Grain Boundary Effect on Bacterial Attachment†

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Abstract

AISI Type 304 L Stainless steel is a widely used material in industries due to its strength and resistance to corrosion. However, corrosion on SS is reported largely at weld or its adjacent areas. Bacteria were observed to colonize preferentially near welds and the reason for this is described as the surface roughness. In the present study, we have evaluated the influence of yet another important metal surface condition ie. substratum microstructure on the bacterial adhesion. 304 L SS weld samples were prepared and machined to separate weld metal, heat affected zone (HAZ) and base metal portions. These coupons were molded in resin so that only the surfaces polished to 3μ finish were exposed to the experimental medium with bacteria, Pseudomonas sp., isolated from a corrosive environment in Japan. The coupons were exposed for varying duration. The area of bacterial attachment showed significant difference with time of exposure and the type of coupons. Generally, the weld metal samples showed more attachment while the base metal the least. The area of attachment was inversely proportional to the average grain size of the three samples. As the bacteria started colonizing, the attachment largely occurred on the grain boundaries of base metal (after 8h, 84.62% and 15.38% of total number of bacteria attached in the field of view (FOV) at grain boundary and matrix, respectively) and on the austenite-ferrite interface in the weld metal (after 8h, 88.33% and 11.77% of total number of bacteria attached in the FOV at boundary and matrix, respectively). Weld area had more grains and hence more grain boundary/unit area than base metal and hence resulted in more bacterial attachment. The SEM observation showed this increased attachment of Pseudomonas sp. resulted in the initiation of MIC on weld coupons by 16 days. Therefore, the results provide data to support the fact that substratum microstructure influences bacterial attachment which, in turn leads to corrosion.

KEY WORDS: (stainless steel welds)(*Pseudomonas* sp.)(bacterial attachment)(microbiologically influenced corrosion)(substratum microstructure)

1. Introduction

The subject of metal microbe interaction is one of immense current interests that encompass a remarkable range of disciplines. One of the most detrimental effects of metal microbe interaction is microbiologically influenced corrosion (MIC). It is well known that when a metal surface comes in contact with a non-sterile fluid, a conditioning film results followed by biofilm. This might result in the initiation of corrosion. The role that sessile bacteria and their associated biofilm play in MIC was in the focus of scientific attention since late 1970s[1]. MIC is reported to occur in various types of materials including austenitic stainless steels[2), 3)]. A majority of cooling water system failures is reported to be around or within weldments [4)-8)]. Weld regions are particularly prone to microbial colonization because welding alters the material surface characteristics[9)].

The microscopic heterogeneity of engineering materials, whether created intentionally or as an artifact, is the basis for their properties. The heterogeneity is evident on the scale of microbes and is an important factor in MIC. The combination of physical and compositional changes brought about by the welding process is believed to facilitate accumulation of organic matter onto the surface followed by bacterial colonization [10), 11)]. Bacteria tend to accumulate more on rough surfaces than smooth and this aspect was given emphasis to foster the belief that welds are prone to increased bacterial colonization.

Stein (1991)[12)] reported that MIC susceptibility of base metal adjacent to weld area cannot be attributed to sensitization but to the microstructure

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produced during the manufacturing process. Geesey *et al.* (1996)[13] reported bacteria preferred to settle on the depressions of oxide film grain boundaries of 316 SS in a flowing water system. Walsh *et al.* (1994)[9] also attempted to relate MIC susceptibility and substratum microstructure. It is well known that the biofilm bacterial activities alter the metal surface condition leading to microbiologically influenced corrosion[14),15]. It depends on the environmental and biological factors as well as the surface characteristics of the materials. The very first step towards biofilm formation is the attraction of bacteria towards the material surface. And one of the very important characteristics of weld is its microstructure. However, till date studies addressing the attachment of bacteria in relation to the substratum microstructure or surface condition are sparse.

Earlier bacterial attachment studies on stainless steel weldments showed that the attachment varies with respect to the region on the weldments [16]. In the present study, we have evaluated using microbiological techniques the influence of substratum microstructure on the bacterial attachment and its possible relationship in the initiation of MIC on SS welds.

2. Materials and Methods

2.1 Experimental coupons

AISI type 304-L stainless steel welds were made by Gas Metal Arc Welding (GMAW) process. (JIS Y 308 L electrode; welding speed was 3mm per second; arc voltage 36 V; welding current 300A and shielding gas 100% Argon). These samples were machined and weld metal, heat affected zone (HAZ) and base metal portions were separated. This was done after etching the sample to differentiate the respective portions. The machined metal coupons (10x 5 mm) were molded in resin in such a way that only the surface to be observed is exposed.

Coupons thus prepared were polished to uniform surface finish with emery papers of various grit sizes to get a 3μ finish. After polishing, coupons were washed thoroughly with detergent followed by rinsing with distilled water. They were degreased with acetone and kept in a desiccator until the exposure tests. The polishing was done to eliminate the effect of surface roughness occurred during welding.

2.2 Bacteria used

Bacterial strain used for the attachment studies was *Pseudomonas* sp., an isolate from a corrosive ground water environment. This strain was reported to cause corrosion of 304 L SS in a short span of time[17]. It was sub-cultured repeatedly two times in Nutrient Broth (Difco: Bacto peptone $5gl^{-1}$ and bacto beef extract $3gl^{-1}$) and log phase (14-16h) culture was used for the experiment.

2.3 Experimental procedure

Coupons were washed in 100% acetone and sterilized using 70% ethanol. They were exposed to UV for 10 minutes and dried in a sterile chamber. The medium used was diluted Nutrient Broth (0.05% v/v in micro filtered distilled water, the concentration of Cl^- was below 0.1ppm). The bacterial density in the medium was intermittently monitored by plating in nutrient agar. The experimental medium was taken in conical flasks and sterilized. To half the number of flasks containing sterile medium, one ml each of the bacterial inoculum (prepared as described above) was added and the rest were kept as such to study the effect of medium on coupons, (the medium control). One more set of the same number of flasks were prepared with sterile distilled water (distilled water control). To each flask, three molded coupons (weldmetal, HAZ and base metal) were introduced aseptically. All sets were kept in an incubator shaker at 28°C at 90 *rpm*. Bacterial attachment on coupons was observed for 16 days. Three flasks each were sampled at intervals 4h, 8h, 12h, 24h, 96h, 144h and 192h. One set of the three flasks was kept until 16 days by replacing 3/4 of the medium with fresh sterile one on the 8th day. These 16 days coupons were used for SEM observation, i.e. to test for corrosion initiation or formation of pits.

2.4 Coupon analysis

Coupons retrieved were air dried inside a sterile chamber and were stained with acridine orange, a fluorescent dye (0.01% w/v in sterile distilled water) before observing under an Epifluorescence microscope (excitation wavelength 330~385nm and emission <420nm). Fifteen different fields on each coupon were selected randomly and the images were recorded using a CCD camera. These images were analyzed for the area of bacterial attachment using image-processing software. Area of attachment is expressed as the percentage of the area of field of view (this was determined as an average of fifteen readings, area of attachment of bacteria/area of FOV x 100). The number of bacteria in the experimental flasks at the time of sampling was determined by plate count method using Nutrient agar (Difco).

2.5 Statistical analyses

The data were analyzed using simple regression analysis, Student t-test, and one-way ANOVA as well as multivariate analysis using repeated measure 2-way ANOVA[18]. The area of attachment data was converted to arcsine values before the analysis of variance and the homogeneity was tested using Cocheran's test for homogeneity. The hypothesis for the repeated measure ANOVA was that there was uniform attachment of bacteria irrespective of the type of the substratum and with time.

2.6 Scanning Electron Microscopic Observation

Coupons exposed for 16 days were observed under SEM. Samples after retrieval were fixed overnight in gluteraldehyde at 4°C. The fixed biofilms were dehydrated using gradient concentrations of ethyl

alcohol, freeze-dried and kept in a dessicator until observation. The surface preparation was done by gold palladium coating. SEM images of the selected fields were taken.

2.7 Test on the influence of substratum microstructure

In order to study the influence of substratum microstructure on bacterial attachment, another experiment was carried out with three sets of AISI type 304-L stainless steel weld metal and base metal coupons. This was studied by micro marking the location of observation on polished surfaces of the coupons and subsequently exposing them to an active microbial culture for a definite period of time. After retrieval, the points of bacterial attachment on the surface were recorded using the markers as reference points. Later, the points of attachment were related to the underlying microstructure of the surface.

The coupons were polished to 3μ finish and five locations in the middle of the coupons were marked with the help of a diamond marker available with the metal micro-hardness tester. One ml each of log phase *Pseudomonas* sp. culture was introduced into the flasks containing sterile 0.05% (v/v) nutrient broth medium. Marked coupons of weld metal and base metal were introduced aseptically into the flasks and kept in an incubator shaker at 28°C at 90rpm. Coupons were retrieved at 4h, 8h and 24h of exposure and were air dried in a sterile chamber and stained with acridine orange (0.01% w/v).

The coupons were observed under an epifluorescence microscope in the bright field and images with the markers were recorded using a CCD camera. The observation of the same fields was repeated once again in the fluorescence mode by carefully changing the filter and the fluorescence images were also saved. Later the coupons were electrolytically etched with 10% oxalic acid as the etchant (6v, current 1A for 35 seconds). The etching process did not disturb the markers on the surface. Coupons after washing were dried under a hot air blower before observing under the microscope in the same magnification in the bright field. Images of the locations with the markers were again recorded. Later, these images were superimposed using the markers as reference. Thus the area of observation on the coupons could be relocated precisely. Superimposition of the images could be carried out using the image processing software. The process of superimposition involved manual operation. However, the precision could be as high as micron level since the microscopic images could be further enlarged before overlapping. From these superimposed images, the points of bacterial attachment on these coupon surfaces could be found out and the influence of grain boundaries on the bacterial attachment could be ascertained. Bacterial cells that are attached on the boundaries or lying very close to it (<1μ i.e. less than the average length of this bacterium) are counted as those attached to the grain boundary and those lying away from this level in the matrix are considered as attached to the matrix. This could be easily detected in the enlarged superimposed images. Thus the number of bacteria attached in the grain boundary and matrix are expressed as % of total number of bacteria found in the FOV (No. of bacteria on grain boundary or matrix/total No. of bacteria in the FOV x 100). The analysis could be performed only on 2 and 8h coupons since by 24h bacteria started forming micro-colonies spreading out of grain boundaries there by making the counting impossible.

2.8 Metal composition

The elemental composition (only the major components such as Fe, Cr, Ni, Mn, S, P and Si) at the grain boundaries and the matrix were analyzed using EPMA (Electron Probe Micro analyzer, Jeol 1800). This was done after electrolytic etching of coupons using 10% oxalic acid as described above.

2.9 Grain size measurement

The grain size of weld metal (only columnar grain width), HAZ and base metal was determined as below. The coupons after polishing to diamond (3μ) surface finish were electrolytically etched using 10% oxalic acid. The micrographs were taken under bright field (Figure 1). From these micrographs, the average width of the grains was measured. The grain size is expressed as the average width of the grains [19].

From this data, the nature of the substratum surface i.e. the larger the grain size, the lesser the length of grain boundaries/unit surface area and the smaller the grain size the larger the length of grain boundaries/unit surface area, was determined.

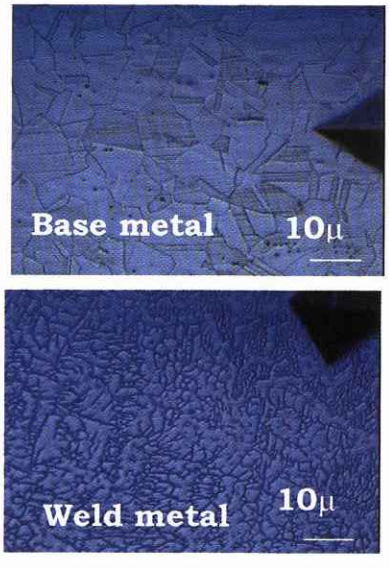

Fig. 1 Microscopic images of base metal and weld metal coupons after electrolytic etching to show the distribution of grains (base metal) and grains and sub-grains (weld metal) on the coupon surfaces

2.10 Potential measurement

In another experiment, two sets of weld metal, HAZ and base metal coupons were exposed to experimental medium, which was same as for the attachment studies. One set was inoculated with *Pseudomonas* sp. and another sterile (control). All flasks with coupons were incubated at 28°C in an incubator shaker at 90 *rpm*. Intermittent potential measurement was carried out for a maximum period of 30 days using a potentiostat. The change in potential of different coupons as a function of exposure time was recorded. The data was compared with the bacterial attachment and corrosion initiation on different types of coupons.

3 Results

3.1 Total Viable Bacterial count

The initial concentration of bacteria in the medium was in the order 10^5 cfu/ml, which increased to 10^8 cfu/ml by 24h and stabilized at 10^{7-8} cfu/ml through out the experimental period.

3.2 Area of bacterial attachment with types of coupons

The area of bacterial attachment increased with time on all three types of coupons, with a maximum at 192h. However, the area of attachment was different in all three types of coupons. Base metal showed the lowest area of adhesion, while the weld metal the highest. However, by 192h, both weld metal and HAZ showed more or less the same area of attachment (Figure 2&3).

The 2-way ANOVA results showed the area of attachment varied significantly between the types of coupons ($F=35.067$, $p<0.0001$) and with the duration of exposure ($F=102.262$, $p<0.0001$). The synergistic effect of exposure duration and type of coupon on the bacterial attachment also was significant ($F=4.925$, $p<0.0001$).

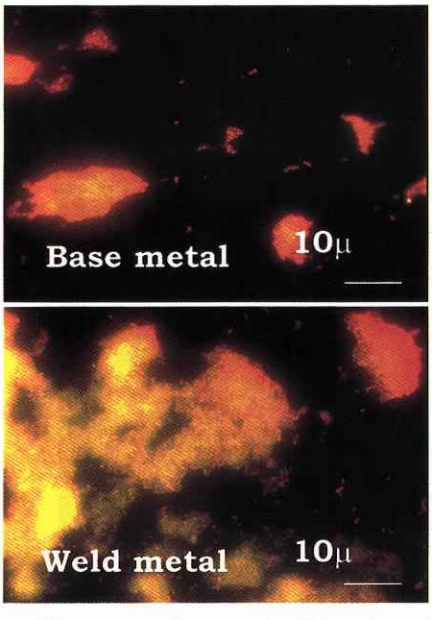

Fig. 2 Epifluorescence images of weld metal and base metal showing bacterial adhesion after 192 h of exposure in the experimental medium with *Pseudomonas* sp.

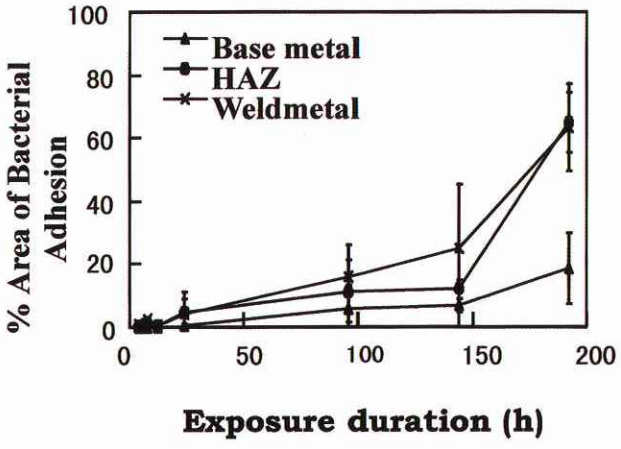

Fig 3. Variation in the percentage area cover of bacteria on weld metal, HAZ and base metal coupons during the experiment. Error bars show standard deviation

3.3 SEM observation

All coupons exposed for 16 days were observed under SEM. The results showed initiation of pits on weld metal coupons with bacteria by the 16-day (Figure 4), while base metal and HAZ did not show pits (entire coupon surface was scanned for the presence of pits). Also, no sign of corrosion could be seen on control coupons (Figure 4).

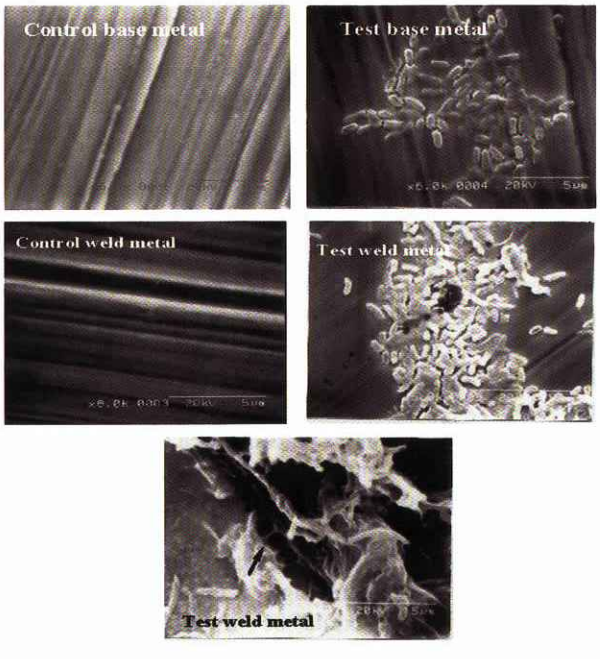

Fig.4 SEM photographs of base metal (top two) and weld metal coupons after 16 days of exposure in the medium with and without (control) *Pseudomonas* sp. Weld metal coupons exposed to bacteria showed pits and a skeleton type of corrosion (arrow in the bottom one) while the control not showing initiation of corrosion.

3.4 Location and intensity of initial bacterial attachment

The superimposed images of the marked locations of the coupons showed during the initial period (4h and 8h) the bacterial attachment occurred on or near the grain boundaries in the case of base metal and austenite ferrite interface in the case of weld metal. By 24h, the bacteria started forming micro colonies spreading away from the grain boundaries to matrix (Figure 5).

The bacterial density on the grain boundary and matrix after 2 and 8h of exposure is given in Figure 6. The bacterial density (represented as the percentage of total number of bacteria present in the FOV) was found to be significantly more on grain boundaries compared to matrix both on base metal and weld samples.

3.5 Grain size and its relationship to the area of bacterial attachment

The grain size data showed smallest grains in the weld metal region (10.83μ±3.7) and the largest in the base metal (27.24μ±3.18) while grains in the HAZ coupon showed an intermediate size (18.05μ±1.15). Thus, the grain size varied significantly between the three types of coupons (one way $ANOVA_{2,151} = 42.07$, $p<0.0001$). The regression analysis with the grain size and area of bacterial attachment showed a significant inverse relationship (Figure 7).

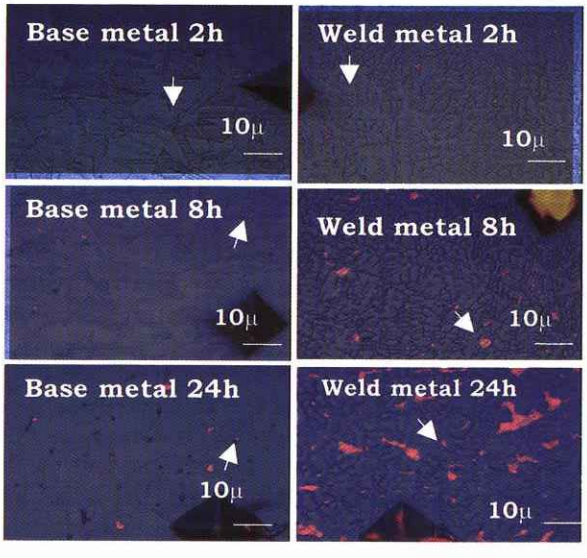

Fig.5 Superimposed images (epifluorescence and bright field) of base metal and weld metal coupons after 2h, 8h and 24h exposure in the experimental medium with *Pseudomonas* sp. Bacterial cells (orange spots shown with arrows) attached on the grain boundaries of base metal and austenite-ferrite interface in weld metal coupons during the initial period of colonization (2h and 8h). By 24h bacteria started developing into micro colonies or patchy biofilm spreading out from the grain boundary to the matrix.

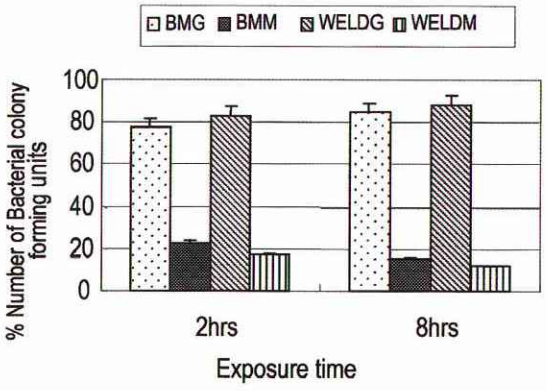

Fig.6 Variation in bacterial density (as % of total cfu in the FOV) at grain boundary as well as matrix of stainless steel weldments after 2 and 8h of exposure in diluted nutrient broth medium with *Pseudomonas* sp. Error bars show SD (n=4).

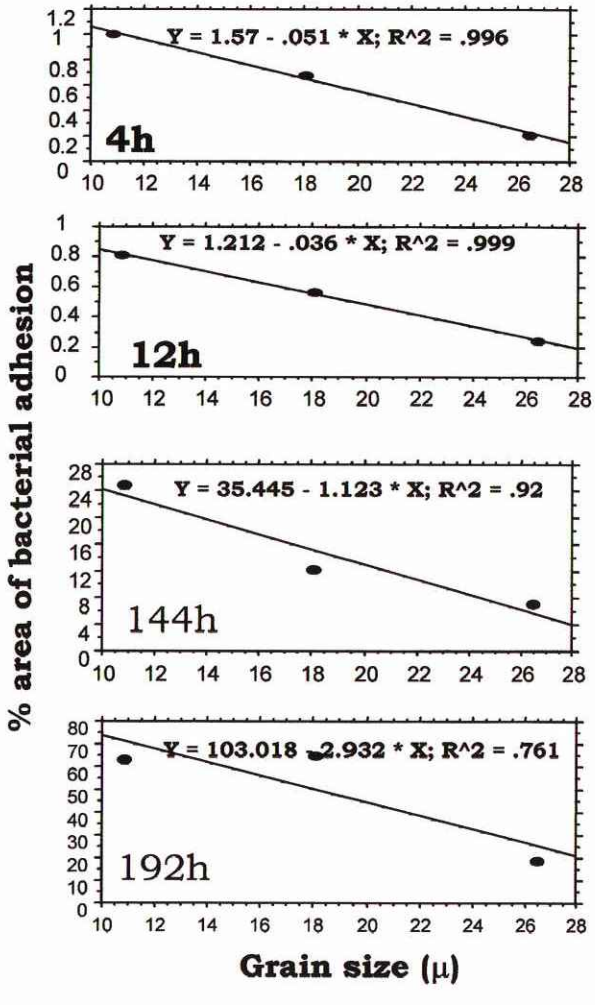

Fig. 7 Results of regression analysis between the average grain size of weldmetal, HAZ and base metal coupons and the percentage area cover of *Pseudomonas* sp.

The significance of this relationship reduced as the exposure time increased. This shows the smaller the grain size, the larger the number of grains per unit area, and the length of the total grain boundary. Hence, the longer the grain boundary/unit area, the greater the bacterial attachment.

3.6 Elemental composition

The EPMA analysis showed some variations in the concentration of elements in the weld metal, HAZ and base metal coupons (Table 1).

While the matrix region of the three types of coupons showed significant difference in Fe, Ni and Mn (one-way ANOVA, p=<0.05), the grain boundary showed significant difference in Cr, Mn and P (one-way ANOVA p=<0.05). With respect to the difference in composition of matrix and grain boundaries, only Cr and Ni were significant (paired one tail t-test, p=<0.05) in the weld region, while in the other two types of coupons no difference in composition could be detected.

3.7 Potential variation

The potential variation observed on the three types of coupons exposed to sterile (control) and with bacteria is given in the Figure 8. For weld metal exposed to bacteria, the potential rose systematically from –130 to 70 mV Ag/AgCl with in 8 days. The base metal coupons exposed to bacteria also showed a rising trend in potential compared to the control coupon. However, HAZ coupons showed comparatively stable values throughout the study period.

Table 1. Elemental composition in weight percentage (± SD) at the matrix (grains) and grain boundaries (GB, austenite-ferrite interface or columnar boundaries in case of weldmetal) of weldmetal, HAZ and base metal coupons of 304 L SS used in the experiment. Measurements were made in Electron Probe Micro Analyzer (JOEL, Superlab 8600).

Element	Weldmetal		HAZ		Base Metal	
	Matrix	GB	Matrix	GB	Matrix	GB
Fe	69.58± 0.26	70.06± 1.92	72.86± 1.22	72.15± 1.46	72.96± 0.58	73.20± 0.32
Cr	20.16± 0.71	23.94± 0.90	19.39± 0.25	21.20± 2.28	19.53± 0.25	19.88± 0.40
Ni	10.76± 0.76	6.48± 0.24	9.34± 0.63	7.60± 2.33	9.08± 0.15	8.81± 0.21
Mn	1.86± 0.08	1.63± 0.05	1.01± 0.12	0.98± 0.02	0.91± 0.09	0.83± 0.05
Si	0.55± 0.05	0.52± 0.03	0.62± 0.08	0.61± 0.03	0.60± 0.03	0.58± 0.06
P	0.02± 0.01	0.02± 0.01	0.03± 0.03	0.04± 0.01	0.03± 0.02	0.01± 0.01
S	0.02± 0.002	0.01± 0.003	0.01± 0.01	0.01± 0.02	0.01± 0.02	0.02± 0.003

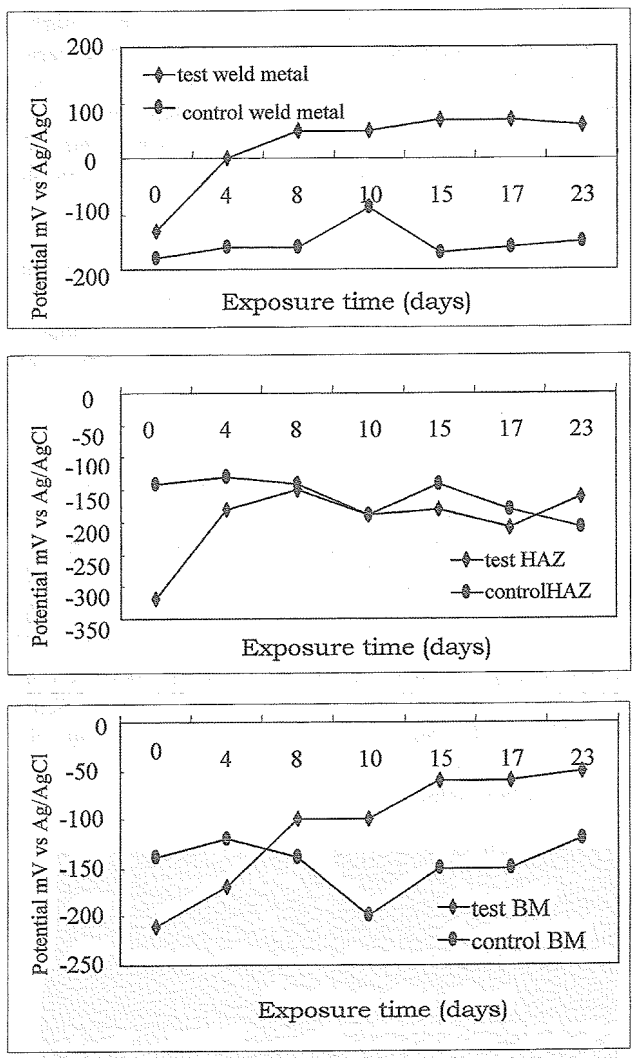

Fig.8 Variation in corrosion potential (mV) on weld metal, HAZ and base metal coupons with and without *Pseudomonas* sp. during the study period.

4 Discussion

4.1 Area of bacterial attachment with types of coupons

The rate of bacterial transport to the substratum surface is reported to be dependent on the concentration of bacteria in the bulk liquid at low concentration[20]. A diluted medium was used in this study. This was to make sure that the effect of medium on the coupons was minimal and to have a slow growth and moderate density of bacteria in the medium through out the period of the experiment. The area of bacterial attachment increased with time and was found to be significantly different in all the three types of coupons studied. Both the type of coupons as well as the period of exposure significantly affected the bacterial attachment. On an average, base metal showed the lowest area of attachment while the weld metal the highest, which agrees with the report of Walsh (1999)[21] and Eshwar & Dexter (1999)[22] while not with Tide *et al.* (1999)[23]. Walsh (1999)[21] reported less preference of base metal

regions by microbes. However, Tide *et al.* (1999)[23] reported no such preference for microbial attachment, as they could not see any significant difference in the bacterial settlement between weldmetal and base metal regions. Bacterial settlement is reported to be influenced by substratum surface roughness and geometry[24] and weld metal regions are reported to have more microbial colonization as the welding alters the material surface condition including surface roughness[9),25),16)].

Biofilm formation on material surface is considered as the initial step of MIC. Soracco *et al.* (1988)[26] reported a correlation between sensitization and MIC. Since we used uniformly polished coupons, influence of surface roughness is not arising even though this would alter the substratum surface to some extent than that of the stainless steel surfaces found in typical industries. The significant variation of bacterial attachment between the types of coupons thus indicates factors other than the surface roughness affecting bacterial attachment. The factors, which could be thought of are the elemental composition (segregation of ions and inclusions), substratum microstructure[25),16)] and surface energy between grain boundary and matrix. Differential charge distribution among the three types of coupons also could be influencing the bacterial attachment.

4.2 Location of initial bacterial attachment

Experiment on the influence of microstructure on the bacterial attachment showed initial attachment of bacteria occurring on the grain boundaries. It was interesting to note that this phenomenon was evident more on weld metal than on base metal coupons. Thus, the present study provides preliminary data on the influence of substratum microstructure on bacterial attachment which, till date was not supported with experimental evidence[27]. Muller *et al.* (1992)[28] showed the influence of substratum surface energy in the attachment of two strains of *Pseudomonas* sp. They have found that the rate of bacterial adsorption on a substratum increased with increasing surface free energy and surface roughness.

In this study, the grain size and the area of bacterial attachment showed a significant inverse relationship (Figure 7). Weld metal region had smaller grains composed of vermicular and columnar grains ($10.87\mu\pm3.7$) contributing to longer grain boundaries per unit area. While base metal had larger grains ($27.24\mu\pm15.59$) thus resulting in shorter grain boundaries per unit area. As the initial attachment occurred on the grain boundaries (Figures 5 & 6), weld metal harbored more cells. This initial colonization influenced the subsequent growth and recruitment. The results are supported by the information given by Walsh (1999)[9] that MIC susceptibility of weld region is influenced by segregation and sub-grain interfaces.

Regarding the reasons why bacterial cells are attracted towards grain boundary, there are two important possibilities. First, the elemental segregation occurring at the grain boundaries and bacteria are attracted towards it. Bacteria are known to get attracted towards specific elements (Hughes and Poole, 1989)[29]. In order to test it, EPMA analysis of the coupons was carried out on the matrix as well as on the grain boundaries. The EPMA analysis showed some variation in the concentration of elements in the weld, HAZ and base metal coupons. While the matrix region of the three types of coupons showed significant difference in Fe, Ni, and Mn the grain boundary showed significant difference in Cr, Mn and P. With respect to the difference in elemental composition between matrix and grain boundaries of the respective coupons, both Cr and Ni were significantly different in the weld coupons, while the other two types of coupons did not show any significant difference in the elemental concentrations. This could be anticipated because the test material what we have used was low carbon grade 304L. Das & Mishra (1985)[30] reported that corrosion in stainless steels could be explained by the widely accepted theory that an excess phase, rich in Chromium is precipitated along the grain boundaries. As a result, these areas lose their chemical resistance leading to susceptibility to corrosion. Many researchers agree that those materials, which are more resistant to MIC, are generally resistant to localized corrosion. It is for this reason, that a higher concentration of chrome equivalent is advised for MIC resistance[31]. In addition, the literature show no special affinity by *Pseudomonas* sp for chromium or nickel that are altered during welding process and concentrated in the grain boundaries (Cr) or in matrix (Ni). But, as mentioned by Walsh (1999)[9], the non-equilibrium thermal cycling during welding distributes a sulfur and phosphorus rich liquid along continuous cell boundaries. Both these elements are favorable for bacteria and there are chances of these influencing the attachment. However, enrichment of these elements could not be seen in the present study.

As the second possibility, differential energy distribution in the grain boundary and matrix could be thought about. As per literature[32], grain boundaries hold more energy than the matrix. Bacteria could be considered as charged colloidal particles[33]. Bacterial cell surfaces are generally polyanionic; i.e. they are negatively charged[34]. Attraction of bacteria towards a surface occurs either by chemotaxis[35], or by physical forces[36]. Reversible attachment occurs as a result of electrostatic, van der Waals or simple hydrostatic forces, followed by the irreversible attachment due to biologically mediated processes, influence of surface free energy, hydrophobicity etc[36]. There is experimental evidence relating surface potential and cellular adsorption to surfaces; these data generally show that there is an inverse correlation between adsorption and cell surface potential[37]. Therefore, chances are more for the cells to be attracted towards the grain boundaries with a high energy level and elemental segregation. However, this result disagrees with earlier reports such as Moreno *et al.* (1993)[38] where they mentioned that

there might not be any grain boundary preference for bacterial attachment. Eashwar & Dexter (1999)[22] opined that in the above said report[38], there is a possibility that the bacterial response to the grain boundaries was overwhelmed by the geometrical and work hardening changes induced by abrasion. Eashwar & Dexter (1999)[22] observed preferential bacterial settlement in the anodic site with a note on the possible effects of electrochemical charge[39] and metal ion release. Little et al (2000)[40] demonstrated attraction of marine bacteria towards established anodic sites. As evidenced by the results of the present study, the grain boundary preference is slowly decreased with time. As the colony grows as a result of the growth of the original colonizer as well as the attachment of new cells, it becomes simply too large to be confined to the grain boundaries that it sprawls out into the matrix area there by loosing the boundary preference by the bacteria.

Food industries use stainless steel as the industrial material due to its durability, resistance to corrosion and easy handling. In the US, a No. 4 surface finish (surface roughness value R_a <1μ) is recommended for stainless steel that comes in contact with food (Boulange, 1996[41], US-3-A-Sanitary standard 01-06, 1974 [42], as reported in Tide et al., 1999)[23]. Thus, the weld beads need to be ground and polished in order to achieve the surface standard. Our study shows that, in spite of polishing to 3μ surface finish, the bacteria tend to attach more on the weld surface than base metal finally resulting in MIC.

SEM observation of the 16-day coupons showed initiation of pits on the weld metal coupons. This is the characteristic visual sign of MIC at welds. Borenstein (1991)[7] reported that welds of type 304-L base metal using 308 L as filler metal are susceptible to MIC and these were exactly the materials that used for this study. During the present study pitting attack was observed more on weld metal than on HAZ and base metal coupons. This result shows that more bacterial attachment leads to more corrosion and more attachment at weld metal could be due to the heterogeneity and grain boundary preference of bacteria. The corrosion potential measurement of coupons exposed to live bacteria also showed an increasing trend on weld metals than on HAZ and base metal. Thus weld metal regions supported more bacterial attachment leading to the initiation of corrosion in a faster pace than on the other types of coupons studied including bacteria free controls.

5. Conclusions

To summarize, the bacterial attachment was found to be significantly more on weldmetal and HAZ coupons than 304-L base metal. This increased bacterial attachment resulted in microbiologically influenced corrosion of weldmetal as evidenced by the pits and skeleton type corrosion. As for the reasons for the increased bacterial attachment on weldmetal, the present study showed the influence of substratum microstructures. The initial bacterial attachment occurred on or near grain boundaries or austenite ferrite interfaces on base metal and weld metal coupons, respectively. The grain size measurement showed, weld metal with smaller grains than base metal and HAZ. The negative correlation obtained between bacterial attachment and grain size thus explains one of the probable reasons for the increased bacterial attachment to weld metal. The grain boundary preference weakened with time. However, the initial attachment played a significant role to determine the subsequent growth of bacteria. Thus the present study showed, in addition to the surface roughness, the underlying microstructure of substratum plays a determinative role in bacterial attachment and grain boundary preference could be one of the main reasons for the preferential bacterial attachment to weld metal.

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