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Influence of metal microstructure on bacterial adhesion

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Abstract

The effect of metal microstructure on the adhesion of a corrosion-causing bacterial strain of *Bacillus* sp. was studied using weld metal, heat-affected zone and base metal coupons of AISI type 316 L stainless steel. Observations were carried out using epiflourescence microscope, and micrographs were taken using CCD camera. The extent of adhesion was calculated by image-processing techniques. For the material tested, coupons with different microstructure showed differences in the area of adhesion in spite of uniform surface condition. The heat-affected zone showed maximum number of attached bacterial cells with the base metal showing the least. The results suggest that the bacterial adhesion to metals is influenced by microstructure and segregation of elements on the metal surface in addition to surface roughness.

Keywords: Metal microstructure, bacterial adhesion.

1. Introduction

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.[1] Case histories published on MIC usually make references to the appearance of corrosion in welded zones, which usually takes the form of pitting. A majority of cooling system failures in many industries is around or within weldments.[2–6] The microscopic heterogeneity of engineered materials, whether created intentionally or as an artefact, is the basis for their properties. Heterogeneity is evident on the scale of microbes and is an important factor in MIC. Weld regions are particularly suitable to microbes in many of the systems tested since welding alters the characteristics of the material surface.[7] The combination of physical and compositional changes brought about by the welding process facilitates accumulation of organics on the surface and subsequent colonisation by bacteria.[8–9] The preference of weld as a site of colonization by bacteria is correlated to the surface roughness.[10] Existing literature hardly mentions anything about the preferential attachment of bacteria onto areas of different microstructure. It is known that the key to the alteration of conditions at a metal surface before the initiation of MIC is the formation of a biofilm. The very first step towards biofilm formation is the attraction of bacteria towards the metal surface and their attachment. Needless to mention that one of the very important characteristics of weld is its microstructure. Hence, the present study was aimed at finding out the effect of microstructure on the adhesion of a corrosion-causing bacterial strain of *Bacillus* sp. on 316 L stainless steel.

Table I
Welding parameters

Material	Composition	Electrode used	Type of welding	Welding speed	Arc voltage	Welding current	Shielding g
SUS 316L	Fe, Cr18%; Ni 12%, Mo 2%	JIS Y 316L	GMAW (one pass) welding	3mm/s	36V	300A	100%Ar

2. Materials and methods

2.1. *Material used*

AISI-type 316 L stainless steel.

2.2. *Welding details*

Weld metal samples were made by gas metal arc welding (GMAW) process. Various parameters of welding are given in Table I.

2.3. *Preparation of experimental coupons*

Welded samples of the material were machined and weld metal, heat-affected zone (HAZ) and base metal portions were separated. 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 only the surface to be observed is exposed.

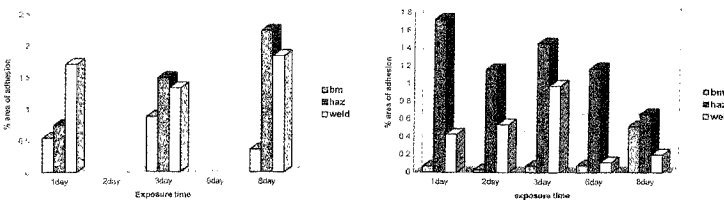
Coupons of two different surface conditions were prepared. One set was as-welded and the other polished to 1500 grit with emery paper to a uniform surface finish.

3. Experimental procedure

Coupon exposure studies were conducted for a period of 8 days. Bacterial strain used for the experiment was an isolate of *Bacillus* sp. from the residual water of an MIC-affected effluent-treatment plant. The medium used was nutrient broth {Difco: (Bacto Peptone: 5g/L; Bacto beef extract: 3g/L)}. The nutrient broth was diluted to 1% (v/v) with microfiltered distilled water before sterilization. The bacteria were cultured in nutrient broth (Difco) and from the log phase culture (18-24 h growth), uniform inoculum was added to each of the experimental flasks. Then the coupons were introduced into the experimental medium aseptically. Experimental flasks were kept in an incubator shaker set at 28°C at 90 rpm. Coupons were retrieved aseptically for observation on the 1st, 2nd, 3rd, 6th and 8th days in addition to the visual observation for changes in surface appearance.

3.1. *Epiflourescence 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 nonflowing sterile distilled water before the surfaces were viewed under



a as-welded b polished

FIG 1 Variation in percentage area of adhesion of *Bacillus* sp. on 316 L stainless steel base metal, HAZ and weld metal as a function of exposure time

epiflourescence microscope. About ten different fields were selected randomly and the images were recorded through a CCD camera. These images were further analysed for bacterial density and area(s) of adhesion using image-processing softwares.

3.2. *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 a desiccator until observation. The surface was prepared by gold palladium coating. SEM images of the selected fields were taken. Microstructure of all the coupons were observed by metallographic microscope after etching with corresponding etchants and pictures were taken.

4. Results and discussion

As-received weld coupons of 316L SS during the initial stages showed bacterial settlement in the following order, i.e. base metal <HAZ <weld metal. As time passed, bacteria colonized more on HAZ than on weld. However, right from the initial stages, polished HAZ coupons showed larger area of bacterial adhesion when compared to weld and base metal (Fig. 1). *Bacillus* sp. preferred to adhere onto HAZ as compared to weld metal and base metal even after polishing to the same surface roughness (Fig. 2). In the case of as-welded HAZ coupons, the area of adhesion is increased as time passed, whereas it was not so in polished coupons. This might be due to the easier sloughing off of adhered cells on polished coupons due to smoother surface. An interesting phenomenon of microcolony formation was seen on polished HAZ coupons (Fig. 2).

In this experiment, base metal, HAZ and weld showed significant differences in the area of adhesion in spite of the uniformly polished surface condition. HAZ harboured more bacterial cells followed by weld metal and base metal. Generally, it could be seen that there is a difference in percentage area of adhesion between as-welded and polished coupons. In addition, as time passed, the percentage of adhered cells decreased, possibly due to depletion of nutrients and sloughing off.

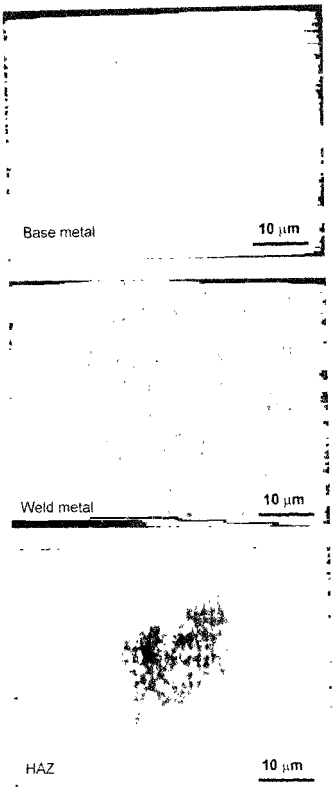


FIG. 2. Variation in adhesion area and pattern of *Bacillus* sp. on polished 316 L stainless steel base metal, HAZ and weld metal.

The low percentage of adhesion on base metal coupons compared to HAZ and weld could be due to the difference in surface roughness. However, in the present study, the three different areas of the weld, viz. weld metal, HAZ and base metal were polished to the same surface finish thereby eliminating the effect of surface roughness. The results still revealed a significant difference in the extent of area to which cells got adhered.

The preferential attachment of bacteria in the HAZ of 316 L SS could be due to the following reasons:

1. Low segregation of elements in HAZ. For example, chances of molybdenum segregation are more in weld metal.[11] It is reported that Mo in trace amount is essential and preferred by bacteria; however, high concentration is toxic.[12] Hence, it may assumed that the concentration of molybdenum in the HAZ region might be more suitable for attracting bacterial species towards it.
2. Another possible reason 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.[13] However, this is not experimentally proven in the present study.
3. Microcolony formation also accounts for increase in the area of adhesion on HAZ. It is evident from the micrographs that there are local aggregations of bacterial cells which can be seen in the form of microcolonies. This type of microcolony formation is common in the case of *Bacillus* sp.[14] This might be due to the formation of filaments as a result of 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.[15] Yet another report indicates about *E. coli* forming filaments up to 300 times the length of a normal cell in certain conditions of metal toxicity.[16] 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 is clear from the micrographs that the surrounding portions of the microcolonies are devoid of or are represented by scattered cells. This can be attributed to nutrient depletion as a result of overutilisation of available macromolecules by the microcolony.

From the point of view of corrosion also, this phenomenon of microcolony formation is important, as there are more chances of formation of differential aeration zones and pitting. An elemental analysis on the spot of microcolony would have given a better picture, which could not be undertaken in the present experiment.

It could be seen that there is a difference in the percentage area of adhesion between as-welded and polished coupons of the same material. This accounts for the influence of surface roughness on bacterial adhesion in addition to microstructural effects. Since base metal, HAZ and weld showed difference in the area of adhesion in spite of the uniformly polished surface condition, the influence of microstructure gets significance. As all other conditions are the same except the microstructure for the three different areas of weld, it may be assumed that the difference in bacterial adhesion density and pattern observed are possibly due to microstructure variation.

5. Conclusion

The adhesion of *Bacillus* sp. on base metal, HAZ and weld metal of type 316 L is influenced not only by surface roughness but also by microstructure as well as segregation of elements as

a result of welding. 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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