Corrosion Science 93 (2015) 48–57

Contents lists available at ScienceDirect

Corrosion Science

journal homepage: www.elsevier.com/locate/corsci

Corrosion of carbon steel by sulphate reducing bacteria: Initial attachment and the role of ferrous ions

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ARTICLE INFO

Article history:
Received 1 November 2014
Accepted 2 January 2015
Available online 7 January 2015

Keywords:
A. Carbon steel
B. Weight loss
B. SEM
C. Microbiological corrosion
C. Pitting corrosion

ABSTRACT

A study of the effect of ferrous ions on the initial attachment of sulphate reducing bacteria (SRB) and subsequent corrosion of carbon steel has been performed. It was found that the initial bacterial attachment process is complex, depending upon time period investigated. Early stages (⩽60 min) of attachment showed no dependence on the iron level in the media. After 60 min, more attachment and exopolymer production was observed in iron rich medium compared to iron deficient medium. The results suggest that monitoring of ferrous ion levels might be helpful in detection of MIC attack by SRB.

1. Introduction

Bacterial attachment and subsequent biofilm formation on material surfaces can influence the deterioration of those surfaces by changing the physical or chemical properties of the interface [1,2]. This change in the corrosion behaviour of materials in the presence of microorganisms is a process known as microbiologically influenced corrosion (MIC) [3]. MIC can cause significant economic losses and affects various industries including maritime, oil and gas, power generation, water distribution and nuclear waste [4,5].

While they are not the only microorganisms responsible for MIC, anaerobic sulphate reducing bacteria (SRB) are by far one of the most widely reported and studied bacteria in relation to MIC [2,6]. SRB are frequently found in natural environments such as in coastal clay soils, polluted marine sites [7], and in off-shore oil extraction equipment [2]. They have been shown to influence the corrosion of metallic materials, especially ferrous alloys, when present in the biofilm formed on these surfaces and are usually associated with pitting corrosion attack [8,9]. The effects of pitting with MIC can be more severe than normal uniform corrosion as the rapid attack can lead to penetration of a material with the potential for structural failure [9,10]. Several mechanisms have been proposed to explain the role of SRB in MIC of metallic materials [11–14], and this is an ongoing active area of research. Some of the unique features of corrosion caused by SRB are that (i) it occurs at neutral pH in anaerobic environment, (ii) oxygen is usually not involved, and (iii) the corrosion products include iron sulphides [2,15].

To prevent the corrosion of metallic materials caused by SRB biofilms, it is necessary to understand the fundamental processes involved in the biofilm development including the initial bacterial attachment. A range of factors have been reported which can generally affect the process of initial bacterial attachment and subsequent biofilm formation, including physico-chemical properties of microorganisms [16,17], microstructure and surface features of substratum [15,18] and environmental conditions [19,20]. Beech and Gaylarde [21] found that extracellular polymeric substance (EPS) plays an important role in the initial attachment of *Pseudomonas fluorescens* and *Desulfovibrio desulfuricans* to mild steel surfaces. EPS has also been shown to bind metal ions selectively [22]. It has been reported that lipopolysaccharides (LPS) in the outer membrane of gram-negative cells like SRB genus *Desulfovibrio*, interact specifically with ferrous [Fe (II)] ions [23]. It was suggested that such interactions could be involved in both initial SRB attachment and subsequent corrosion of ferrous metals in the aqueous environment [24]. Gaylarde and Johnston [25] showed that preventing the attachment of SRB to a metal surface decreased the

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http://dx.doi.org/10.1016/j.corsci.2015.01.006
0010-938X/

rate of corrosion of that metal. Beech et al. [15] studied the initial (1 and 3 h) attachment and subsequent corrosion of mild steel in the presence of two different species of SRB. While they found differences between SRB species in their aggressiveness towards mild steel under identical growth conditions, no correlation was observed between the initial number of SRB cells attached and the subsequent corrosion of mild steel coupons. Similarly, Noor et al. [26] found that the corrosion rate of a carbon steel measured at two different temperatures had no correlation with the planktonic or sessile SRB cell numbers. These divergent observations mean that it is difficult to draw any direct conclusions and suggests that more research is needed to help clarify the role of initial bacterial attachment in MIC of carbon steel.

The concentration of ferrous ions in the medium has been found to be a significant parameter in the corrosion of mild steel mediated by SRB. A large amount of work in this area was undertaken during the 1950s to 1970s looking at the effect of ferrous ions on SRB corrosion and on the effect and nature of iron sulphide (FeS) films on MIC [27–30]. Reports by King and co-workers [29,30] for example showed that the rate of corrosion of mild steel and iron was proportional to the concentration of ferrous ions in the test media. It was shown that the amount of ferrous ions present changed the type of FeS film that formed and increased levels of ferrous ions caused a breakdown of the FeS film which led to higher corrosion rates. A number of mechanisms have been suggested for the role of ferrous ions on corrosion caused by SRB including Fe/FeS galvanic coupling, cathodic depolarisation, hydrogenase regulation, and also changes to the physical nature of the FeS film formed [31–33]. The influence of ferrous ions on SRB corrosion appears to be a complex phenomenon and further studies on the exact mechanism and contribution of ferrous ions and/or FeS film on SRB influenced corrosion are needed to better understand their role.

The majority of reports on the influence of ferrous ions in SRB corrosion have focused on bacterial attachment on time scales of days to weeks corresponding to more mature biofilm formation [34–36]. However, the initial (on the hour to sub-hour scale) SRB attachment, a key step in the subsequent biofilm formation, has not been studied. Our investigation aimed to study the initial attachment of the SRB species *D. desulfuricans*, to 1010 carbon steel and to determine if there was any relationship between the initial SRB attachment and longer term (28 days) corrosion of carbon steel and the role of ferrous ions in these processes. The influence of metal microstructure, specifically grain boundaries, on the initial SRB attachment was also studied.

2. Experimental procedure

2.1. Metal sample preparation

Carbon steel (1010) coupons, with the chemical composition of 99.38% Fe, 0.10% C, 0.44% Mn, 0.02% Si, 0.01% S, 0.02% P and 0.03% Al, were cut with dimensions of 25 mm × 25 mm × 3 mm from a plate sample using an automatic abrasive wheel cutting machine with water cooling (Struers Secotom-50, Australia). The coupons were polished using an automatic grinding and polishing machine (Struers Tegramin-25, Australia). First the coupons were ground sequentially up to 1200 grit silicon carbide paper to obtain a smooth surface finish. Finally, the coupons were polished through a sequence of fine polishing with 9 and 3 μm diamond suspensions to a final 0.04 μm finish using silica suspension (Struers OPU, Australia). The polished coupons were ultrasonically cleaned with acetone for 10–15 min, rinsed with distilled water followed by ethanol, and then dried under warm air. Prior to testing, coupons were ultrasonically cleaned with acetone followed by sterilisation via immersion in absolute ethanol (100%) and then aseptically dried within a level 2 physical containment (PC2) cabinet.

The surface roughness of a material has been reported to affect the attachment of bacterial cells [37]. The surface roughness of the polished coupons was therefore characterised using a 3D optical profilometer (Contour GTK1, Bruker, Germany), as described in detail elsewhere [18,38]. The average surface roughness (Sa) values were consistent across the polished coupons and measured to be within the nanometer range, i.e. $\leqslant 2.6 \pm 0.4$ nm.

2.2. Preparation of test media and bacterial culture

Bacterial attachment and subsequent corrosion tests were conducted in ATCC 1249 Modified Baar's (MB) medium in the presence (Fe + MB) and absence (Fe–MB) of ferrous ions. To prepare the Fe + MB medium, which is the medium recommended by ATTC for the strain of SRB used in this study, the following components: 4.1 g $MgSO_4 \cdot 7H_2O$, 1.0 g NH_4Cl, 1.26 g $CaSO_4 \cdot 2H_2O$, 0.5 g K_2HPO_4, 5.0 g tri-sodium citrate, 5.67 g sodium lactate, and 1.0 g yeast extract were added for each litre of MilliQ water. The pH was adjusted to 7.5 ± 0.1 using a 3.5 M KOH solution, and sterilised by autoclaving at 121 °C and 103 kPa for 20 min. After cooling, 0.1 ml of 5% $(NH_4)_2Fe(SO_4)_2$ solution, sterilised by filtration using syringe filters (0.02 μm in pore size), was added to 5.0 ml of the previously autoclaved medium. This is equivalent to 195 mg/L of iron ions concentration in the medium. The Fe–MB medium has exactly same composition as the Fe + MB except that $(NH_4)_2Fe(SO_4)_2$ was not added.

The sulphate-reducing bacterium (SRB) used in this study, *Desulfovibrio desulfuricans* (*D. desulfuricans*) ATCC 27774, was obtained from the American Type Culture Collection (ATCC), USA. For each experiment, bacteria were taken from uniform stock stored in 15% glycerol at −80 °C and grown in 40 mL of sterile Fe + MB medium in a 50 mL Eppendorf tube under anaerobic conditions. The anaerobic conditions were maintained using Compact W-Zip Seal Pouches (AG0060C, Oxoid) with AnaeroGen Sachets (AN0025A, Oxoid) and were verified using the anaerobic redox indicator resazurin (BR0055B, Oxoid). Cultures were incubated at 37 °C on a rotary shaker at 110 rpm for four days. For the inoculation medium, a 1 mL aliquot of the four day old *D. desulfuricans* was introduced into 500 mL of fresh sterile Fe + MB medium and incubated for three days at 21 °C within a level 2 physical containment (PC2) cabinet under anaerobic conditions. The number of bacterial cells was determined using a haemocytometer under a light microscope at 400× magnification. The results showed that after three days, the number of bacterial cells in the medium was equivalent to $\sim 2.14 \times 10^7$ per mL. The bacterial culture was then centrifuged for 15 min at 2000 RCF (Centrifuge 5804R, Eppendorf, Germany) to collect a bacterial pellet. After centrifuging, the supernatant was discarded and the bacteria were resuspended in an equivalent volume of fresh sterile Fe + MB and Fe–MB respectively.

2.3. Initial bacterial attachment studies

For initial bacterial attachment studies, the prepared metallic coupons were aseptically introduced into individual sterile polypropylene containers (LS26-60L, ProSciTech, Australia) containing 45 mL of the test medium of interest (Fe + MB and Fe–MB) inoculated with *D. desulfuricans*. Control experiments were performed by immersing metallic coupons in abiotic test media. Triplicate steel coupons were tested for each exposure time, after which the tests were repeated to give $n = 6$ for each set of conditions. During tests, the coupons were fully immersed in the medium with the polished side facing upward. Coupons were removed from the medium after 15, 30, 60, 240, 480 and 720 min to observe the early stages of bacterial attachment. All experiments were carried out in