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Effect of Surface Condition on Attachment of Bacteria to Stainless Steel Welds†

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Abstract

Microbiologically influenced corrosion (MIC) is ubiquitous. Welds are reported to be prone to MIC due to the altered material surface characteristics. This leads to the notion that bioadhesion is influenced by the substratum microstructure. Little is known quantitatively about the preferential adhesion of bacteria on areas of varying microstructures. One of the important characteristics of weld is its microstructure and an important determinant in the biofilm formation is bioadhesion. Thus, a study addressing both these factors would provide the probable reason why the welds suffer preferential MIC attack. Experiments were carried out to study the effect of microstructure on the adhesion of a gram-positive bacterium, Bacillus sp. isolated from the residual water of an MIC affected effluent treatment plant. Weld samples (weld metal, HAZ and base metal separately) of two different materials viz. 316L and 304L stainless steel were tested. Area of bacterial adhesion showed significant difference between base metal, HAZ and weld metal of both the materials tested. Weld metal and/or HAZ harbored more bacteria in both the materials tested, with base metal showing the least. Also, a significant difference in percentage area of adhesion was observed between as welded and polished coupons of the same material. Since base metal, HAZ and weld metal of both the materials showed difference in area of adhesion in spite of the uniform surface condition, the influence of microstructure gathers significance. This preferential adhesion contributes very much to corrosion and can be considered as one of the factors causing MIC attack on welds.

KEY WORDS: (Bacterial adhesion) (*Bacillus* sp.) (Stainless steel welds) (Microstructure) (Microbiologically influenced corrosion)

1. Introduction

A requisite event in biofilm accumulation is the adsorption of bacterial cells at a substratum. If these adsorbed cells find suitable environmental conditions, biofilm formation will occur through continued adsorption and growth of adsorbed cells. Biofilms can be deleterious, when they induce corrosion (Mueller et al., 1992)[1]. Microorganisms growing on surfaces perform a variety of metabolic reactions, the products of which may promote the deterioration of the underlying substratum. These reactions refer to biocorrosion or microbiologically influenced corrosion (MIC) when the underlying substratum is a metal or metal alloy (Geesey 1991)[2]. Microbiologically influenced corrosion (MIC) is a serious problem in a number of industries including power generation, petrochemical, pulp and paper, gas transmission and shipbuilding. The role that sessile bacteria and their associated biofilm formation play in MIC was one of the chief factors for the increased interest in microbiologically related problems during the period from the late 1970s to the present (Obuekwe et al 1981)[3]. Since the turn of the century, MIC has been referred as the cause for deterioration of various materials. The combination of unexpected attack and rapid failure make MIC a matter of considerable concern in many applications (Walsh 1999)[4]. Though there is agreement among researchers in the involvement of microbes in corrosion, there is little consensus on the mechanisms involved in this process (Walsh and Willis, 1995)[5]. But, it is noticeable that there are consistent reporting of increased incidence of MIC at and near welds (Kobrin, 1986; Garner 1979; Garner, 1982)[6-8].The formation and succession of biofilms depend on the environmental and biological factors as well as the surface characteristics of the materials. MIC is reported in various types of materials. Also, it is well known that microbes settle preferentially on different materials. High purity metals frequently have low mechanical strength resulting in the use of alloying elements to improve mechanical, physical, fabrication and corrosion characteristics (Corrosion basics- an introduction (Houston, Tx: NACE, 1984,p 49)[9]. Alloy structures are inhomogeneous, compositions are discontinuous, and properties are anisotropic from an atomic scale to a macroscopic scale. The microscopic heterogeneity of engineered 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. Weld regions are particularly attractive to microbes in many

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surface characteristics (Walsch et al. 1994)[10]. There are reports on the influence of alloy composition, manufacturing specifications such as surface finish and heat treatments and presence of protective coating on susceptibility to MIC (Borenstein 1991)[11]. In another report, the same author states that subsurface tunnelling has been observed along ferrite stringers in weld areas of stainless steel. Frequently, pitting of stainless steel is located in the HAZ, fusion line and adjacent base metal of welds (Borenstein, 1991)[11]. The topological, chemical and microstructural alterations resulted by welding may increase the corrosion susceptibility and introduce the potential or galvanic corrosion or other electrochemical events at the welded region (Enos and Tailor 1996)[12]. A majority of cooling system failures in many corrosion resistant alloys are around or within weldments (Borenstein 1991;Kohler 1991; Hayner et al 1988; Borenstein 1988; Borenstein and Lindsay 1987;)[11, 13-16].

The mitigation of MIC initiation under solution annealed condition which alters microstructure has been studied. Kearns and Borenstein (1991)[17] state that welds having filler metal composition matching the base metal have lower corrosion resistance than fully annealed base metal due to lack of homogeneity and the microsegregation of chromium and Molybdenum. Chemically depleted regions can be much more susceptible to localised attack (Kearn and Borenstein, 1991)[17]. The combination of physical and compositional changes brought about by the welding process is believed to facilitate accumulation of organics onto the surface and subsequent colonisation by bacteria (Walsh et al, 1992; Videla and Characklis 1992)[18-19]. Since the report of Olsen and Szybalski (1950)[20], the preference of weld as a site of colonisation by bacteria is evident and was correlated to the surface roughness. Kikuchi and Matsuda (1996)[21] in a review, pointed out that HAZ may show a changed internal structure and composition and may in some circumstances act as a location where corrosion can be easily and preferentially initiated. Stein (1991)[22] reported that MIC susceptibility of base metal related to weld area cannot be attributed to sensitisation but to the microstructure produced during the manufacturing process. Walsch et al (1994)[10] described the attempts to relate MIC susceptibility and microstructure. The case histories published on MIC usually make references to the appearance of corrosion in welded zones, which usually takes the form of pitting. There are also scattered reports on relationship between sensitisation state of the steel and MIC (Ibars et al 1992)[23]. This is one of the facts that points towards the correlation between the susceptibility to this type of corrosion and to the microstructural state in which the metals are found. Existing literature hardly mentions anything about the preferential attachment of bacteria on areas of different microstructure. It seems that more research must be done on this aspect so that the microstructural state of the materials that undergo MIC is determined in a more precise and quantitative way. In other words, it is necessary to know whether microstructure difference provides any cue for either attracting bacteria to the surfaces or in any way to modify the activity of the attached bacteria. It is known that the key to the alteration of conditions at a metal surface before the initiation of microbiologically induced corrosion is the formation of a biofilm. The very first step towards biofilm formation is the attraction of bacteria towards the material surface by complex means. And one of the very important characteristics of weld is its microstructure. Hence, a study involving both the microstructure and bacterial adhesion would throw more light on the question why welds suffer preferential MIC attack. If preferential adhesion in relation to microstructural features were the key to preferential MIC at weldments, then prevention of bacterial adhesion or selective elimination would be a potential weapon to mitigate MIC. Hence, the present study was planned to find out the effect of microstructure on adhesion of a corrosive bacterial strain of *Bacillus* sp. on three different materials.

2. Experiment

2.1 Materials

AISI Type 316 L stainless steel and 304 L stainless steel were used.

2.2 Welding details:

Weld metal samples were made by the Gas Metal Arc Welding (GMAW) process. Different parameters of welding are given in Table 1.

Material:	SUS 316L	SUS 304L
Electrode used:	JIS Y 316L	JIS Y 308L
Type of welding:	GMAW (one pass)	GMAW (one pass)
Welding speed:	3mm/s	3mm/s
Arc voltage:	36V	36V
Welding current:	300A	300A
Shielding gas:	100% Ar	100%Ar

Table 1. Welding parameters for preparation of experimental coupons

2.3 Preparation of experimental coupons:

Welded samples of the materials were separated to weld metal, HAZ and base metal portions by machining. Machining was done after marking and making sure the weld metal, HAZ and base metal

portions by etching with the corresponding etchants. The machined metal coupons were moulded in resin such that the surface to be observed only is exposed. Coupons of two different surface conditions were prepared. One set was as welded and the other polished to 1500 grit with emery paper to a uniform surface finish.

2.4 Methodology:

The experimental medium for coupon exposure studies was 1%(v/v) nutrient broth. The nutrient broth {Difco: (Bacto Peptone: 5g/L; Bacto beef extract: 3g/L)} was diluted to 1% (v/v) with microfiltered distilled water before sterilization. Bacterial strain used for the experiment was an isolate of *Bacillus* sp. from the residual water of a MIC affected effluent treatment plant. This strain is reported to cause pitting of stainless steel welds in the laboratory (Kikuchi et al, 1998)[24]. The bacteria were cultured in nutrient broth (Difco) and from the log phase culture (18-24hrs growth), uniform inoculum was added to each of the experimental flasks.

The experimental coupons prepared as described before were cleaned, degreased and sterilized. Then, they were introduced into the experimental medium aseptically. Inoculated flasks containing the coupons were kept in an incubator shaker set at 28°C and 90-rpm. Coupons were retrieved aseptically for observation on the 1st, 2nd, 3rd, 6th and 8th day in addition to the visual observation for the changes in surface appearance.

2.5 Observation:

2.5.1Epiflourescence microscopic observation:

Staining solution was prepared by dissolving acridine orange (for staining nucleic acids) in sterile distilled water to give a concentration of 0.01% (w/v). Coupons retrieved aseptically were air-dried in a sterile chamber and were stained with this solution. All stained coupons were rinsed in non-flowing sterile distilled water before the surfaces were viewed under epiflourescence microscope. Statistically significant numbers of fields were selected randomly and the images were recorded through a CCD camera. These images were further analysed for bacterial density expressed as area(s) of adhesion using image-processing software.

2.5.2 Scanning Electron Microscopic (SEM) observation:

Samples for SEM observations were kept at 4° C overnight for fixation in gluteraldehyde. The fixed biofilms were dehydrated using gradient concentrations of ethyl alcohol, air dried and kept in dessicator until observation. The surface preparation was done by gold palladium coating. SEM images of the selected fields were taken.

2.5.3 Optical microscopic observation:

Microstructure of all the coupons were observed by metallographic microscope after etching with corresponding etchants and pictures were taken.

3. Results and Discussion

Base metal coupons harboured very less bacteria compared to HAZ and weld metal in the case of as welded coupons of 316L stainless steel. Eventhough initially, weld metal showed more attachment compared to HAZ, as time passed, a clear trend of HAZ getting more settlers was seen. Generally, the trend was Base metal< Weld metal <HAZ since the 3rd day of exposure. As the exposure time reached about 8 days, HAZ showed more area of adhesion than weld metal. However, base metal showed the minimum adhered cells throughout the experiment (Fig. 1,2). Right from the initial stages, polished HAZ coupons were showing more area of adhesion compared to weld metal and base metal. It was very well evident that base metal was preferred less by *Bacillus* sp. compared to weld metal and HAZ even after polishing to the same surface roughness (Fig. 1).

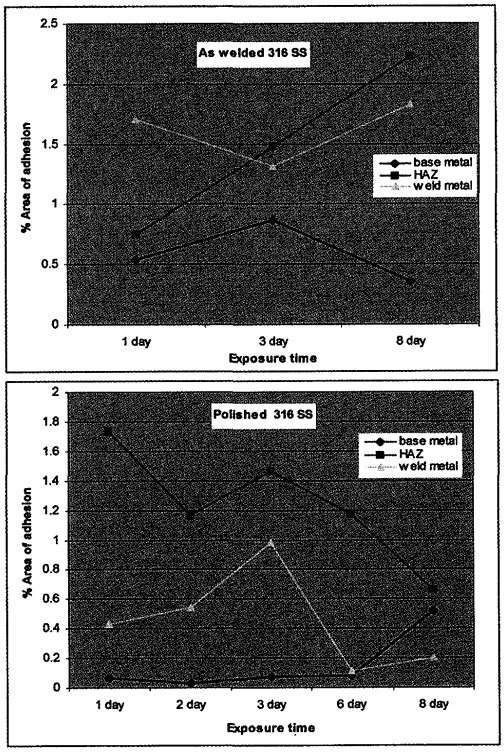

Fig. 1 Variation in % area of adhesion of B*acillus* sp. on 316 L stainless steel base metal, HAZ and weld metal as a function of exposure time

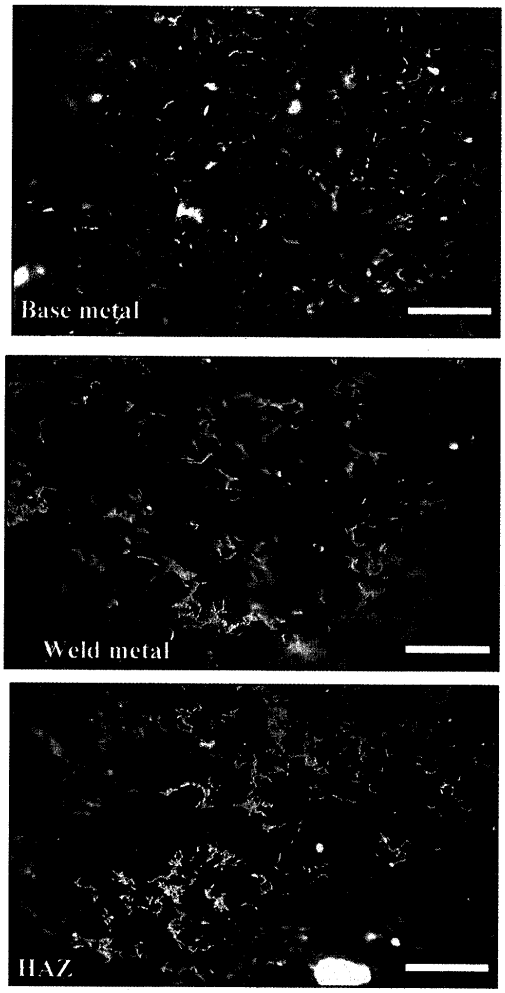

Fig. 2 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to as welded 316 L stainless steel coupons (marker= 10 micron)

The low percentage of adhesion in the case of as welded base metal coupons compared to HAZ and weld metal could be due to the differential surface roughness. But, in the present study, the three different areas of the weld viz. weld metal, HAZ and base metal after polishing to the same grit level also showed significant difference in the bacterial adhesion area. In general, the extent of area of bacterial adhesion was more in as welded coupons compared to polished ones. This, in spite of the possible underestimation due to the uneven surface during imaging is evident in the case of 316 L SS. The preferential attachment of bacteria in the HAZ of 316L SS could be due to the following reasons: a) The low segregation of elements in HAZ, for example, chances of molybdenum segregation are more in weld metal (Kearns and Borenstein, 1991)[17]. It is reported that molybdenum in trace amount is essential and preferred by bacteria, however, high concentration is toxic (Beveridge et al.1997)[25]. Hence, it could be assumed that the concentration of molybdenum in the HAZ region might be more suitable for the attachment of bacterial species towards it. b) Another reason which could be cited as a possibility for preference of the HAZ by *Bacillus* sp. is the tendency of bacterial cells to adhere to the grain boundaries. There are reports that bacteria show preferential attachment over grain boundaries (Muller et al. 1992)[1]. However, this is not experimentally proven in the present study. c) Microcolony formation also accounts for the increase in area of adhesion on HAZ. It was evident from the micrographs that there were local aggregations of bacterial cells, which could be seen in the form of filamentous microcolonies. This type of filament formation is common in case of *Bacillus* sp.

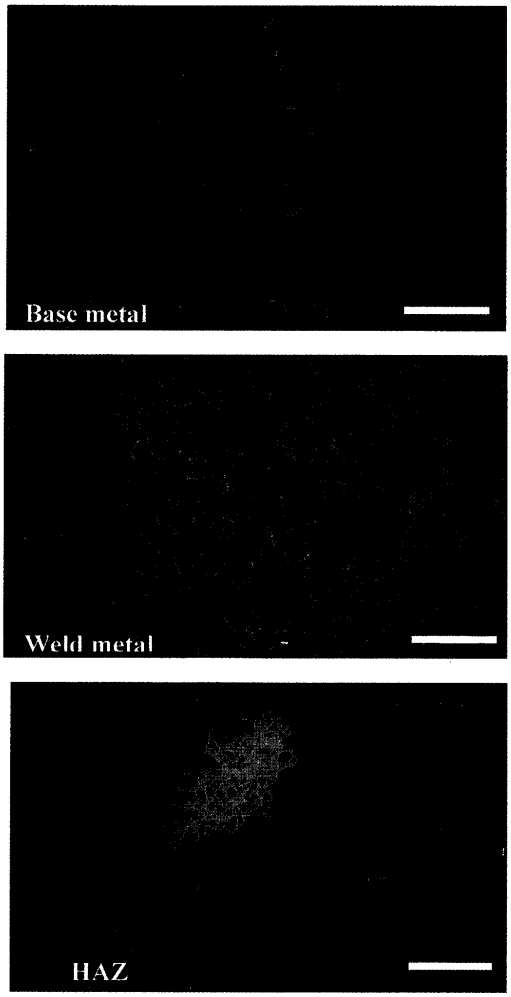

Fig. 3 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to polished 316 L SS coupons (marker= 10 micron)

This might be due to the inhibition of septa formation or cell division under certain extreme conditions. It is reported that in bacteria, inhibition of cell division but not growth may lead to the formation of filamentous cells (Hughes and Poole, 1989)[26]. Yet another report mentions about *Escherichia coli* forming filaments upto 300 times the length of a normal cell in certain conditions of metal toxicity (Rosenberg, 1965)[27]. The probable reason for the observation in the present study could be the presence of chemical species inhibiting septa formation or cell division in the HAZ region, since this phenomenon is not seen in any other coupons tested. In addition, it was clear from the micrographs that the surrounding portions of the microcolonies were devoid of or represented by scattered cells. This could be attributed to a nutrient depletion as a result of over utilisation of available macromolecules by the microcolony. From the point of view of corrosion also, this phenomenon is important, as there are more chances of formation of differential aeration zones and pitting.

In 304L stainless steel, as welded coupons showed an adhesion pattern in the increasing order from base metal, HAZ and weld metal. This was the same throughout the study period (Fig.4,5). In the case of polished coupons also, the trend was the same. Weld was harbouring more bacteria followed by HAZ and base metal (Fig.4,6). Aggregation of ions, and inclusions, is more in the weld region gradually decreasing to base metal through HAZ and bacterial adhesion followed the same order. In other words, the heterogeneity of the surface played a significant role in adhesion. Welding alters the size, shape, amount, composition and distribution of microstructure constituents in the fusion zone and the heat affected zone (Walsch and Willis, 1995)[28]. During welding of 304L SS, 308L was used as the electrode. This was different from 316L SS and also molybdenum is absent in 304L. Therefore, it could be assumed that the effect of molebdenum toxicity and the microcolony formation were lacking in 304L SS unlike in 316 L SS. Only the heterogeneity of welds and segregation of elements might have played the role and decided the trend in 304L SS.

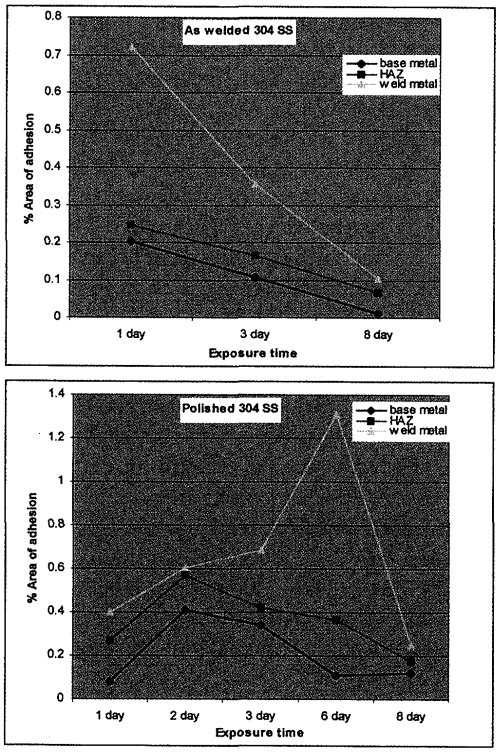

Fig. 4 Variation in % area of adhesion of *Bacillus* sp. on 304 L stainless steel base metal, HAZ and weld metal as a function of exposure time

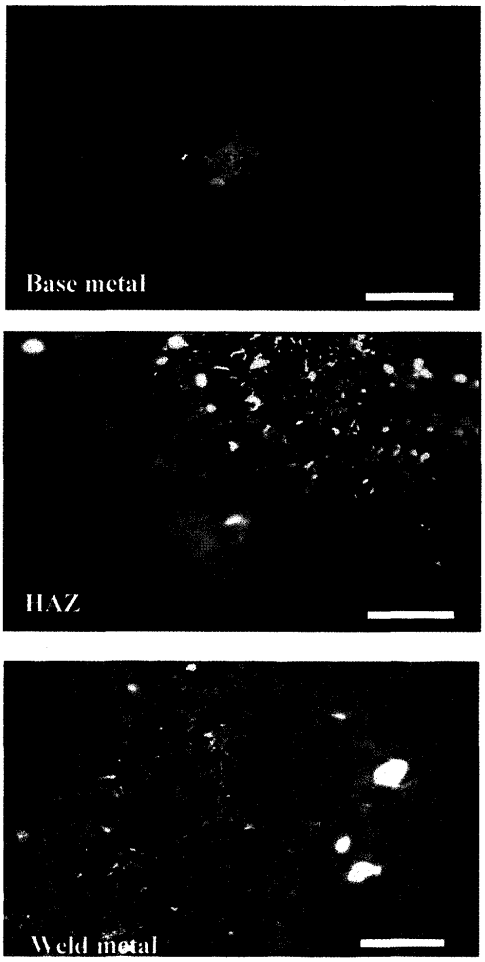

Fig. 5 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to as welded 304 L SS coupons (marker=10micron)

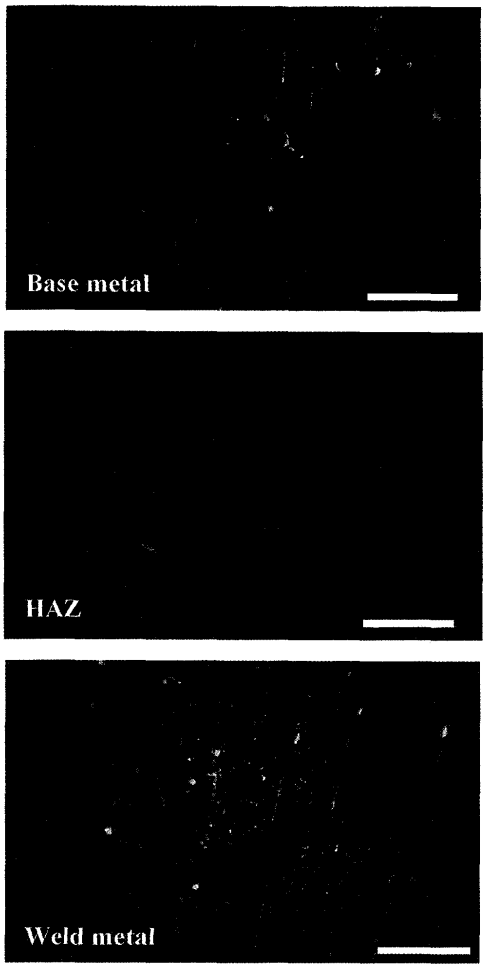

Fig. 6 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to polished 304 L SS coupons (marker=10micron)

There is also another important factor which could be cited as a possibility of more attachment on the weld and HAZ region, compared to base metal, i.e. the differential charge distribution in the regions of weld metal, HAZ and base metal. This can influence formation of conditioning film. Bacteria are generally negatively charged bodies with variable cell surface hydrophobicity and can be regarded as living colloidal particles in relation to their behaviour at surfaces (Marshall and Blainey, 1990)[29]. Therefore, they could be attracted towards the more positive weld or HAZ region compared to base metal. Also, the adhesion of bacteria to surfaces is influenced by the inherent properties of the substratum surface such as the surface charge, surface free energy etc. and the way in which these properties are modified by the molecular adsorption at the surface or the conditioning film. A chemotactic response to any nutrient gradient established near a surface could also affect the attraction of bacteria towards it. The possible presence of conditioning film between the metal surface and bacterial cell surface might not be imparting a significant change in the scenario as the formation of conditioning film could be influenced by the surface characteristics of the substratum.Yet another possibility is the differential wettability. The difference in wettability among the three different regions that in turn depends on the net charge too may play a role in bacterial adhesion. These differences might be in a microscale. But, the microscopic heterogeneity of many materials whether created intentionally or as an artefact, is quite clear on the scale of microbes and is an important factor in MIC (Walsh et. al., 1993)[30] However, these aspects require further laboratory studies.

4 Conclusions

The most significant observation in the present study was the difference in area of adhesion among weld metal, HAZ and base metal regions of the weld. This was true for both the materials tested viz. 316 L and 304 L stainless steels. HAZ or weld metal showed more bacterial adhesion compared to base metal, which showed the lowest in both the cases. The trend remained the same even in the case of uniformly polished surfaces, significantly revealing the influence of microstructure or surface chemistry.

It could also be seen that, there was a difference in percentage area of adhesion between as welded and polished coupons of the same material. This very well accounts for the difference in surface roughness. Generally, as the time of exposure increased, the percentage of adhered cells decreased. This might be because of the death and detachment, since the experiment was conducted for a short span and hence without replacement of medium.

Since in both the materials tested base metal HAZ and weld metal showed difference in area of adhesion in spite of the uniformly polished surface condition, the question of influence of microstructure gets significance. To conclude, the adhesion of *Bacillus* sp. on base metal, HAZ and weld metal of 304L and 316L stainless steels is influenced not only by the surface roughness but by the microstructure or surface chemistry as well. This preferential adhesion contributes very much to corrosion and can be considered as one of the factors causing preferential MIC attack on welds.

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