ATTACHMENT OF BACTERIA TO CARBON STEEL WELDMENTS†

Kurissery R SREEKUMARI*, Masayoshi OZAWA, Kenji TOHMOTO*** and Yasushi KIKUCHI******

Abstract

Adhesion of Bacillus sp. onto carbon steel weldments has been investigated. Carbon steel coupons were exposed to pure culture of Bacillus sp. in a diluted nutrient medium and initial period of bacterial adhesion was monitored. The area of bacterial adhesion was determined by image processing software. Results revealed differential attachment over different parts of the weld, such as weld metal, HAZ and base metal portions. Initially, ie. till 24 hrs, the adhesion of bacteria showed a pattern of weld metal> HAZ> base metal eventhough the surface roughness was uniform. As time passed, this pattern is lost and HAZ harboured more bacteria than weld metal. Generally, base metal showed the lowest area of adhesion. From the present study, it is inferred that the difference in surface condition influences the bacterial adhesion of carbon steel initially. This preferential adhesion is deleterious and might contribute to preferential MIC attack of carbon steel weldments.

KEY WORDS: (Bacterial adhesion) (carbon steel weldments) (microbiologically influenced corrosion)

1. Introduction

The involvement of microorganisms in the deterioration and destruction of materials can be through biofouling, biodeterioration and biocorrosion or microbiologically influenecd corrosion (Thompson et al, 1994; Borenstein 1994; Gaylarde and Videla, 1995)[1-3]. Microbiologically influenced corrosion (MIC) refers to corrosion that ensue loss of metal by the presence and activities of microbes. MIC can occur in any aqueous environments, because of the omni present nature of microbes in fluid systems coupled with adequate nutrients and corrosive byproducts (Natarajan 1997)[4]. Most of the industrially used metals and alloys including carbon steels are readily attacked by microbes. Among the different mechanisms by which bacteria influence corrosion of metals, biofilm formation on material surfaces is considered very much significant. Most reported cases of MIC involve localized form of attack, implying the reason to be the discontinuous nature of biofilms formed over the surfaces and the associated differential aeration cells. The case histories published on MIC usually make references to the appearance of corrosion in welded zones (Borenstein 1991;Kohler 1991; Hayner et al 1988; Borenstein 1988; Borenstein and Lindsay 1987)[5-9]. Many failures of welded carbon steel products have been attributed to the selective corrosion of the weld. Localized corrosion of carbon steel weldments were reported from various environments, steel types and weld procedures (Stephen and Pistorius, 2000)[10]. Weld regions are particularly attractive to microbes in many of the systems tested because welding alters the material surface characteristics (Walsch et al. 1994)[11].

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)[12-13]. 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. To investigate whether there is a relationship between the microstructure of the substratum and bacterial adhesion which contributes to preferential MIC attack of weldments was the target of this study.

2. Materials and Methods

Carbon steel welds were prepared by the Gas Metal Arc Welding (GMAW) process as per the welding parameters given below:
Electrode used: JIS YCWI; Type of welding: GMAW (two pass); Welding speed: 3mm/s; Arc voltage: 36V; Welding current: 300A; Shielding gas: 100% Ar.
Welded samples were machined and weld metal, HAZ and base metal portions were separated. Machining was done after marking the weld metal, HAZ and base metal portions by etching. The machined metal coupons were molded in resin such that the surface to be observed only is exposed. Two different sets of coupons were tested. One set was as -welded and the other, polished to 1500 grit with emery paper for uniform surface finish.
Exposure studies were conducted with the above-described coupons. The medium of exposure was 1% (v/v) nutrient broth. Medium was taken in a conical flask,

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* Foreign Researcher
** JAPEIC Tarasaki Center
*** Technical Assistant
****Professor

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and sterilized. Coupons for exposure were degreased in acetone and sterilized before immersion. 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)[14]. Coupons were introduced into the medium aseptically. Experimental flasks were kept in an incubator shaker set at 28^{0}C and 90-rpm.

Coupons were retrieved for observation aseptically on the first day, 2nd day, 3rd day, 6th day and 8th day in addition to visual observation for any change in surface appearance.

Coupons taken out from the medium were air dried inside the sterile chamber and were stained with acridine orange fluorescence dye (0.01% w/v), prior to observation. About ten different fields were selected randomly, the images were captured through a CCD camera and were saved for further computation using image processing software. In another set of experiment, similar coupon exposure tests were conducted for 24 hrs with intermittent sampling at 4,8,16 and 24 hrs. This was to confirm the preference of different portions of weld by *Bacillus* sp. before the corrosion products start accumulating on the surface.

3. Results

The area of bacterial adhesion on as welded coupons showed a pattern of weld metal> HAZ>base metal (Fig.1,2).

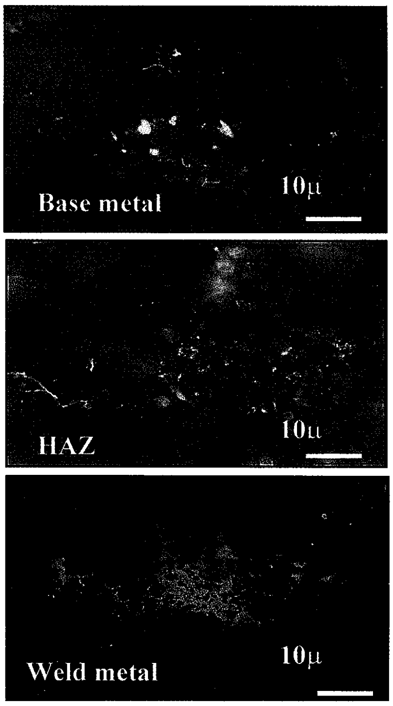

Fig.1 Epiflourescence photomicrographs showing *Bacillus* sp. attached to as -welded carbon steel coupons

Visual observation of the coupons showed shearing off of the corrosion product alongwith the cells in the case of weld and HAZ by 8th day. Corrosion products on base metal were still intact as the initiation was delayed. As a consequence the adhesion area in base metal showed dominance on 8th day. Carbon steel polished coupons started corroding in the initial stage of exposure itself owing to the removal of oxide film during polishing.

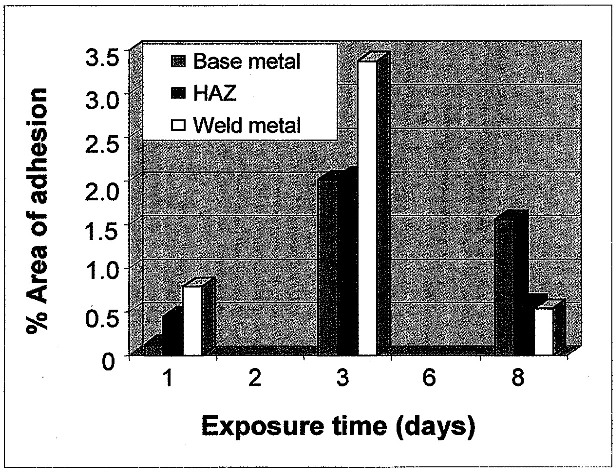

Fig.2 Variation in % area of adhesion of *Bacillus* sp. on as welded carbon steel weld metal, HAZ and base metal coupons as a function of exposure time

Both HAZ and weld metal showed corroded surfaces on the first day itself. However, base metal started corroding only on the third day of exposure. In the case of polished coupons, HAZ was harbouring more bacterial cells throughout the study period. The base metal showed its peak of adhesion on the third day(Fig 3,4).

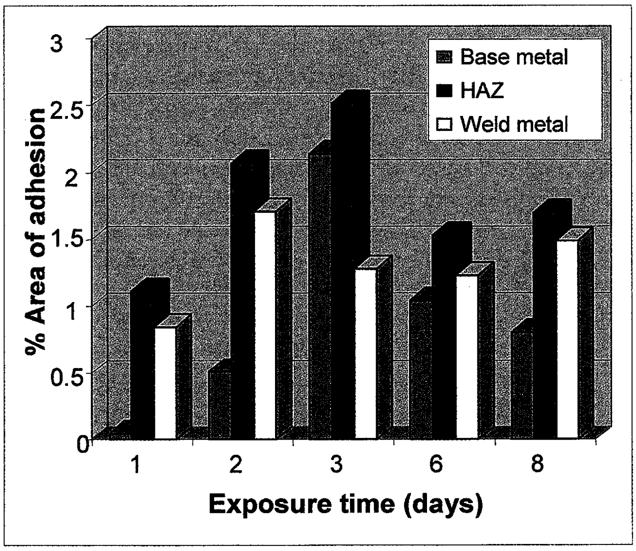

Fig.3 Variation in % area of adhesion of *Bacillus* sp. on polished carbon steel weld metal, HAZ and base metal coupons as a function of exposure time

The one-day experiment carried out with polished carbon steel coupons revealed that by 4hrs itself weld metal

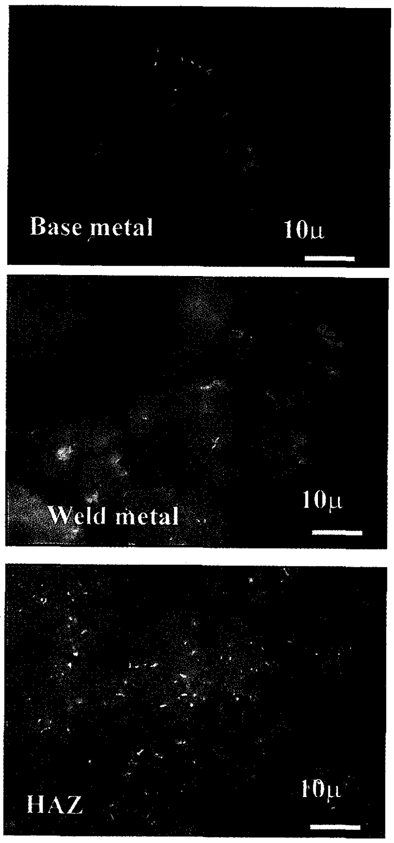

Fig.4 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to different polished coupons

started corroding. HAZ followed the weld metal and started corroding by 16 hrs of exposure (Fig.5,6).

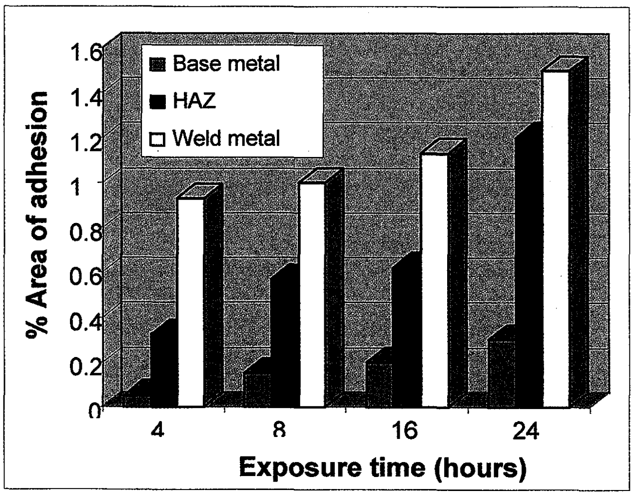

Fig.5 Variation in % area of adhesion of *Bacillus* sp. on polished carbon steel weld metal, HAZ and base metal coupons as a function of expoure time

Base metal coupons were retaining their shining surfaces further. Initially, i.e till 24 hours, weld metal samples were showing more area of bacterial adhesion might be due to the surface roughness caused by the accumulated corrosion products. But, once all the coupons started corroding and accumulating corrosion products, HAZ was predominant in bacterial adhesion showing a preference of *Bacillus* sp.to the corrosion products accumulated on HAZ.

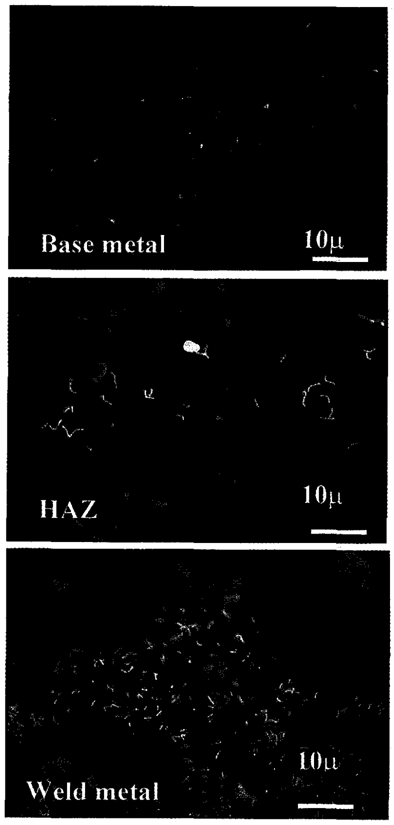

Fig. 6 Epiflourescence photomicrographs showing *Bacillus* sp. adhered to different coupons after 8 hrs. exposure

4. Discussion

When a fresh material surface comes in contact with a non-sterile aqueous medium, conditioning film start forming. The conditioning film in turn plays a decisive role in the bacterial attachment to the surfaces. It is well known that bacteria prefer a sessile mode of life than planktonic. Once a nutrient rich surface is available, they get attracted towards it through chemoreceptive mechanisms, resulting in adhesion (Geesey 1991)[15]. Various factors could influence the formation of conditioning film and thereby bacterial adhesion such as elemental seggregation or inclusions; differential charge distribution and/or differential wettability on the substratum surface.

In the case of present experimental coupons, the microstructure as well as the elemental segregation differ among the three regions. Formation of oxides will be more in the weld region, reducing gradually as moved away from weld metal to base metal through HAZ. Thus, conditioning film formation could be influenced by this factor thereby resulting in a different structure and composition of conditioning film in the weld metal, HAZ and base metal regions. This in turn influences bacterial adhesion. It is rather difficult to infer the influence of any individual factor such as microstructure or inclusions in long term exposure studies as the surface condition changes once the coupons start corroding. Sreekumari et al (2000)[16] reported that base metal, HAZ and weld metal of 304 L and 316 L stainless steels showed a difference in area of bacterial adhesion in spite of their uniformly polished surface condition, thereby showing the influence of substratum microstructure on bacterial adhesion. In the present study also, polished HAZ and weld metal showed more bacterial adhesion than polished base metal, thereby showing the influence of microstructure on adhesion of *Bacillus* sp. It has been suggested that certain microstructures formed during weld thermal cycle, specifically bainite, are less corrosion resistant than others (Kowaka, 1990)[17]. It has also been proposed that a galvanic couple develop between the different microstructures in the weldment, promoting selective corrosion of certain parts. However, regarding MIC in carbon steel, there are no reports as such indicating the influence of microstructure. The difference in adhesion of bacteria among the different microstructures as shown by this study, would certainly be influencing the electrochemical nature of the surface thereby leading to accelerated corrosion by contributing to the otherwise possible corrosion effect.

5. Conclusions

The test species, *Bacillus* sp. showed preferential adhesion over different parts of carbon steel weldments viz. weld metal, HAZ and base metal. Base metal portion was the least preferred substratum compared to weld metal and HAZ. The heterogeneity in the weld region due to oxide formation and differential distribution of elements alongwith their segregation might be the reason for the increased bacterial attachment at these particular regions. In addition to these effects, the nature and composition of the corrosion products also might have influenced bacterial attachment once the coupons started corroding. This differential attachment of bacteria could be deleterious from the point of view of corrosion also and could be viewed as one of the reasons contributing to the preferential MIC attack on welds.

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