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Is metabolic activity by biofilms with sulfate-reducing bacterial consortia essential for long-term propagation of pitting corrosion of stainless steel?

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Concentric electrodes have been fabricated from 304 stainless steel in which a small anode (0.031 cm^2) and large cathode (4.87 cm^2) are induced by the application of a current density of 11 $\mu A\ cm^{-2}$ at the anode. It has previously been shown that reproducible pitting and maintenance of a galvanic current occurs only in the presence of a consortium of bacteria containing sulphate-reducing bacteria (SRB). Actively corroding systems that had maintained a galvanic current for at least 24 h after the applied current was removed were treated with inhibitors of the SRB. The only inhibitor tested which had any marked effect on the galvanic current was sodium molybdate which is a known inhibitor of corrosion as well as SRB.

Keywords: sulphate-reducing bacteria; microbial corrosion; inhibitors; metabolism; pitting; 304 stainless steel

Introduction

The involvement of microbial biofilms in the corrosion of metals is now readily accepted with many papers detailing the actions of microorganisms on metals including copper [1], aluminum [4], mild steel and stainless steel [13]. The involvement of the sulphate-reducing bacteria (SRB) in the pitting of metals has been the primary focus of research interest. Most of the papers on microbially influenced corrosion (MIC) of steels have examined the effect of the SRB on mild steel. Until recently there has been very little published, showing that SRB pit stainless steel in the laboratory. The involvement of the SRB in pitting of stainless steels in natural environments is, however, well detailed. Despite the wealth of papers published on SRB corrosion of mild steel alone there is still no clear agreement on the mechanisms involved. Hamilton [11] suggested that MIC is probably not the result of a single organism acting via a single mechanism, but rather a consortium acting by several mechanisms. Recently this has been shown in the case of SRB corrosion of 304 stainless steel [3] where induced pitting of the anode of a concentric electrode was maintained only in the presence of a mixed consortium of the SRB *Desulfovibrio vulgaris* and a *Vibrio* sp. A galvanic current could not be maintained in the presence of either of the bacteria when grown in a pure axenic culture. The galvanic current was not a transient effect as it was reproducible and could be maintained for over 200 h.

Angell *et al* [3] also showed that SRB appeared to have an effect on the cathode by creating a high charge transfer resistance (Rct), greater than 100 $K\Omega\ cm^2$. This was the case in both the mixed culture and the pure axenic cultures. However, in the presence of the mixed culture of the SRB and the *Vibrio* sp, a low Rct value less than 1 $K\Omega\ cm^2$ was seen only on the anode. This may be in keeping with the suggestion by Crolet *et al* [7] that the action of SRB in pitting of mild steel is dependent on their metabolism and the production of acids. In the work of Angell *et al* [3] it was thought that there needed to be interplay between the two species acting as a consortium on the anode, whereas the SRB alone was responsible for action seen at the cathode.

Remedial action employed in cases of SRB-induced corrosion in systems with evidence of attack normally involves the double action of applying a biocide and also a corrosion inhibitor. It has been shown that compounds such as molybdate have a double effect, inhibiting both the SRB and the corrosion [15]. However, little work has been done on the effect of a biocide alone on established corrosion. Luo *et al* [14] showed that treatment with glutaraldehyde of a consortium containing SRB initiating MIC on mild steel in the short term resulted in an increase in the corrosion rate. However, this effect was short lived and eventually the corrosion rate dropped to that seen in the sterile control that was unaffected by the treatment. It was proposed that glutaraldehyde increased membrane permeability that allowed acids present within the cell to leak out and temporarily cause an increase in the rate of corrosion.

There have been few reports on the use of the conventional SRB inhibitors in corrosion studies. Angell *et al* [2] showed that sodium azide added to a concentric electrode in which the current had been maintained for 48 h had no effect on the corrosion. Postgate [17] cited the work of Sorkin who observed that sodium azide at 0.1–1 mM ml^{-1} inhibited the growth of *Desulfovibrio* while actually stimulating the rate of sulphate reduction in hydrogen. This is unusual as sodium azide is thought to act on the ultimate electron transfer from cytochrome *a* to oxygen. In sulphate-reducing bacteria the terminal electron acceptor is normally sulphate or occasionally nitrite, nitrate or iron. White and

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Smith [18] showed that 18 mM 2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide inhibited respiration in *Hemophilus parainfluenza* by blocking electron transport between cytochrome *b* and c_1. Unpublished data from this group showed that even in the presence of 180 mM 2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide in a lactate/acetate SRB medium *Desulfovibrio vulgaris* was able to produce iron sulphide, however no measure of growth was made.

Both carbon monoxide and copper (II) chloride inhibit the periplasmic hydrogenase enzyme in *Desulfovibrio vulgaris* [10]. Conversely, in *Desulfovibrio desulfuricans*, which has a cytoplasmic hydrogenase, only carbon monoxide was effective in inhibiting the hydrogenase; copper (II) chloride had no effect [9].

This paper deals with the effects of inhibitors on actively-pitting communities containing the sulphate-reducing bacteria using the concentric electrode system described above.

Materials and methods

The bacteria were a culture of *Desulfovibrio vulgaris*, and an unidentified *Vibrio* sp, which is a facultative anaerobe. Also, *Vibrio natriegens* has been shown by some reports to be involved in the MIC of metals [8]. The SRB tolerates low levels of oxygen. No quantification was carried out on the level of oxygen tolerance, but the SRB were not killed by exposure to air during the plating procedure described below. It also grew in previously degassed MPN tubes opened and left open to the atmosphere in a laminar flow hood. The growth medium used in the reactors was a modified artificial seawater containing (g L^{-1}): sodium chloride, 23.00; magnesium chloride, 4.88; sodium sulphate, 3.83; calcium chloride, 0.925; potassium chloride, 0.65; potassium bromide, 0.09; boric acid, 0.024; strontium chloride, 0.023; sodium fluoride, 0.0028; ammonium chloride, 0.094; yeast extract, 0.0018; sodium lactate, 0.075; ascorbic acid, 0.0019; potassium orthophosphate, 0.0468; sodium carbonate, 0.189.

Concentric electrodes were machined from 304 stainless steel rod, providing a small anode with a large surrounding cathode. The anode had a surface area of 0.031 cm^2 and the cathode had a surface area of 4.87 cm^2. The anode was electrically isolated from the cathode by a PTFE^R spacer. Care was taken in the machining of each component of the concentric electrode to ensure a snug fit. Electrical wire was soldered to the back of each electrode before the whole assembly was mounted in a cold cure epoxy. The electrical wire was passed through a 1.5-cm length of 16-gauge silicone tubing. Half the silicone tubing was sealed into the epoxy, the other half was used to secure a piece of glass tubing to allow positioning of the electrode within the reaction vessel. The electrical wires were passed through the center of the tubing to the outside of the vessel. A 600-grit abrasive paper was used to polish the mounted concentric electrode. The polished electrode was then degreased by sonicating it in acetone before it was air-dried.

The reaction vessel comprised a 600-ml glass vessel with a 316 stainless steel top. Five ports were provided in the top and were sealed with compression fittings, allowing for: medium addition and removal, gas sparging and venting, a circulating loop to aid mixing in the vessel and ports for a titanium counter electrode and calomel reference electrode. The reference electrode was electrolytically connected to the vessel via a Lugin's capillary. The medium feed contained a drip tube to prevent bacterial growth back to the medium reservoir and cross contamination of the vessels.

Bacterial inoculations were carried out at 0, 24 and 48 h. For the initial part of the experiment the system was run as a batch reactor to enhance bacterial attachment to the concentric electrode. The reactor vessel was sparged with a 95% nitrogen/5% hydrogen gas mixture to ensure anaerobic conditions. During this period mixing was carried out using a magnetic stirrer operating at approximately 100 rpm. Following the batch phase, flow was initiated at 1 ml min^{-1} giving a dilution rate of 0.1 h^{-1}. As electrochemical measurements were being made on the flowing system the magnetic stirrers were replaced by a circulation loop in which medium was pumped from the bottom of the vessel to the top.

To initiate the pitting for the first 72 h, while the bacteria were cultured under static conditions, a galvanic current was applied to the concentric electrode. The current was applied via a galvanostat so that an anodic current density of 12 μA cm^{-2} was maintained. At the end of this period the current was removed and the resultant current flowing between the anode and cathode was monitored. After a galvanic current had been maintained in the mixed culture system for between 24 and 48 h, inhibitors were added to the culture vessel and medium reservoir. Previous work with the system [3] showed that stable galvanic currents maintained for the first 24 h were not lost (longest monitoring period 200 h). In light of this fact the only controls deemed necessary for this work were sterile controls which would show abiotic effects on the corrosion current by the inhibitors. Before addition of an inhibitor, electrochemical measurements were made to calculate the polarization resistance (Rp) of each of the anodes and cathodes using dc polarization. The Rp value was then measured again at several time points after addition of the inhibitor. The Rp value is the inverse of the corrosion current (I corr), a measure of the rate of corrosion. Table 1 shows the inhibitors used, their mode of action and concentration.

Each experimental run consisted of three vessels, two of which were inoculated with the bacteria and showed the maintenance of the galvanic current, the other vessel was maintained as a sterile control.

At the end of the experiment the concentric electrodes were removed from the vessels and bacterial viability was assessed for each of the anodes and cathodes. Cells from the anode were recovered by extensive swabbing of the surface with cotton swabs. These swabs were then placed in 10 ml of the lactate/acetate medium described below and cells were resuspended by vortex mixing for 1 min. Cells from the anode were then recovered by scraping the metal surface with a sterile razor blade and resuspending the cells in 10 ml of the same medium as for the cathodes. Viability was assessed by both MPN tubes using a lactate/acetate medium containing (g L^{-1}): sodium acetate, 2.8; sodium lactate (60% syrup), 3.5; yeast extract, 1.0; ascorbic acid, 0.1; magnesium sulphate pentahydrate, 0.5; sodium sulphate, 0.5; dipotassium orthophosphate, 0.5; ammonium

Table 1 Mode of action and concentration of inhibitors used

Inhibitor	Concentration ($g\ L^{-1}$)	Mode of action (suspected)
Glutaraldehyde	0.500	Total kill of all microorganisms
Sodium molybdate	0.100	Inhibition of growth of SRB
Copper (II) chloride	0.033	Inhibition of periplasmic hydrogenase of SRB
Carbon monoxide	Saturated	Inhibition of periplasmic and cytoplasmic hydrogenase of SRB

chloride, 0.5; iron (II) sulphide, 0.1; sodium chloride, 7.0; sodium thioglycolate, 0.1. This same medium was used for routine culturing of the SRB; *Vibrio natriegens* was maintained on Marine agar 2216 (Difco, Detroit, MI, USA). Plate counts were also made on Iverson's medium [12] incubated anaerobically, allowing the counting of both black SRB colonies and white colonies of the *Vibrio natriegens*.

Results and discussion

Sterile controls run with each inhibitor showed that no galvanic current was maintained in the absence of the mixed community of bacteria. All the Rp values were between 10 and 100 $K\Omega\ cm^2$ in line with values reported previously for non-pitting concentric electrodes [3]. After addition of the inhibitors there was no deviation from these values outside of this above range.

The resultant current density plots for the various treatments are shown in Table 2, which correlates treatment with galvanic current before and after treatment and shows whether viable SRB were recovered from the electrodes at the end of the experiment. Numbers for *Vibrio natriegens* were similar to those reported for the SRB.

Glutaraldehyde and copper (II) chloride resulted in the death of the sulphate-reducing bacteria, whereas the other two treatments, carbon monoxide and sodium molybdate seemed only to result in a reduction of numbers in the biofilm, compared to previous studies [3], which these studies replicated in every way except for the addition of the inhibitors, where 10^4–10^7 CFU cm^{-2} SRB were isolated on both the anodes and cathodes. Glutaraldehyde was chosen due to its bactericidal effect. Copper was present as copper (II) ions that are bactericidal [5]. In the original work which cited copper (II) chloride as an inhibitor of the periplasmic hydrogenase of *Desulfovibrio vulgaris*, the action was determined by assaying hydrogenase activity and took no account of possible cell death. The action of molybdate could also be questionable as in this experiment it was used against a biofilm, not planktonic cells, and the greater resistance of biofilm cells to bactericidal compounds is now well documented [6].

The current densities summarised in Table 2 show that the only treatment that effected current density of an actively-corroding cell was sodium molybdate. Sodium molybdate is a group VI oxyanion and is routinely used in the petrochemical industry as a corrosion inhibitor. Here it is thought that its action in stopping corrosion was due to its passivating effect on the electrochemical cell and not to its effect on the bacteria, although it did have an effect on the number of viable bacteria isolated from the electrode.

The polarisation resistance (Rp) measurements performed before and after treatment showed that the only marked variation in the values before and after treatment were seen with sodium molybdate. This treatment was capable of inhibiting not only the bacteria but also the corrosion. Before the addition of the molybdate the Rp value for the anode was 6.61 $K\Omega\ cm^2$ and this rose to 10.41 $K\Omega\ cm^2$ after the treatment. The Rp value for the cathode was constant around 125 $K\Omega\ cm^2$ before and after treatment. This suggests that the inhibitor acted against the anodic reaction which is generally considered to be the oxidation of metallic iron to iron (II). Molybdate is considered to inhibit corrosion by forming iron (II/III) molybdate on the metal surface at a passive potential [16]. The results seen in the increase in the Rp value for the anode could be accounted for by formation of iron molybdate complexes passivating the anode and therefore increasing its Rp value.

The lack of any action on the corroding systems by the inhibitors of the SRB, other than molybdate, suggests that after the 24 to 48 h, in which the corrosion was established, bacterial activity was no longer necessary for maintenance

Table 2 Galvanic currents ($\mu A\ cm^{-2}$) before and after inhibitor treatment along with presence of viable cells (CFU cm^{-2}) at the end of the experiment on the electrodes (resolution 0.03 $\mu A\ cm^{-2}$)

Inhibitor	Galvanic current—before	Galvanic current—after	Viable SRB
Glutaraldehyde	2	2	none
Sodium molybdate	1	0	100
Copper (II) chloride	2	2	none
Carbon monoxide	1	1	10
Untreated control[a]	3	3	1.7×10^7

[a]Data from [3]

of the galvanic current. It appears that neither inhibition of the hydrogenase by carbon monoxide nor cell death by glutaraldehyde was incapable of destroying the current. It is therefore suggested that the bacteria are active in establishing or 'fixing' the electrochemical cell that results in formation of a pit, either by acting on the cathode or anode or a combination of the two, allowing the cell to become established. However, once that cell is established, it becomes self-regulating and capable of continued existence even after the bacteria have been killed or inhibited. Earlier work [3] showed that the bacterial consortium is necessary for establishment of the maintained galvanic current, but the bacterial community is necessary only in the early stages of pit formation.

Further work on the system is currently being undertaken to look at the effect of inhibitors added at the early stages of the process, when the applied current is removed, to detect the point at which bacterial action is necessary. Work is also planned to decide the mechanisms by which the members of the consortium are active.

Conclusions

(1) Further evidence has been provided for the reproducible nature of the system in that a galvanic current is maintained in the presence of a mixed community of bacteria containing a SRB and *Vibrio natriegens*.
(2) Viable bacteria or bacterial metabolism is not necessary for the maintenance of the galvanic current after it has been maintained for 24 to 48 h.
(3) Sodium molybdate was active as a corrosion inhibitor in its action of passivating the anodic reaction of an active corrosion cell, as well as inhibiting growth of the sulphate-reducing bacteria.

The evidence presented that active microbially influenced pitting corrosion is not necessarily inhibited by the killing or inhibition of the bacteria involved is very important when regimes for tackling MIC problems are addressed. Clearly both a corrosion inhibitor and a bacterial inhibitor are necessary for the effective treatment on actively-pitting systems. Treatment with either a corrosion inhibitor or a biocide without the other could be ineffective, in that, the presence of active bacteria after treatment with a corrosion inhibitor could result in the bacteria serving to facilitate the establishment of further active corrosion cells. Alternatively, it has been shown that killing or inhibiting only the bacteria involved in an actively pitting area does not necessarily result in inhibition of the corrosion cell already established.

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