

Coupon exposure studies:

Total viable bacterial count: Total viable bacterial count (TVC) remained in the normal level throughout the study period (Figure3 A and 3B).

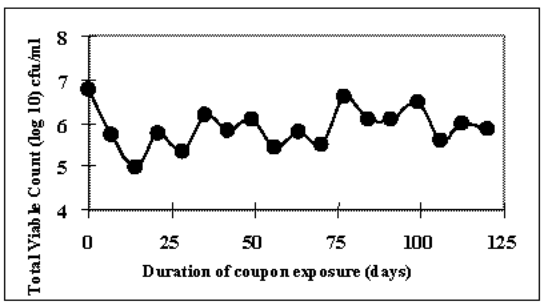


Fig.3A Variation in total viable count of *Bacillus* sp. in the medium during the experiment

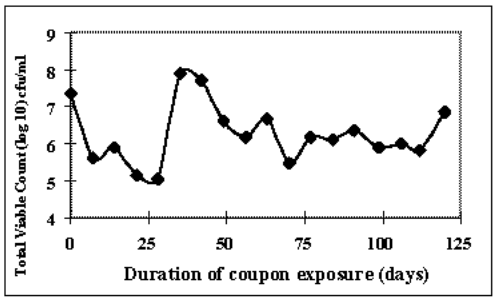


Fig.3B Variation in total viable count of *Pseudomonas* sp. in the medium during the experiment

The graph denotes the TVC of the medium in the flask in which the coupons for observation after 120 days were kept. The TVC was maintained at a level of $10^{5\sim7}$ throughout the study period in all the experimental flasks.

Scanning Electron Microscope observation: Coupons retrieved after different intervals were observed under Scanning Electron Microscope. The results are shown in Fig. 4 to Fig.6.

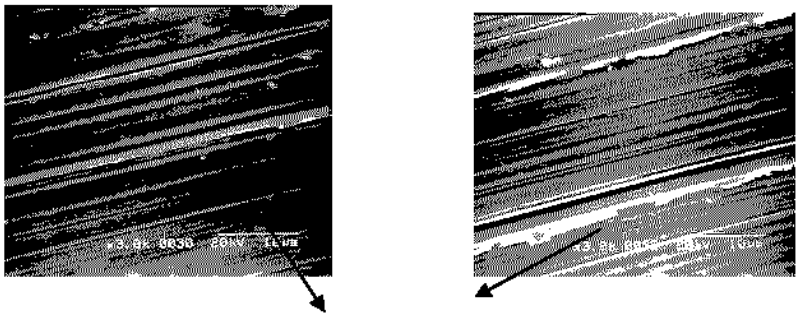


Fig.4 SEM images of surface of AISI type 304 SS after exposure to 120 days in sterile nutrient medium(control)

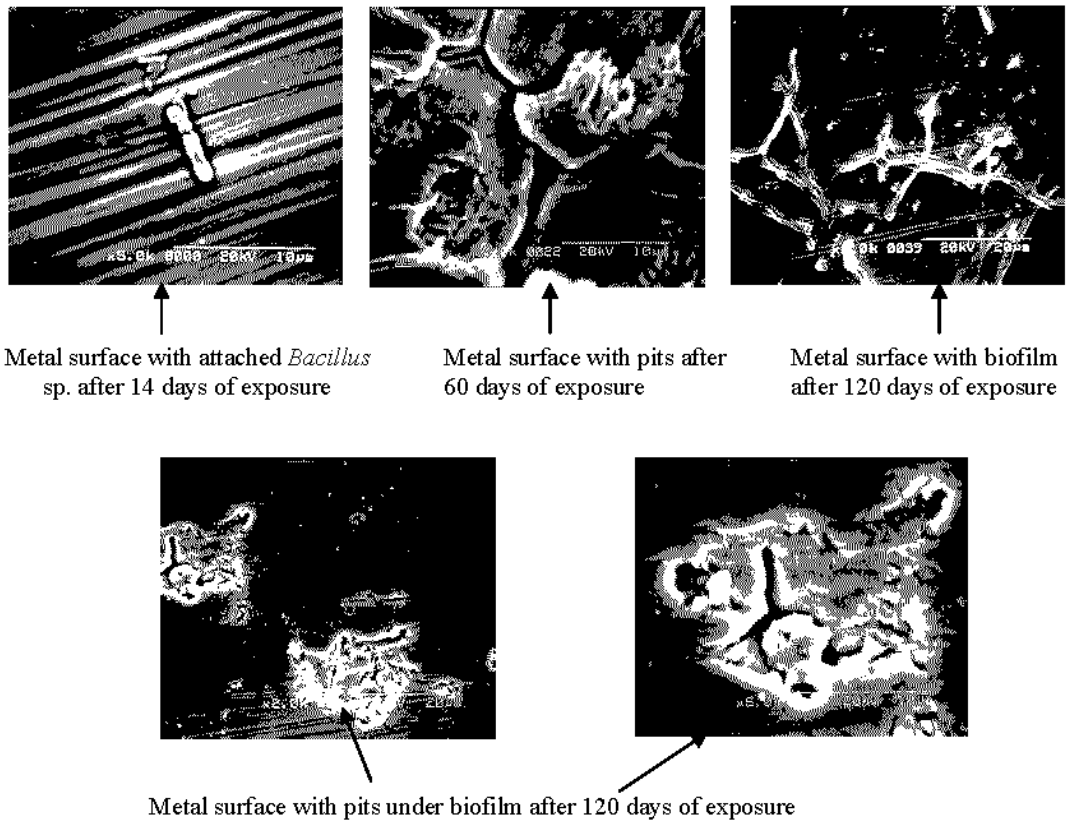


Fig.5 SEM images of surface of AISI type 304 SS after different period of exposure to *Bacillus* sp.

Compared to sterile controls (Fig.4), coupons exposed to bacteria have shown pitted surfaces revealing bacterial influence on corrosion. As shown in the SEM images (Fig.5) pitted surfaces were observed on 60days in the case of *Bacillus* sp. On detailed observation, the type of pitting was similar to the pitting usually observed in scales. Significantly, coupons exposed to *Pseudomonas* sp. showed heavily pitted surface by 60 days (Fig.6). Pitting was observed even on 14th day though the pits were in initial stages. The pits were similar to characteristic MIC pits. Most of the pits were seen adjacent or associated with biofilms. Control samples were devoid of pits or corroded surfaces till the termination of the experiment i.e 120 days.

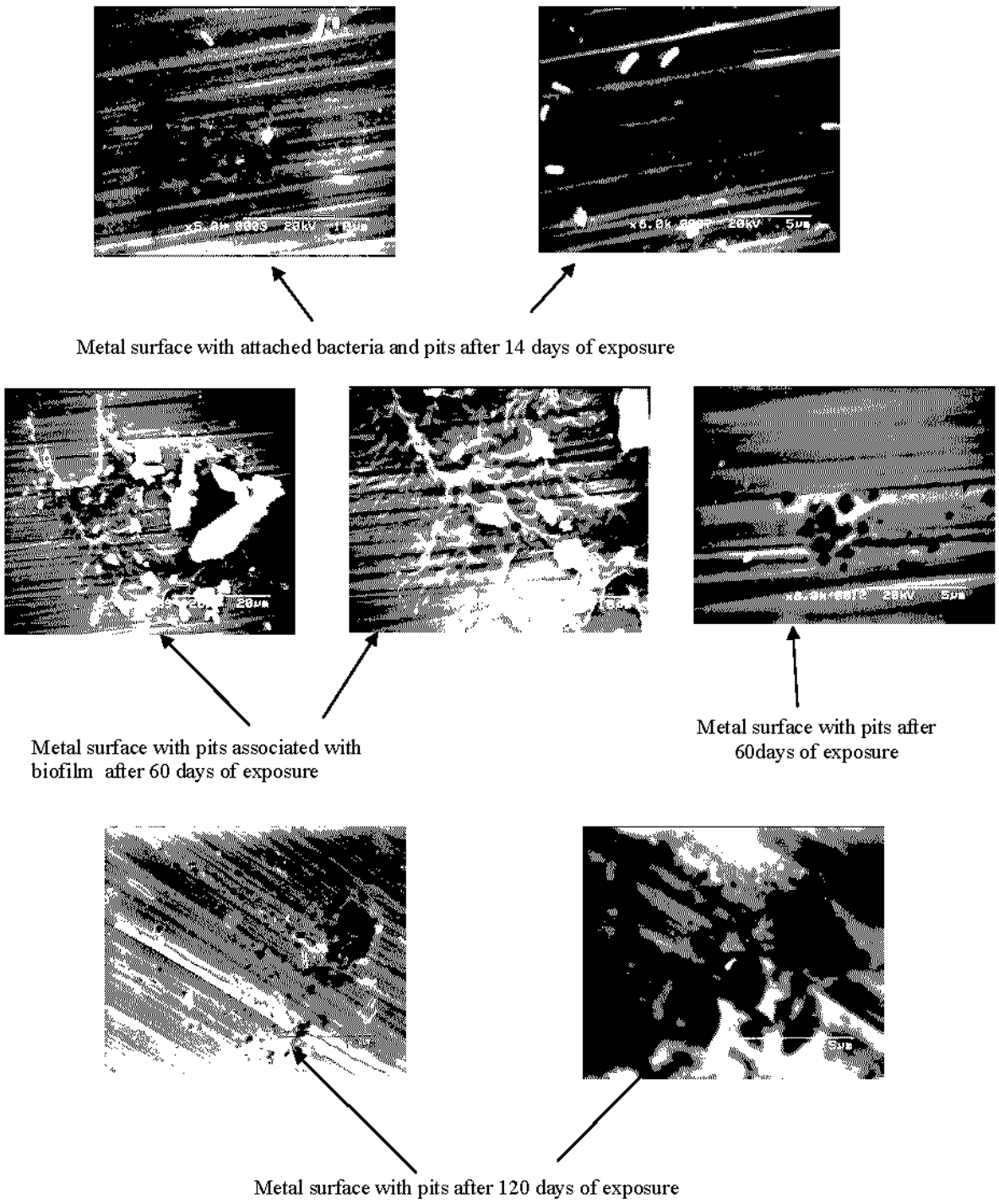


Fig. 6 SEM images of surface of AISI type 304 SS after different period of exposure to *Pseudomonas* sp.

The appearance of pits differed in the case of *Bacillus* sp and *Pseudomonas* sp. In the case of *Bacillus* sp., pits were widely spread and looked like the scales being pitted. Coupons started showing pitted surfaces by 60 days. In the case of *Pseudomonas* sp., from 14 days onwards smaller but round pits were observed which had grown in number and size towards the termination of the experiment. Hence, it is inferred from the results that *Pseudomonas* sp was more harmful compared to *Bacillus* sp. as its effect was seen earlier and the pits were typical MIC pits by appearance. The surface appearance after pitting differed in the case of different bacteria

suggesting the possibility of a difference in mechanism. This aspect is not looked into in detail in the present study. However, from the literature (Borenstein,1994) it is known that formation of oxygen concentration cell is a possible mechanism of localized corrosion in stainless steel. *Pseudomonas* sp is known to be a prolific biofilm former and exopolymer producer. Physiologically, they are aerobic, having a strict respiratory type of metabolism with oxygen as the terminal electron acceptor (Holt et al, 1994) Once a biofilm is formed and the metabolism proceeds oxygen depleted micro zones could be formed. Biofilm is never uniform, it has a characteristic patchy nature (Borenstein, 1994) Hence, the possibility of oxygen concentration cells over the surface of stainless steel coupons is well expected in the case of exposure to *Pseudomonas* sp. which in turn leads to pitting as seen in Fig.6. *Bacillus* sp. on the contrary, could be aerobic or facultatively anaerobic, leaving room for a respiratory or fermentative type of metabolism (Holt et al, 1994). Hence the chances of formation of corrosive metabolites is more in the case of *Bacillus* sp. leading to the appearance of pits similar to etch pits, might be due to dissolution by the acidic metabolites. However, in the case of slime samples, presence of SRB was confirmed. Though not proven experimentally during this study, there are chances of formation of an anaerobic environment due to the metabolism and film formation of *Bacillus* sp. SRB would thrive well inside and consequently might assist corrosion of the material.

Conclusions

1. Presence of both aerobic and anaerobic bacteria in the failure site points towards the chances of microbiologically influenced corrosion.
2. Surface appearance of coupons exposed to sterile medium in comparison to that of coupons exposed to different bacteria reveals the microbial influence on corrosion.
3. Difference in appearance of pitting in the case of *Bacillus* sp. and *Pseudomonas* sp. might be due to difference in the mechanisms of localized corrosion. These mechanisms are theoretically explained.

Acknowledgements

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