Pergamon

Corrosion Science, Vol. 37, No. 7, pp. 1085–1096, 1995
Elsevier Science Ltd
Printed in Great Britain
0010-938X/95 $9.50 + 0.00

0010-938X(95)00016-X

MICROBIALLY SUSTAINED PITTING CORROSION OF 304 STAINLESS STEEL IN ANAEROBIC SEAWATER

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Abstract—A system has been developed in which a small anode and large cathode can be induced in a concentric stainless steel electrode. A current of approximately 11 μA cm^{-2} was applied for 72 h while bacteria were allowed to colonize the electrode in a closed system, in artificial seawater under anaerobic conditions. Once the applied current was removed, the resultant galvanic current was monitored and a flow of nutrients resumed to an open system. Only a co-culture of SRB and *Vibrio* sp. maintained a current of approximately 3 μA cm^{-2} for >200 h. No current was maintained with pure axenic cultures or in sterile controls. Bacterial counts revealed that the resultant current was dependent on the number and type of bacteria on both the anode and cathode. It would appear that SRB are necessary on the cathode, leading to high charge transfer resistance above 100 kΩ cm^2, while a mixed consortium is necessary on the anode giving low charge transfer resistance below 1 kΩ cm^2. These results would appear to give further evidence for the previously proposed cathodic depolarization theory as a mechanism for MIC and for another anodic reaction involving a mixed consortium.

INTRODUCTION

Corrosion and microbial corrosion in particular have been the focus of research for many years providing a wealth of information with several mechanisms being suggested. Menzies[1] lists five such methods as follows: (1) the production of corrosive metabolic products, (2) the production of differential aeration and concentration cells, (3) depolarization of cathodic processes, (4) disruption of natural and other protective films, and (5) the breakdown of corrosion inhibitors.

The bulk of the literature on MIC details the anaerobic corrosion of mild steel, caused by the sulphate-reducing bacteria (SRB) and characterized by the presence of black iron sulphide. The action of the SRB is not limited to mild steel and has been reported for copper and its alloys and stainless steels.[2,3] Both latter metals have a greater resistance to corrosion than mild steel and it is generally considered harder to induce corrosion in these metals, particularly stainless steel. This is due in part to the natural formation of a passive layer normally less than 50 Å thick which prevents the metal reverting to the thermodynamically stable oxide.[4] The structure and composition of the passive layer are not entirely established. Uhlig and Revie[5] proposed an amorphous structure of chemisorbed oxygen bonding perpendicular to the surface and electrostatic bonding between oxygen anions and metal cations parallel to the surface. The passive film can be described in terms of a thin, highly doped *n*-type semiconductor layer.[6] The doping of the passive layer is increasingly *n*-type with the addition of chromium to the base alloy.[6] The passive layer can breakdown under the following, usually localized, environments:[7] (i) dilute and concentrated HCl, HBr,

Manuscript received 12 September 1994; in amended form 17 November 1994.

and HF, and salts that hydrolyse these acids, (ii) oxidizing chlorides such as $FeCl_3$, $CuCl_2$, or NaOCl, (iii) sea water, except brief exposures or when cathodically protected, and (iv) some organic acids, including oxalic, lactic and formic acids.

The ennoblement, or increase in the open circuit potential (OCP), has been reported when stainless steel is exposed to marine environments[8] as well as in fresh and brackish waters. Ennoblement is characteristic of an increased probability of localized corrosion occurring as the OCP approaches the pitting potential E_{pit}. It has been shown experimentally[9] that ennoblement is the result of an acceleration of the rate of oxygen reduction under a biofilm. Little *et al.*[10] further showed that ennoblement did not occur in stainless steel when the metal–biofilm interface was anaerobic. Little *et al.*[7] noted that ennoblement did not occur for three stainless steels exposed to Pacific Ocean water for three months.

During corrosion processes, metal ions are lost from the surface into solution at the anode of an electrochemical cell. This needs to be counterbalanced by the transfer of the excess electrons to a cathodic area of the metal substratum, where a secondary reaction can accept the electrons. Several mechanisms have been suggested for the pitting of steels by SRB. Initially it was suggested that when oxygen was absent, protons or hydrogen sulphide might act as an electron acceptor at the cathode:

$$2H^+ + 2e^- = 2H = H_2; \tag{1}$$

$$2H_2S + 2e^- = 2HS^- + H_2. \tag{2}$$

It is important to note that HS^- will only be formed in waters with a pH >7, otherwise H_2 will be formed. The subsequent oxidation of the molecular hydrogen by SRB would accelerate polarization of the cathode by absorbed hydrogen and produce sulphide and the potential for metal sulphide corrosion products:

$$4H_2 + SO_4^{2-} = 4H_2O + S^{2-}; \tag{3}$$

$$Fe^{2+} + S^{2-} = FeS. \tag{4}$$

The theory outlined above has been termed the cathodic depolarization theory. The theory cannot account for the levels of corrosion seen in experiments as determined by weight loss. Further evidence for the cathodic depolarization theory was given by Pankhania *et al.*,[11] who proved that SRB can oxidize cathodic hydrogen and use it as a source of metabolic energy. Later it was shown that corrosion could be caused by the presence of iron sulphide itself. It was therefore proposed that sulphide could act as the cathode and that the role of the bacteria would therefore be (a) to regenerate (or depolarize) iron sulphide, (b) to produce more iron sulphide by their growth reaction, or even (c) to bring fresh iron sulphide surfaces constantly into contact with the steel by their movement.[12]

Both of the above theories concentrate on the action of the SRB at the cathode. Recently it has been suggested that SRB can regulate the pH of their environments by changing their metabolism.[13] These differences in pH could account for the initiation of an anode at a site of low pH and a cathode at areas of higher pH. Campaignolle *et al.*[14] could simulate pitting with a concentric electrode in which an induced small anode was surrounded by a large cathode. It was shown that pitting corrosion of AISI 1020 mild steel was only sustained in the presence of SRB. Later unpublished research revealed that the culture used in these studies was not pure

SRB but had become contaminated by a *Vibrio* sp. at some unknown point in time. The galvanic current generated was proportional to the number of SRB isolated from the anode, suggesting that the anode could be the driving force of this type of corrosion.

This paper details experiments using similar stainless steel concentric electrodes in which pitting is sustained only in the presence of a co-culture of SRB and a *Vibrio* sp. It was also noted that there was a 10-fold increase in the number of SRB at the anode than at the cathode on concentric electrodes. This result would suggest that the bacteria are somehow involved in the anodic process rather than the cathodic alone, as previously reported. This maintained current was reproducible and therefore provides a system in which MIC of stainless steels can be studied in the laboratory.

EXPERIMENTAL METHODS

Bacteria

The bacteria used in the study were the SRB *Desulfovibrio vulgaris* (ATCC 25979) and a *Vibrio* sp. from an unknown source currently used by this group. The latter bacteria was a Gram negative, motile rod being facultatively anaerobic and oxidase positive. It was therefore identified as a member of the family *Vibrionaceae* and genus *Vibrio*. No match was found with the MIDI system database and further identification was not deemed necessary. Bacterial inocula were grown in 9 ml of lactate/acetate SRB medium[15] at 30°C for 72 h giving approximately 10^8 cfu ml^{-1}. Three inoculations were performed at 24 h intervals into the closed reactor systems. An artificial, defined, seawater medium[14] was used, containing (in $g\,l^{-1}$: 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; plus 1.5 ml of vitamin solution. The medium was made in 16 l batches and filter-sterilized through sartobran capsular filters (Sartorius, New York) into sterile glass carboys. The medium was sparged with a mixture of 95% nitrogen and 5% hydrogen for 24 h before the start of the experiment.

Concentric electrode and reactor design

Concentric electrodes (Fig. 1) were fabricated from 304 stainless steel such that a small central anode (surface area 0.031 cm^2) was separated from a large surrounding cathode (surface area 4.87 cm^2) by a Teflon spacer. Electrical wires were soldered to the back of both the anode and cathode and the whole electrode mounted in epoxy resin (Buehler, Lake Bluff, IL). The working surface of the electrode was polished to a 600 grit finish with Carbimet Discs (Buehler, Lake Bluff, IL), degreased in acetone and sterilized with 70% isopropanol.

The reactor had a working volume of 600 ml and was maintained at ambient room temperature (25 ± 1°C). Steady state conditions were obtained by the addition of fresh medium and removal of waste medium via peristaltic pumps (dilution rate, $D = 0.1\ h^{-1}$). Medium mixing, in the reactors, was achieved via another peristaltic pump that circulated the bulk phase. During the initial inoculation the reactor was run as a closed system to promote bacterial attachment and growth. Normally at this time a current was applied between the anode and cathode of approximately 11 $\mu A\ cm^{-2}$ (at anode) via a Sycopel Scientific Inc. DD10M potentiostat (Tyne and Wear, U.K.). When the current was removed, the same potentiostat was used as a zero resistance ammeter to measure the current flowing between the anode and cathode (galvanic current). While operated as a closed system, further mixing was facilitated by a magnetic stirrer (100 rpm) that was stopped after the initial inoculation/colonization phase of the experiment to allow electrochemical measurements to be made. Throughout the course of the experiment a mixture of 95% nitrogen and 5% hydrogen was sparged through the medium reservoir and each vessel at a flow rate of 30 $ml\ min^{-1}$, to maintain anaerobic conditions. After the initial 72 h inoculation period, the applied current was removed and a flow of nutrients initiated. During the time that an open system was maintained, after the applied current was removed, the resulting galvanic current between the anode and cathode was measured as noted above.

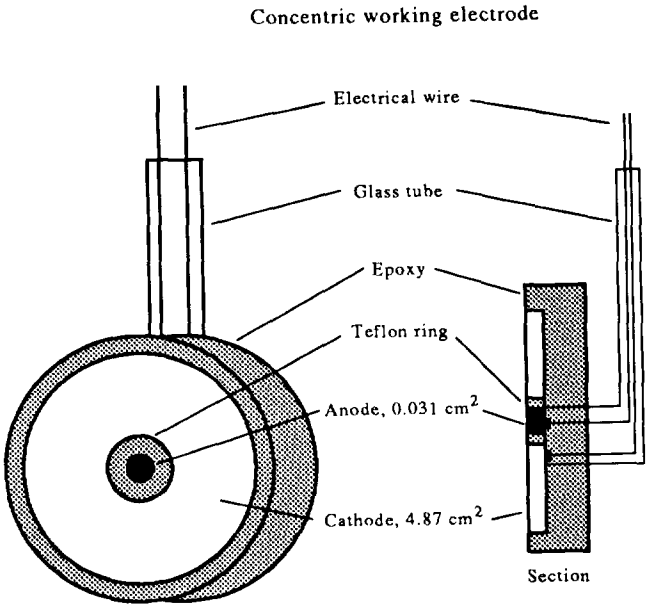

Fig. 1. Schematic of 304 stainless steel concentric electrode design showing the small central anode and large surrounding cathode, separated by a Teflon spacer.

Normally four experiments were run at a time with either axenic or mixed cultures, as well as sterile controls. The sterile control was treated the same as the inoculated system except that no bacterial inoculations were made. Each set of results discussed are from a single electrode chosen as representative from a number of separate runs performed.

Electrochemical measurements

The working electrode was held in place by a glass rod installed in the top of the vessel through which the wires were passed. The concentric electrodes were positioned within 2 cm of the titanium counter electrode. A saturated calomel reference electrode (Orion, MA) was connected via a Luggin's bridge. Electrochemical impedance spectroscopy (EIS) analysis was performed using Zplot software (Scribner Associates Inc. VA), a Solartron 1255 HF frequency response analyser and a Solartron 1286 potentiostat/galvanostat (Schlumberger, Farnborough, U.K.). The applied voltage amplitude was 5 mV at frequencies between 5 mHz and 10 kHz. Five frequencies were examined per decade. Analysis was carried out on both the anodes and cathodes every 24 h over the 72 h period after the applied current was removed.

Bacterial counts

At the end of the experiments the redox potential of each vessel was measured and the bacteria extracted from the cathodes and anodes by scraping cellular material and corrosion products. The removed bacteria was suspended in 9 ml of sterile anaerobic lactate/acetate SRB medium. Bacterial counts were done on this suspension using the most probable number (MPN) technique with lactate/acetate SRB medium. Further counts to differentiate the *Vibrio* from the SRB were done by the Miles and Misra plate count technique[16] on Iverson's medium.[17] Plates and tubes were then incubated anaerobically at 30°C.

EXPERIMENTAL RESULTS AND DISCUSSION

Current densities

Figure 2(a) shows the current density at the anode plotted against time for a mixed culture of the *Desulfovibrio vulgaris* and the *Vibrio* sp. It can be seen that an applied current was maintained at approximately 10 μA cm^{-2} for the first 72 h, while

the bacteria were allowed to grow under batch conditions. Once the applied current was removed and the flow of medium started in the vessels containing the mixed culture of the *Desulfovibrio vulgaris* and the *Vibrio* sp. together, there was a gradual decrease in the galvanic current, for approximately 6 h, until it remained steady at approximately 3 μA cm^{-2} for a further 40 h. This electrode showed clearly discernible pits (1 or 2 $<$ 0.5 mm in diameter) on the anode which were found to form tunnels within the metal. The initial spike in the current seen when the applied current was removed is thought to be due in part to the fact that the applied current was controlled at a set level. Therefore, if corrosion was trying to proceed at a higher rate it would be limited. It was thought that possibly when the applied current was removed the resultant galvanic current was no longer restricted, resulting in a large

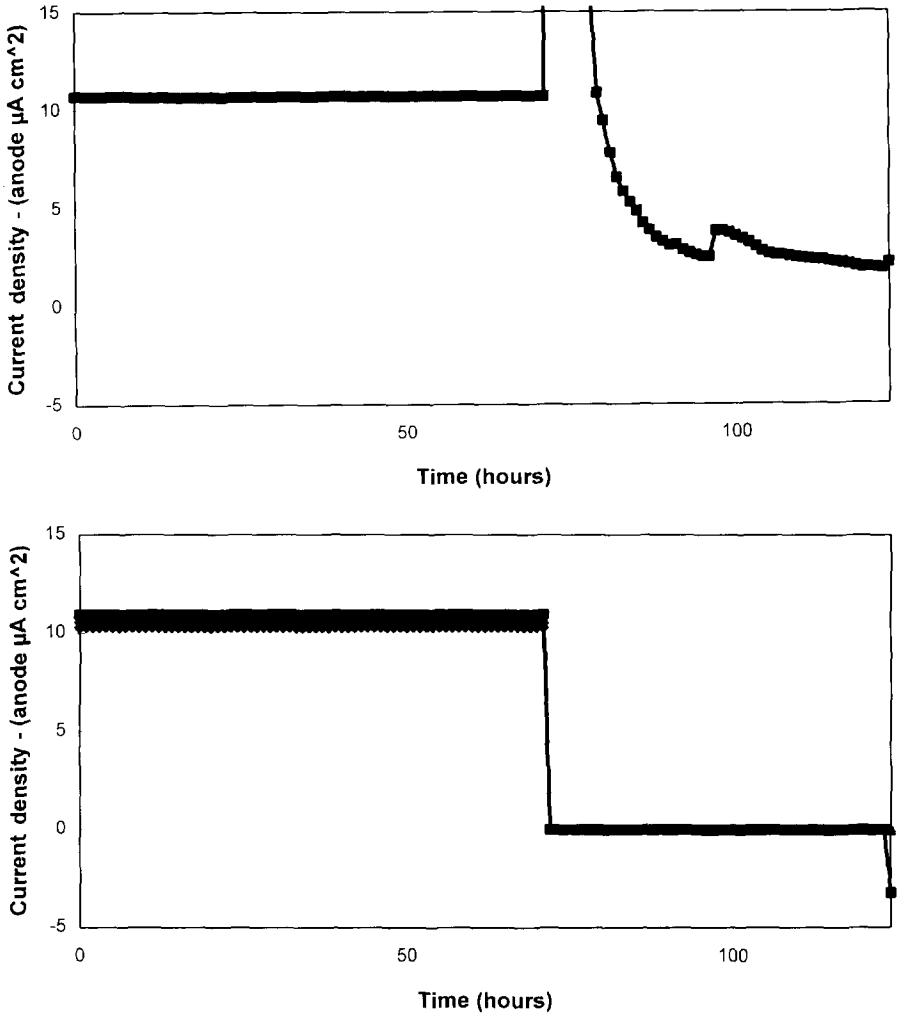

Fig. 2. Time plots of measured current flowing between anode and cathode for: (a) mixed culture of *Desulfovibrio vulgaris* and *Vibrio* sp. with a current maintained after applied current is removed, and (b) ◆ sterile, ■ axenic SRB and ▲ axenic *Vibrio* sp., showing no current maintained after applied current is removed.

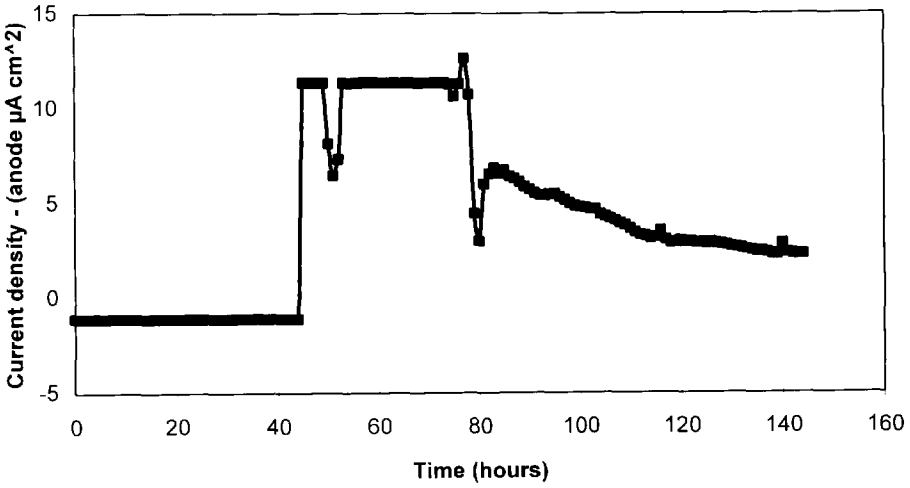

Fig. 3. Time plot of measured current flowing between anode and cathode for a mixed culture of *Desulfovibrio vulgaris* and *Vibrio* sp. with colonization occurring for 48 h prior to current being applied for 48 h, showing maintenance of current after applied current was removed at approximately 80 h.

spike, until equilibrium was maintained. The spike at 100 h was a result of the anode being disconnected from the cathode (via the ZRA) allowing electrochemical measurements to be made. Again it is possible that a build up of current occurred during this time, while the current flow was interrupted.

During the same experiment (Fig. 2(b)) it can be seen that there was no such maintenance of the galvanic current in the sterile control. In the absence of any bacteria after the applied current was removed, it is evident that the current rapidly dropped to below the limit of detection (0.01 μA cm^{-2}). This drop often resulted in a negative current being observed, showing that the 'cathode' had become anodic to the 'anode'. This negative current was negligible and at the limit of detection. In previous unreported experiments using mild steel in the sterile vessels, this reversed current was often quite large (approximately 3 μA cm^{-2} at the cathode). A possible explanation could be that anaerobic conditions were not maintained and general corrosion occurred, which, due to the relative sizes of the anode and cathode, resulted in a greater current being generated at the cathode. This small current seen with the stainless steel could be a similar process. The decrease in the current would be expected due to the greater resistance of stainless steel to general corrosion than mild steel. In the stainless steel systems shown in Fig. 2(b) there was no evidence of visible pitting on either the anode or the cathode. The whole anode was recessed as a result of general corrosion due to the applied current. Figure 2(b) also shows that with both of the axenic cultures the results for the galvanic current were the same as those seen in the sterile controls, in that it was not maintained.

A subsequent experiment (Fig. 3) in which the bacteria were allowed to grow for 48 h before the current being applied for a further 42 h also showed that with the *Desulfovibrio vulgaris* and the *Vibrio* sp. a constant anodic current density in the region of 3 μA cm^{-2} was recorded. This result shows that the current does not need to be applied during the initial colonization period and does not need to be applied

for 72 h. This suggests that the result is not due to bacterial selection during the initial attachment process of the bacteria colonizing the anode and cathode. Rather, it appears to be an effect that can be exerted by the applied current on a mature biofilm. It is likely that the current results in either a change in metabolism of the community or a change in the bacterial membranes, allowing an anodic effect to be asserted. Again the sterile control and the axenic cultures showed no maintanance of the galvanic current and no discernible pits, the plots were similar to those shown in Fig. 2(b) with a rapid decline to zero once the current was removed.

The numbers of bacteria isolated from the anodes and cathodes for each bacterial treatment are shown in Table 1 along with the average resulting galvanic current. In the experiments where a galvanic current was maintained, the numbers isolated, per unit area, from the anode were at least 10 times as high as those from the cathode. With the *Vibrio* sp. in the mixed culture, the anode had 100 times as many bacteria as the cathode. This higher level of colonization could be a result of the applied current with bacteria carrying a net negative charge being attracted to an area carrying a positive charge. Results by Campaignolle *et al.*[14] suggested that for similar experiments, using mild steel, the resultant current was proportional to the number of *Desulfovibrio vulgaris* isolated from the anode. As noted earlier subsequent work revealed that this was not an axenic culture but was contaminated with a *Vibrio* sp.

Figure 4 shows a plot for an experiment in which two vessels with a mixed culture of the *Desulfovibrio vulgaris* and the *Vibrio* sp. In one of the vessels with the mixed culture there was a steady decline in the current over the first 30 h as it approached the limit of detection (Fig. 4). It did not, however, persist, as seen in the other experiments with the mixed culture. This steady decline was in marked contrast to the abrupt decline seen in the sterile control. The bacterial counts from the anode and cathode were three orders of magnitude lower at the anode and one order of magnitude lower at the cathode than those in which the current was maintained (Table 1). During this run it was also seen that one of the mixed cultures did not maintain the current as expected. As shown in Table 1 no SRB were isolated from the

Table 1. Bacterial numbers (cfu cm^{-2}), charge transfer resistance (R_{ct} $k\Omega$ cm^2) and galvanic current density (μA cm^{-2}) for concentric stainless steel coupons with various treatments

Coupon treatment	SRB	*Vibrio*	R_{ct}	Galvanic current
Sterile anode	—	—	40	—
Sterile cathode	—	—	92	
Axenic SRB anode	3.2×10^3	—	56	—
Axenic SRB cathode	2.0×10^1	—	120	
Axenic *Vibrio* anode	—	1×10^8	14	—
Axenic *Vibrio* cathode	—	1×10^7	85	
Mixed anode	1×10^7	1×10^8	0.439	$3\,\mu A\ cm^{-2}$
Mixed cathode	1×10^6	1×10^6	184	
Mixed anode (Fig. 4)	3.2×10^4	1.6×10^7	1.074	Slow decline to zero
Mixed cathode (Fig. 4)	2×10^5	2.8×10^5	74	
Mixed anode (Fig. 4)	3.2×10^4	2.06×10^7	0.786	—
Mixed cathode (Fig. 4)	—	2.1×10^6	23	
Mixed anode (Fig. 5)	3.7×10^7	1.98×10^8	1.168	$1\,\mu A\ cm^{-2}$
Mixed cathode (Fig. 5)	3.1×10^5	4.62×10^6	83	

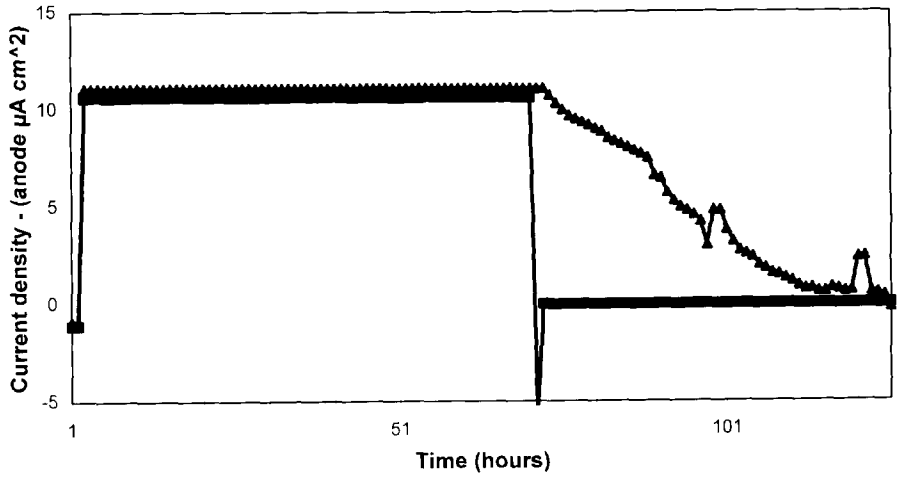

Fig. 4. Time plot of measured current flowing between anode and cathode showing (▲) a slow decline to zero after current was removed for a mixed culture of *Desulfovibrio vulgaris* and *Vibrio* sp., compared to the sharp decline (■) mixed culture as above but with no maintenance of the current due to no SRB colonisation of the cathode.

cathode. This would suggest that the presence of SRB on the cathode is necessary for the maintenance of the current.

Long term testing

To ascertain whether the observed phenomenon was a transient effect, further

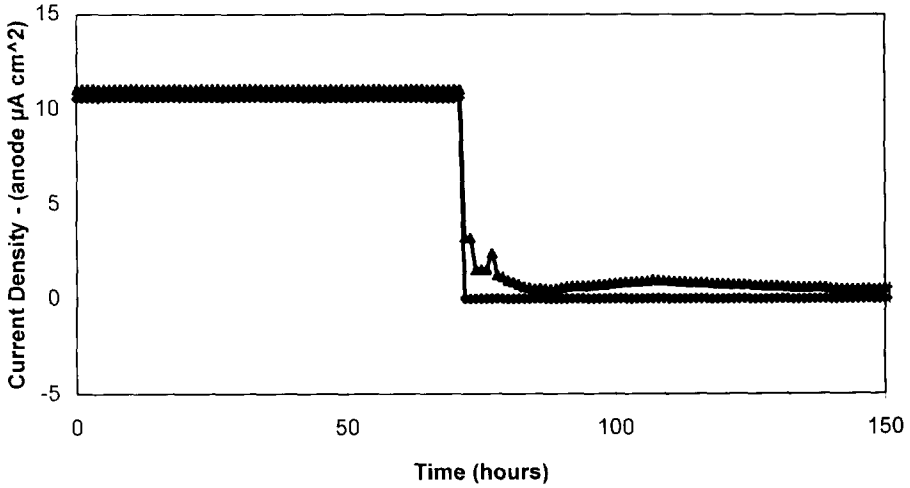

Fig. 5. Extended time plot for 165 h, of measured current flowing between anode and cathode showing (▲) maintenance of a low current after current was removed for a mixed culture of *Desulfovibrio vulgaris* and *Vibrio* sp., compared to the sharp decline (◆) for the sterile control. Note the low current is thought to be due to the low number of bacteria isolated from the surface of the electrode and the decline in current was accompanied by a rise in the R_{ct} value of the anode.

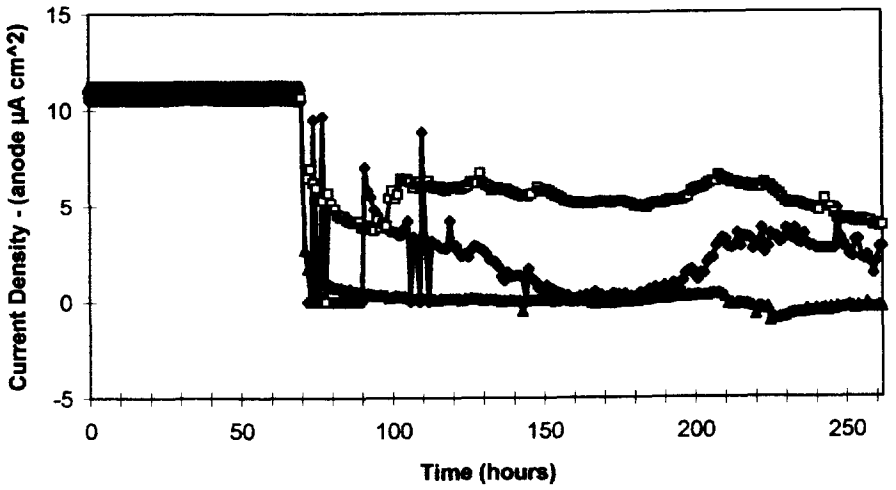

Fig. 6. Extended time plot of measured current flowing between anode and cathode showing (◆) and (□) maintenance of current for over 200 h after applied current was removed for a mixed culture of *Desulfovibrio vulgaris* and *Vibrio* sp., compared to the sharp decline (▲) for the sterile control.

experiments were carried out to determine whether the resultant galvanic current could be maintained for a longer period of time. As shown in Fig. 5, no resultant current was seen in the sterile control. The mixed culture vessel showed a low resultant galvanic current of approximately 1 μA cm^{-2} that persisted for approximately 90 h before it rapidly declined below the limit of resolution. Table 1 indicates that in the mixed culture of bacteria low levels of the *Desulfovibrio vulgaris* were isolated from the anodes and cathodes compared to those experiments which yielded a maintenance of a high galvanic current. Conversely, the numbers of the *Vibrio* sp. isolated from both the anodes and cathodes was reasonably high with 10^8 and 10^6, respectively. It needs to be noted that these counts were taken after the experiment had been running for 200 h and the current had not been maintained for the latter 40 h. It is, therefore, likely that the low current that persisted was due to the low number of SRB on the coupon and the decline at 160 h corresponded to a decline in bacterial numbers. The cause of this speculated bacterial decline is uncertain.

A further experiment was conducted in which the resultant galvanic current was monitored in excess of 200 h after the applied current was removed. The cultures used were: (i) and (ii) mixed SRB plus the *Vibrio* sp.; and (iii) the sterile control. Figure 6 shows that no current was maintained in the sterile control, whereas in both the cultures with the SRB in a consortium, a current was maintained for the period examined. For (ii) this current was not stable for the whole period, with a drop occurring between 125 and 200 h, but it never dropped to zero. This clearly showed that the current can be maintained in a mixed culture containing SRB for extended periods of time (>200 h) showing that it is not merely a transient effect.

Electrochemical impedance spectroscopy and polarization resistance

Figure 7 shows the Bode plots for the anodes (7(a)) and cathodes (7(b)) both with and without a maintained current. The Bode plots for the cathodes in either case

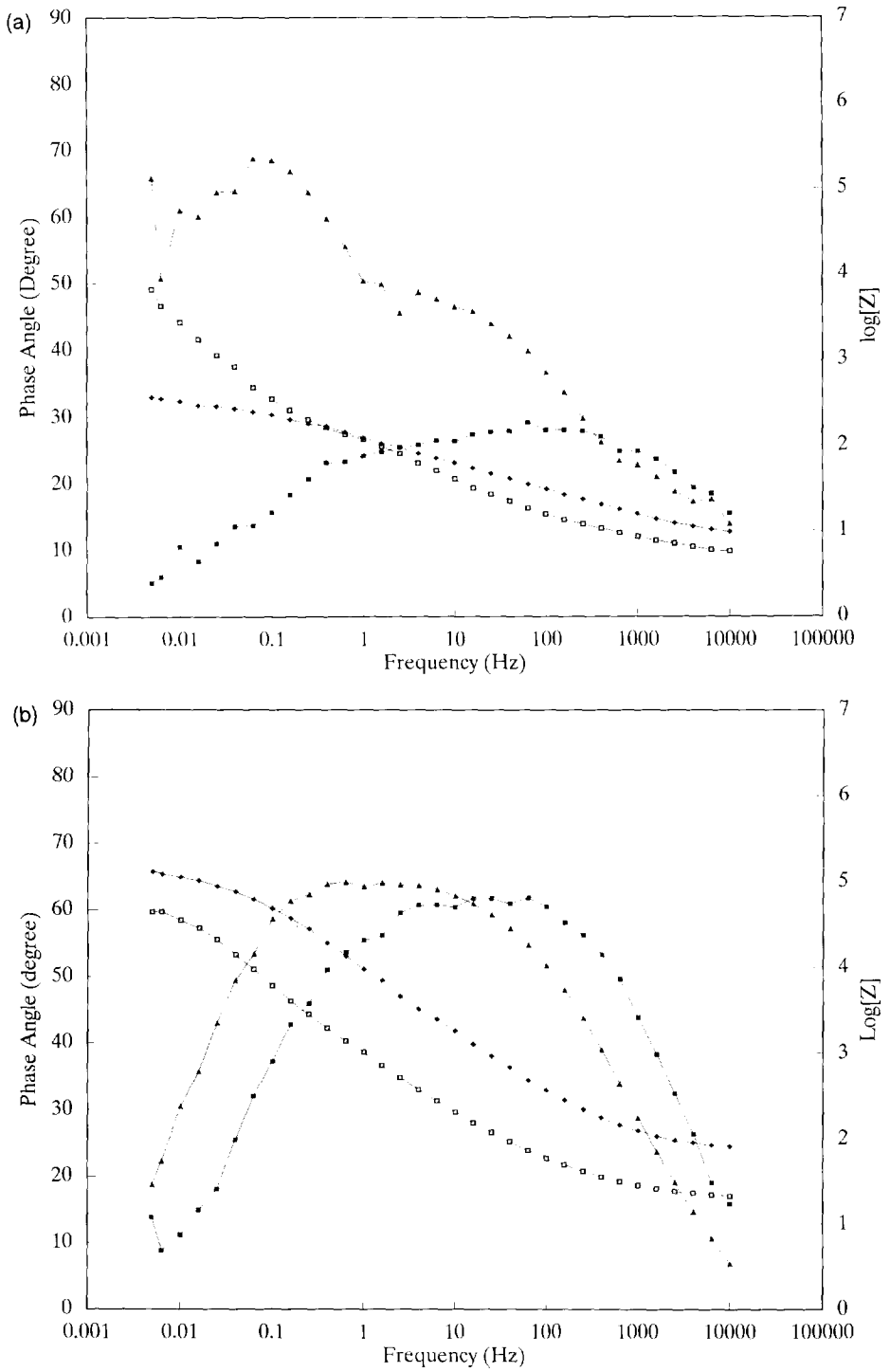

Fig. 7. (a) Bode plots for anodes (■) and (◆) with current maintained, (▲) and (□) with no current maintained. (b) Bode plots for cathodes (■) and (◆) with current maintained, (▲) and (□) with no current maintained.

show little of interest except that in the instance where a galvanic current was maintained, the log [Z] values were higher. In the case of the anodes in the systems where no current was maintained it appears that there are two time constants. Also, where the current was maintained the impedance was lower than in the case where the current was not maintained.

From the Bode plots of log[Z] vs frequency it is possible to calculate the charge transfer resistance (R_{ct}) of the respective anodes and cathodes (Table 1). Based on the results, several trends could be discerned in relation to the pitting of 304 stainless steel. The results are part of a complex pattern for which all the pieces have yet to be assembled. A few reproducible and discernible features were evident. Where the corrosion current was maintained the R_{ct} values at the anode were always below 1 kΩ cm^2. In only one case was an R_{ct} value seen below this level in a vessel in which the current was not maintained. This was a sterile control in which no anodic pitting was seen by either the maintenance of the galvanic current or actual visual pits on the anode. Usually the R_{ct} values for the cathodes were highest when either a mixed or axenic culture of the SRB was present. The values for these were normally $>$100 kΩ cm^2. It did appear that when the anodic R_{ct} was lower than 1 kΩ cm^2 the value of the galvanic current was relative to the cathodic R_{ct} values, with higher R_{ct} values resulting in larger galvanic currents.

In one experiment, shown in Fig. 5 in which the current dropped off after approximately 90 h after the applied current was removed (160 h total time), a marked rise of the R_{ct} value for the anode from 286 Ω cm^2 at 145 h, while the current was being maintained, to 2.051 kΩ cm^2 at 200 h, when the current had stopped, occurred. Here the results for the anode correspond with the predicted pattern, that when the value rises above 1 kΩ cm^2 no current is maintained.

In the presence of SRB either as an axenic or mixed culture a high value for the cathodic R_{ct} is seen which is normally higher than that seen for either the sterile or *Vibrio* controls. This would suggest that the SRB are exerting an effect on the cathode. The low anodic R_{ct} value was normally only seen in the presence of the mixed cultures (one exception as noted above). It would also appear that it is this anodic R_{ct} value being low that is responsible for the galvanic current when SRB are present on the cathode creating a high R_{ct} value. This low anodic R_{ct} value appears to be the result of an interplay between the two bacteria and between an anodic response and a cathodic response to the bacteria. In the case of the axenic SRB lower numbers were present on the anode than seen in the mixed culture. It is therefore possible that the role of the *Vibrio* sp. is to enhance the biofilm formation and help the SRB to colonize the surface, leading to the low R_{ct}. This would necessitate the SRB acting both to enhance the cathode and the anode by different reactions. It is possible that there is an interplay between the two species which results in the lower anodic R_{ct} values such as the production of a metabolic acid.

CONCLUSIONS

A system has been described in which it is possible to maintain reproducibly a galvanic current in 304 stainless steel. The maintenance of the current is due to the presence of a mixed population of bacteria containing SRB. The pitting process is initiated by the application of a galvanic current to a small anode. Without a mixed culture of bacteria this current results in the uniform corrosion of the whole anode.

When a mixed culture of bacteria is present, including the SRB *Desulfovibrio vulgaris*, small, visually discernible pits are formed. These pits have steep sides and often form a network of tunnels within the metal. The following general conclusions regarding the involvement of the SRB can be drawn:

(1) An axenic culture of the SRB cannot on its own maintain the current leading to pitting; a co-culture, in this case a *Vibrio* sp., is necessary and the current can be maintained in excess of 200 h (Fig. 6). Here the bacteria are not responsible for the initiation of the pit but rather for its localization and maintenance. (This is not to say that bacteria cannot initiate pitting, but in these experiments the initiation was enhanced by the applied current.)
(2) The action of the SRB seems to be to increase the charge transfer resistance of the cathode in excess of 100 $k\Omega\,cm^2$ (Table 1) and failure of the SRB to colonize the cathode leads to a lack of maintenance of the galvanic current (Table 1; Fig. 4).
(3) The presence of a mixed culture on the anode leads to a lowering of the R_{ct} value below 1 $k\Omega\,cm^2$ that, in combination with the elevated cathodic R_{ct} in the presence of the SRB, leads to the maintenance of the galvanic current.
(4) Bacterial colonization of the concentric electrode can precede the application of the galvanic current (Fig. 3).

Acknowledgements—This work was supported by Grant N0014-93-1-0326 from the Office of Naval Research. The authors wish to acknowledge Xavier Campaignolle, Krissy Hunnell and Sharon Cowden for their technical assistance in this work.

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