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RELATIONSHIP BETWEEN BACTERIAL COLONIZATION AND CATHODIC CURRENT DENSITY ASSOCIATED WITH MILD STEEL SURFACES

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Cathodic protection (CP) is an effective way to control corrosion of marine structures. In addition to the inorganic film formed during cathodic polarization, microorganisms can become firmly attached to the surfaces and interfere with the cathodic process. In the present experiments the role of two eubacteria was examined in mono and coculture in the formation of the calcareous film under CP in both galvanostatic and potentiostatic mode. The presence of a biofilm interacted with the scale deposition process, which led to a significant increase in the cathodic current demand. This increase was effected by two phenomena, *viz.* the decrease of local pH due to bacterial fermentation products and the oxidation of cathodically-produced hydrogen by the sulphate-reducing bacteria. In addition, the study of a preexisting biofilm placed under CP led to the same conclusions.

KEYWORDS: cathodic protection, sulphate-reducing bacteria, biofilm

INTRODUCTION

Cathodic protection (CP) using sacrificial anodes or impressed current has been recognized as an effective way to prevent corrosion in seawater. The beneficial effect of the scale formed under cathodic polarization includes an increase in the "throwing" power of the CP system and a decrease in the cathodic current demand required, and consequently the cost.

In addition to inorganic deposits, adhesion of non-biotic and biotic material can be influenced by or can influence cathodic polarization. Previous studies have shown that scales formed in artificial seawater differ from those found in natural seawater and that this is largely due to the organic content of the seawater (Edyvean, 1984). Furthermore, the presence of such biofilms in natural conditions has been demonstrated to affect the free potential and to modify the cathodic properties of stainless steels under cathodic polarization (Mollica *et al.*, 1984; Johnsen & Bardal, 1985).

Biofilms formed in natural seawater generate new physical, chemical and hydrodynamic conditions on surfaces and promote the initiation of localized corrosion. The physical presence of the biofilm can create differential aeration cells and metabolic activities such as production of extracellular enzymes, organic acids and exopolymeric substances. It has been suggested that the biofilms in natural environments modify the activation-controlled part of the cathodic oxygen reduction but the precise mechanism is not known (Scotto *et al.*, 1985). Thus, biological activity in the bulk phase as well as on surfaces can interfere

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with the electrochemical reactions occurring during CP and induce changes in the cathodic current demand required to maintain a fixed potential on carbon steel.

In natural conditions cathodic protection is rarely applied to surfaces immediately following their immersion. Thus a significant biofilm forms before the structure is cathodically polarized. Calcareous and biological deposits have often been observed on cathodically protected surfaces exposed to natural seawater environments. Such a preexisting biofilm may be of great importance for both the establishment and the efficiency of a CP system. Furthermore, the presence of a significant bacterial population buried in the calcareous scale can lead to the initiation of underdeposit corrosion.

The aim of this study was to investigate the effect of bacteria as mono- and cocultures on cathodic protection applied in a potentiostatic or in a galvanostatic mode on carbon steel exposed to a synthetic seawater medium, and to investigate how a preexisting biofilm can interfere with cathodic polarization.

MATERIALS AND METHODS

In all experiments, electrodes were cut from X52 carbon steel sheets and embedded in an epoxy resin. The electrodes were then polished with decreasing grain from 120 to 1000 mesh, degreased and rinsed with alcohol and acetone. The edges of the electrodes were masked using an insulating epoxy lacquer to expose 4 cm^2 to the various media.

The study of the growth of bacteria conducted on clean surfaces required 24 coupons placed in 6 reaction vessels (4 coupons per vessel). The assembled systems were sterilized with ethylene oxide gas then filled with 1.2 l of aerobic synthetic seawater medium (ASTM D1141) flowing continuously through the vessels at a rate of $5\%\cdot h^{-1}$. Immediately after contact with the medium, the carbon steel electrodes were polarized to a cathodic potential of –950 mV/SCE using a titanium counter electrode. A facultative anaerobe, *Vibrio natriegens*, was inoculated into one vessel, and a coculture of this with a sulphate-reducing bacterium (SRB) *Desulfovibrio vulgaris* into another. The two remaining vessels were left sterile although the electrodes were likewise polarized. In addition to the four vessels with polarized electrodes, a further two vessels with four electrodes each were subjected to the medium flow. In this instance however, all the electrodes remained at the free corrosion potentials. One of these vessels was inoculated with the *Vibrio*/SRB coculture and the other was left sterile.

In the experiments conducted on the precolonized surfaces, four coupons were placed in each of the six vessels and again the same systems were sterilized with ethylene oxide. The same bacterial consortium of *V. natriegens* and *D. vulgaris* was then inoculated. Biofilms were allowed to form on the surfaces for 3 d then a cathodic polarization –950 mV/SCE was applied to all coupons. At this time four coupons were removed from one vessel and the bacterial populations both in the bulk phase and on the surfaces were determined. A cathodic potential of –950 mV/SCE was then applied to the remaining electrodes.

Finally in the last experiment, a cathodic polarization was applied in galvanostatic mode. The set-up was similar to the one described in the previous experiment including the flow rate and the ASTM seawater medium. A constant current of 50 $\mu A.cm^{-2}$ was applied to carbon steel coupons immersed in vessels inoculated with *V. natriegens* or with a coculture of *V. natriegens* and *D. vulgaris*. Selection of the current magnitude was chosen based on the assumption that at such current the oxygen reduction would be the major cathodic reaction and that no other cathodic reaction such as hydrogen evolution could take place. As references, two vessels with four coupons each were maintained under sterile conditions. In both cases, cathodic current demand as well as the open circuit potential for the unprotected coupons were followed during the whole experiment using a DD 10M/I potentiostat (Sycopel Scientific Incorporated).

Chemical Analyses

Calcium and magnesium contents of the deposits were analyzed using an atomic absorption spectrophometer after dissolving the deposit in a 0.5 M acetic acid solution. X ray diffraction analyses were performed on a Siemens D500 Power X ray diffraction spectrometer at 40 KV and 30 mA for the cathodic tube. Scans were made from 10° to 60° as 2°. Peak patterns were identified by matches with files of standard minerals.

Microbiological Analyses

Attached bacteria were removed by fixing a small glass extractor cylinder to the coupons *via* a butyl rubber O-ring. A 2 ml volume of sterile saline medium was added to the extractor and the tip of an ultrasonic probe (Model W140, Heat systems-Ultrasonics Incorporated) immersed in the liquid. Three pulses of 3 s each were applied at 25% power. Enumeration of the cells in the liquid removed from the surfaces was then performed using acridine orange direct counts (Gerhardt, 1981).

RESULTS

Effect of Bacterial Consortia on the Cathodic Current Demand

The cathodic current demand versus time plot for all protected coupons is shown in Figure 1. In the absence of bacteria, a steady state current demand of 50 μA.cm^{-2} was reached after 72 h. In the presence of a monoculture of the slime- producing bacterium, *V. natriegens*, the current demand increased with time reaching 120 μA.cm^{-2} at the end of the experiment. This increase was considerably higher in the presence of *D. vulgaris*, reaching 300 μA.cm^{-2} after 192 h cathodic polarization. In the absence of cathodic polarization, potential measurements indicated a decrease for all coupons in the open circuit potential (data not shown). The open circuit potential was slightly lower in the inoculated medium than in the sterile medium.

A white deposit on coupon surfaces and a mixture of both organic and inorganic matter was observed in the vessels inoculated with *V. natriegens* (Fig. 2a). Under sterile conditions, a similar white deposit was associated with the coupon surfaces (Fig. 2b). In the vessel inoculated with the bacterial coculture this deposit appeared slightly different, consisting primarily of a grey calcareous deposit and large blackened areas. In both cases, X-ray diffraction analyses confirmed the presence of calcium carbonate and magnesium hydroxide, as aragonite and brucite respectively. Iron sulfides were also detected in the medium inoculated with SRB. Sodium chloride as halite from the marine medium was identified from all coupons.

In the absence of cathodic polarization, coupons exposed under both sterile conditions and in the presence of bacteria exhibited a significant layer of corrosion products. X-ray diffraction analysis identified this material as hydrated magnetite, Fe_3O_4, H_2O and iron oxyhydroxides as goethite (αFeOOH) and lepidocrocite (γFeOOH).

The calcium and magnesium contents of the deposits are shown in Figure 3. As can be seen, under sterile conditions with cathodic polarization (vessels a and b), the Ca/Mg ratio was 2.6, while in the presence of a *V. natriegens* monoculture (vessel c), this ratio was

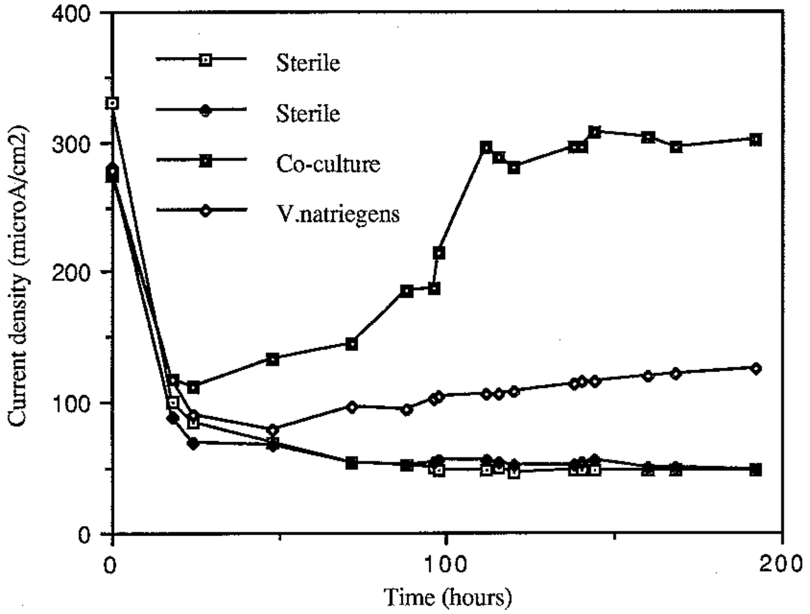


Fig. 1 Current density *versus* time (cathodic potential = –950 mV/SCE).

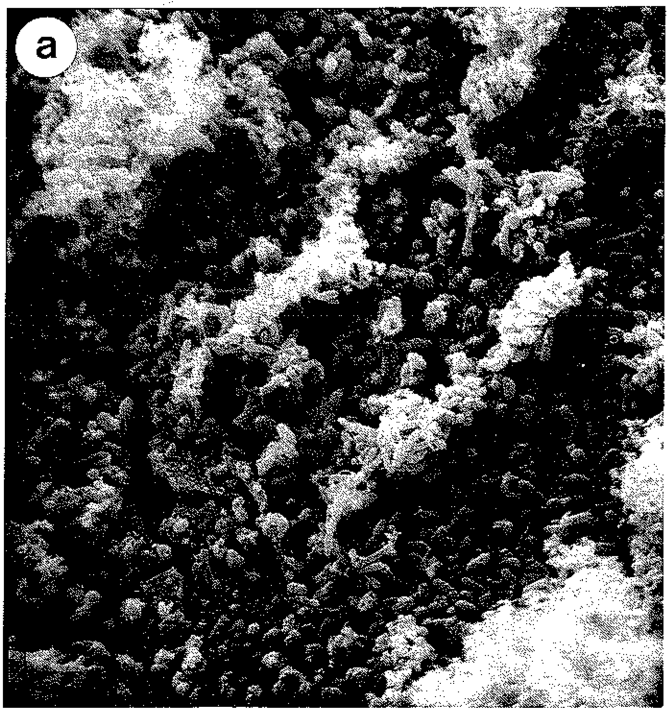


Fig. 2a SEM of the calcareous scale formed in the medium inoculated with *V. natriegens* (×1300).

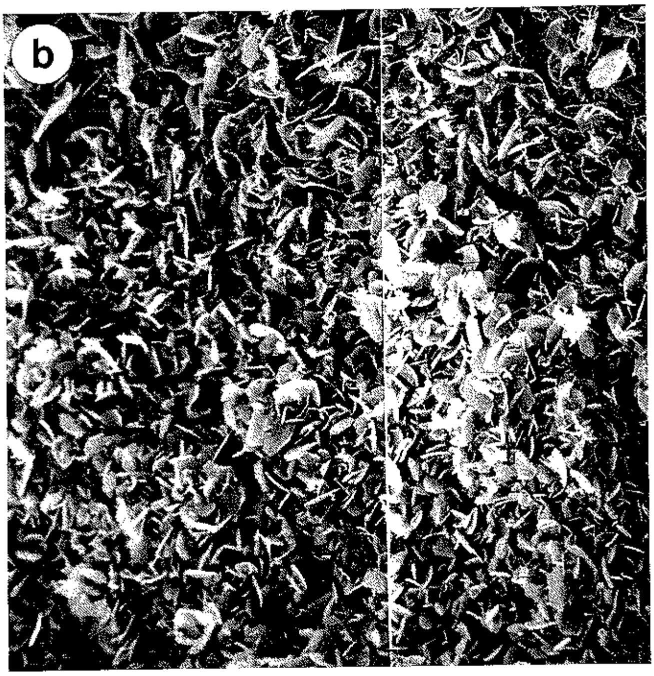


Fig. 2b SEM of the calcareous scale formed under sterile conditions (×1300).

12.4, primarily due to an increase in the calcium content and a decrease in the magnesium content of the deposit. In the presence of SRB (vessel d), less calcium was found on the surfaces and the Ca/Mg ratio decreased to 1.63. In the absence of polarization the Ca/Mg ratio was higher, both in the presence of bacterial and under sterile conditions, with less magnesium detected in the former case. In the presence of *V. natriegens*, X-ray diffraction analyses indicated a significant decrease in the brucite content of the deposits (Fig. 4).

Bacterial total counts performed on two coupons per vessel indicated no contamination in vessels a, b and f and population densities of 1.3×10^7 cells·cm^{-2}, 1.5×10^8 cells·cm^{-2}, and 9.4×10^8 cells·cm^{-2} for the vessels c, d, and e respectively.

Scanning electron microscopy performed on coupons exposed in vessel d inoculated with the coculture showed two kinds of morphologically different microorganisms on the surfaces, indicating the presence of both *V. natriegens* and *D. vulgaris* on the protected coupons. In vessel c with *V. natriegens* alone, the bacterial cells appeared to be trapped within the calcareous deposit. More polymers seemed to be produced by these microorganisms under cathodic polarization (Fig. 2c).

The pH in the bulk phase changed with the experimental conditions. The initial pH remained constant for the three vessels maintained under sterile conditions (a, b, f). In those vessels inoculated with bacteria, a decrease in the pH values from 8.0 to 6.8 was noticed after the first day. The pH remained stable for the remainder of the experiment.

Cathodic Protection on a Colonized Surface

The cathodic current demand versus time is shown in Figure 5. An increase was observed for both vessels inoculated with bacteria after 140 h. The cathodic current

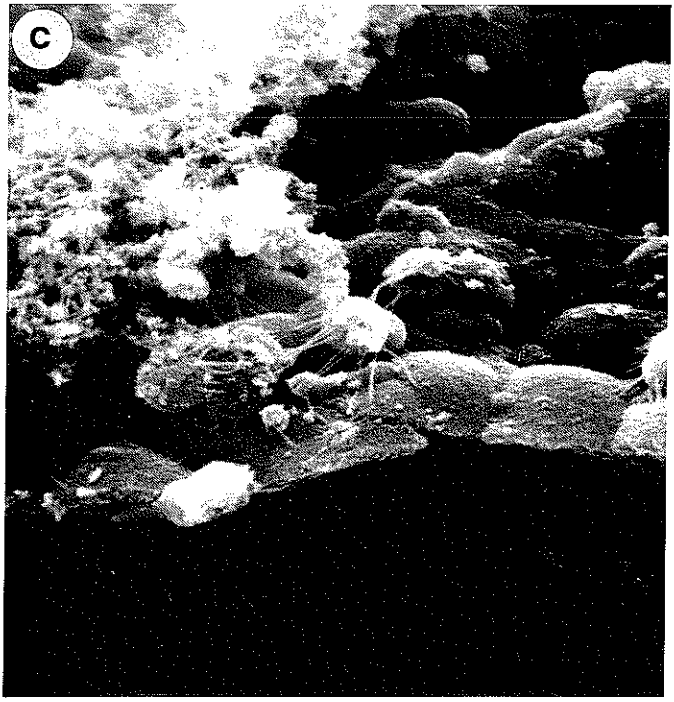
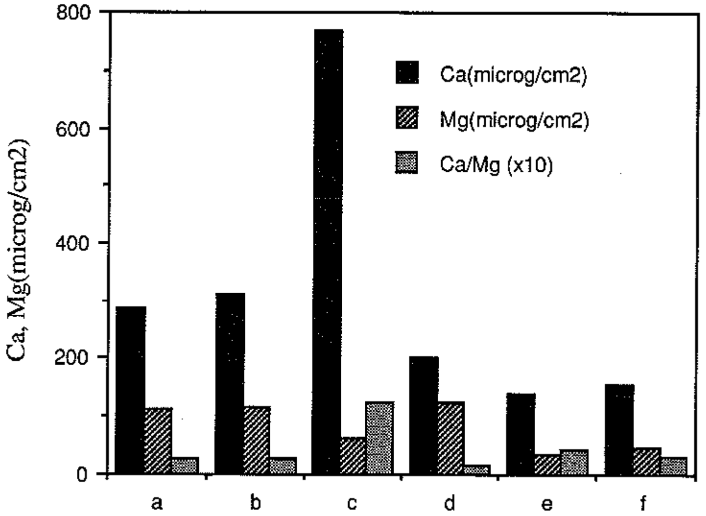


Fig. 2c Bacterial attachment on the cathodically protected surfaces (×4000).



a,b:sterile +CP. c: Vibrio natriegens +CP.d: Coculture +CP. e: Vibrio natriegens, No CP. f: Sterile, No CP

Fig. 3 Calcium and magnesium contents of the deposits (cathodic potential = –950 mV/SCE).

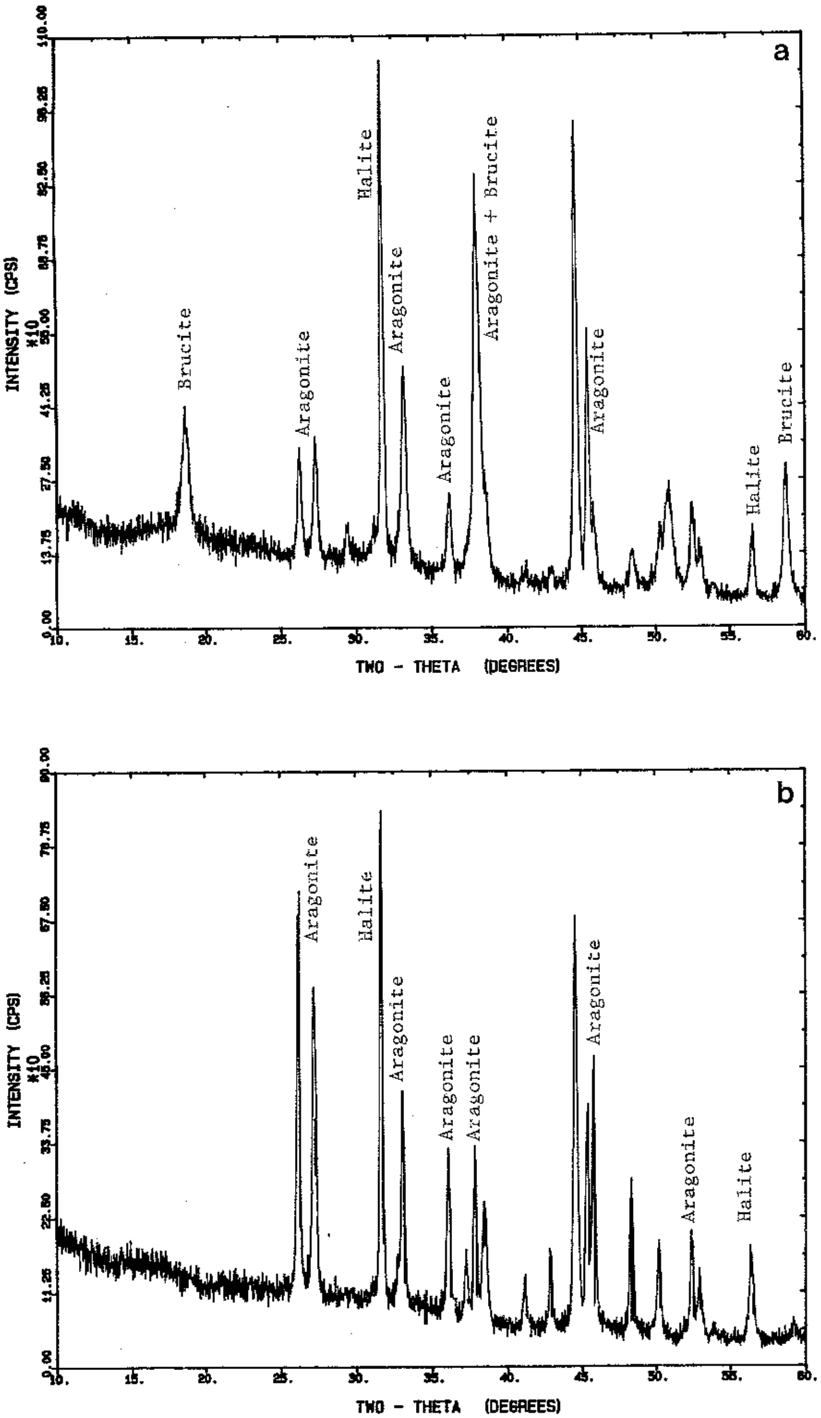


Fig. 4 X-ray diffraction analyses of the deposits. a = under sterile conditions; b = with *V. natriegens.*

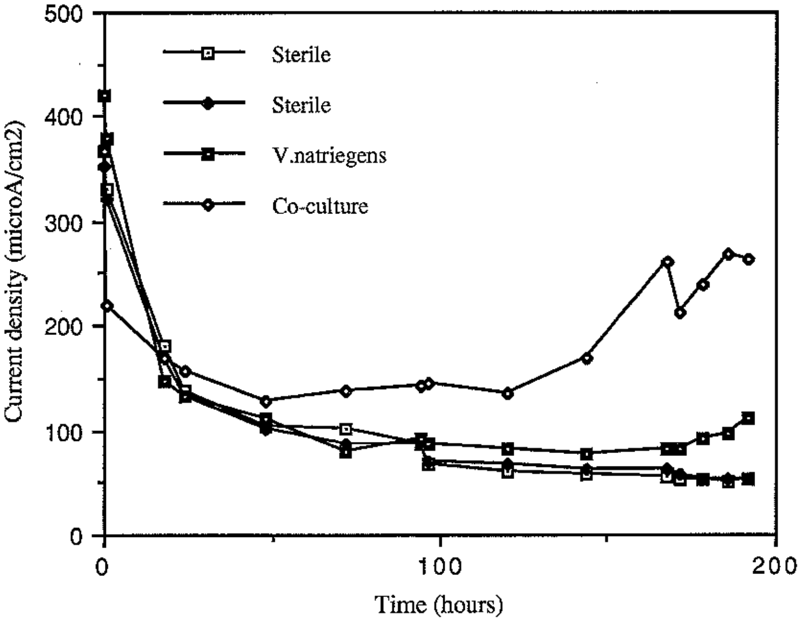


Fig. 5 Current density *versus* time on biofilmed surfaces (cathodic potential = –950 mV/SCE).

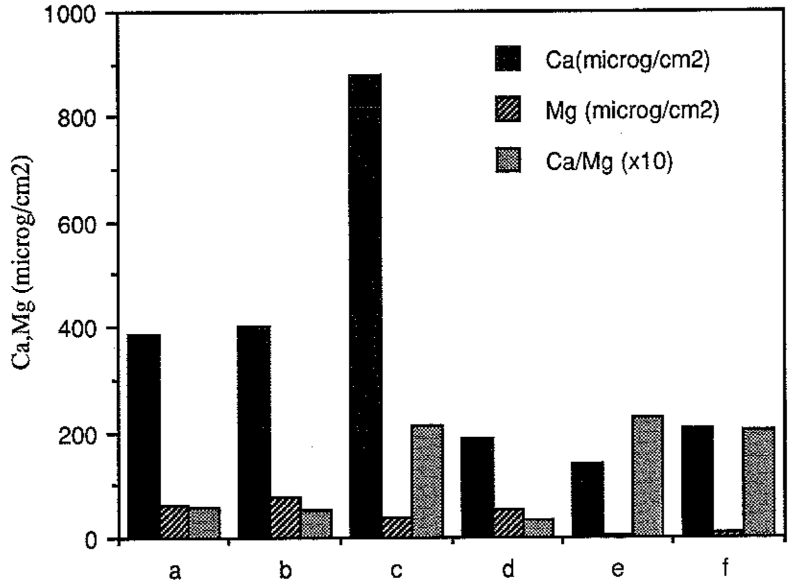
reached 270 μA.cm^{-2} in the vessels inoculated with the coculture. A slight increase in the current demand after 144 h was noted also on coupons exposed to the monoculture of *V. natriegens*. This current demand then reached 120 μA.cm^{-2} at the end of 8d experiment. In the absence of cathodic protection, a decrease in the open circuit potential was observed for both coupons.

After allowing a biofilm to form on the surfaces, direct epifluorescent microscope counts indicated a bacterial population of 1.2 × 10^{8} cells·cm^{-2} after 72 h. After 192 h this population was slightly lower at 1.5 × 10^{7} cells·cm^{-2}. On protected coupons, 3.5 × 10^{7} to 6.2 × 10^{8} cells·cm^{-2} were observed on coupons exposed to *V. natriegens* and the coculture, respectively.

Calcium and magnesium analyses are shown in Figure 6. Similar amounts of calcium were found both on the protected and non protected coupons compared to the previously described uncolonized surfaces. However, lower amounts of magnesium were found with preexisting biofilms under cathodic polarization. Iron sulfides were also detected on the surfaces exposed to the coculture.

CP Applied in a Galvanostatic Mode

Cathodic potentials versus time under a constant current are plotted in Figure 7. Again, a monoculture of *V. natriegens* and a coculture of *V. natriegens* and *D. vulgaris* significantly affected the cathodic potential required to maintain a given current. At the end of the 8 d experiment, these potentials were approximately –1225 mV/SCE and –1275 mV/SCE with *V. natriegens* and the coculture respectively, and –1150 mV/SCE in the absence of bacteria (Fig. 7). Chemical analysis of the deposits revealed higher calcium and magnesium contents under sterile conditions than in the presence of bacteria



a,b: sterile . c: V.natriegens+CP. d: Coculture +CP.
e: Coculture ,No CP (3days). f: Coculture,No CP(8days).

Fig. 6 Calcium and magnesium contents of the deposits on biofilmed surfaces (cathodic potential = –950 mV/SCE).

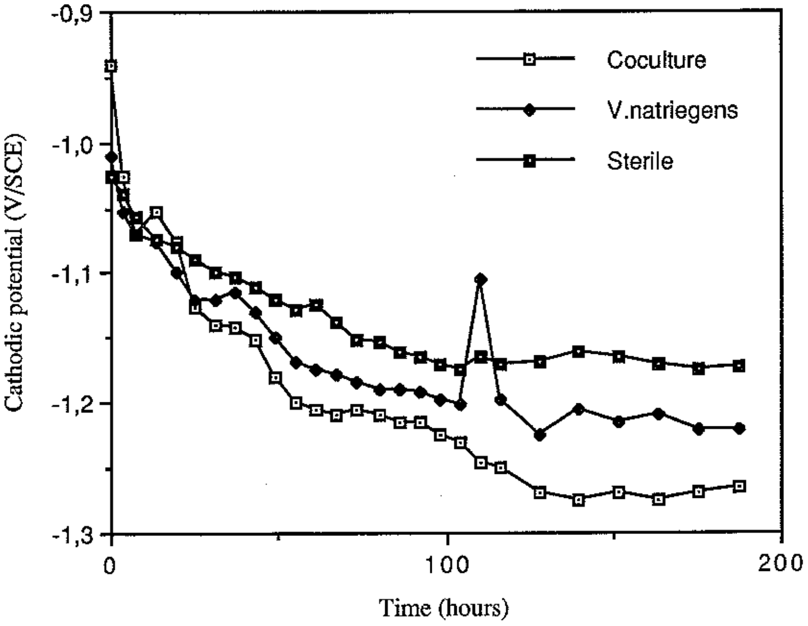


Fig. 7 Cathodic potential *versus* time (constant current = 50 μA.cm^{-2}).

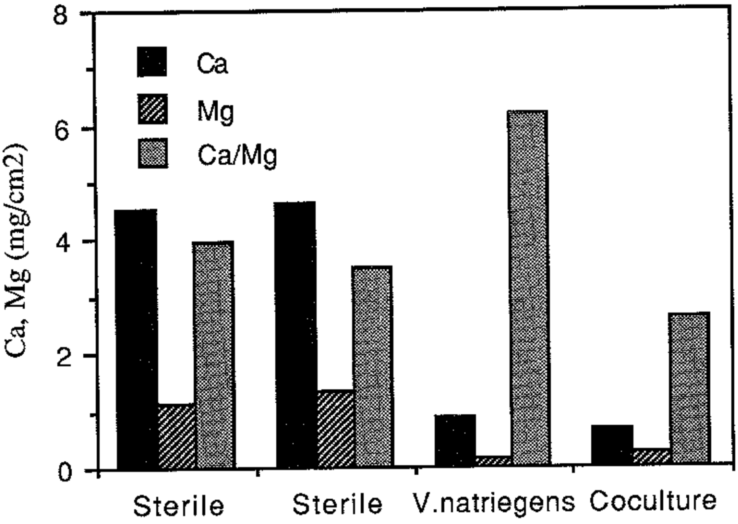


Fig. 8 Calcium and magnesium contents of the deposit (constant current = 50 μA.cm^{-2}).

(Fig. 8) (4.63 mg·cm^{-2} and 0.82 mg·cm^{-2} for calcium *vs.* 0.26 mg·cm^{-2} and 0.11 mg·cm^{-2} for the magnesium respectively). X-ray diffraction analyses confirmed these findings, demonstrating the existence of a thin, homogeneous film of brucite and aragonite under sterile conditions and a heterogeneous film in the presence of bacteria.

Bacterial densities determined by direct-count epifluorescence microscopy showed a bacterial density of 1.0×10^7 cells·cm^{-2} on protected coupons.

DISCUSSION

Applying a cathodic potential to metallic surfaces in seawater drives both oxygen reduction and hydrogen evolution. Both of these reactions lead to the formation of hydroxyl ions, OH^- and hence to an increase in the pH adjacent to the cathodic surfaces. Such an increase in the alkalinity causes the precipitation of magnesium and calcium salts as brucite, aragonite and calcite leading to the formation of a calcareous deposit on the surfaces. These deposits increase the throwing power of the cathodic polarization, decreasing the cathodic current demand and, consequently, the cost of CP. Both the chemical composition and the structure of this deposit greatly influence the effectiveness of CP. Many parameters affect this deposition, including flow velocity (Lee & Ambrose, 1986; Hack & Guanti, 1989) the calcium and magnesium content of the seawater (Culberson, 1983), temperature (Kunjapur *et al.*, 1985) dissolved organic matter (Edyvean, 1984) and pressure. However, interfacial pH (Culberson, 1983) is the most significant factor and any factor which affects this can modify the deposition kinetics, along with the nature and the stability of the scale.

In synthetic sterile seawater, a steady state current was obtained after a few days, indicating the formation of a homogeneous deposit on the surfaces. In the media inoculated with bacteria, a continuous increase in the cathodic current demand was observed. Furthermore, this increase was significantly higher in the presence of a coculture containing SRB than with the facultatively anaerobic bacterium alone. For each condition, with the exception of the sterile medium, a decrease in the pH to 6.8 was measured in the bulk phase. *V. natriegens* is capable of producing large amounts of volatile organic acids, including acetic and butyric acids as products of their metabolism (Dowling *et al.*, 1991). At the flow rate used in the present experiments, this resulted in a decrease in the bulk phase pH and at the metal-solution interface. Thus both the kinetics and the stability of the calcareous deposits can be affected by the medium pH, as indicated by the Ca/Mg ratio as shown in Figure 3.

In the presence of acid producing bacteria, a significant decrease in the magnesium content was observed. Magnesium hydroxide, $Mg(OH)_2$, normally precipitates in seawater when the pH exceeds approximately 9.5 (Culberson, 1983; Lin & Dexter, 1988). The lower pH observed during the present studies prevented magnesium deposition and/or decreased its stability as a magnesium hydroxide deposit. The effects of bacterial activity on the surfaces were confirmed by X-ray diffraction studies, which showed a significant reduction in brucite on the surface. In addition, magnesium has been shown to interfere with calcium precipitation. Magnesium inhibits both the nucleation and the crystal growth of calcite, but inhibits only the nucleation of aragonite precipitation (Bischoff, 1968; Berner, 1975).

These effects are consistent with the observed increases in cathodic current demand and with the enrichment of the calcium deposits with respect to magnesium. The influence of bacteria on the cathodic current demand has been demonstrated also by Little *et al.* (1988), who described the role of acid producing bacteria on the cathodic current demand of stainless steel in a synthetic seawater medium. The present results are also in agreement with other previous studies (Fiksdal & Guezennec, 1988; Guezennec, 1991) which showed a significant effect of volatile fatty acids on the calcium/magnesium ratio associated with cathodically protected stainless steels exposed to synthetic or natural seawater.

The significant increase in the cathodic current demand observed with the coculture is related to the growth of SRB under both the scale formed during cathodic polarization and the anaerobic conditions generated by the *V. natriegens* biofilm. This type of bacterial consortium could then induce an increase in the current demand as a result of both the use of the catholically produced hydrogen and the iron sulfides formed on the surfaces. X-ray diffraction analyses indicated the presence of significant amounts of iron sulfides together with aragonite and brucite. The effects of acid production may also have an important bearing, as discussed above.

Thus cathodically produced hydrogen can promote the growth of microorganisms in marine sediments (Guezennec *et al.*, 1988; Guezennec, 1991) or under other anaerobic conditions. Hydrogen evolution on the cathodic surfaces has been observed at current densities up to 100 $\mu A.cm^{-2}$ (Dexter & Lin, 1991a). In the present studies, a cathodic current above 300 $\mu A.cm^{-2}$ was reached after a few days with the coculture, and iron sulfides were detected in the deposits. It therefore follows that under discontinuous CP application (for example, due to a temporary system failure), SRB growth could cause severe damage to structures. It is also possible that hydrogen evolution could lead to extensive bubble formation, thereby contributing to disruptions in the calcareous deposits. Indeed, the presence of SRB on the cathodically protected surfaces together with the formation of cathodic iron sulfides may play a major role in the CP efficiency and could significantly increase the current demand required to maintain a given potential.

Similar observations can be made with the experiment conducted using CP applied in a galvanostatic mode. A decrease in the cathodic potential was observed as a result of the presence of either a monoculture or the coculture. This decrease was higher in the latter case, suggesting a similar role for the SRB as consumers of cathodic hydrogen.

It was of interest to determine how a *preexisting* biofilm might affect the cathodic polarization. A biofilm present on metallic surfaces can act as a barrier for oxygen diffusion towards the surfaces and interfere with the cathodic reaction (Humble, 1948). A biofilm can also induce an interfacial pH shift to less than 5 as was shown by Dexter and Lin (1991a). Thus both with a bacterial monoculture and a coculture of *V. natriegens* and *D. vulgaris* a significant increase in the cathodic current demand was observed after a few days. However, this current was not significantly different from that found associated with the previously described exposed surfaces. In recent work by Dexter and Lin (1991b), it was shown that both natural marine populations and laboratory cultured biofilms caused a calcareous deposit to form more uniformly on cathodically protected surfaces. Similar observations were made in the present study and SEM analyses suggested a homogeneous distribution of scales on the surfaces. Chemical analyses showed more calcium and less magnesium compared to experiments conducted with exposed, uncolonized surfaces. Again, it can be suggested that low pH values within the biofilm prevented magnesium deposition and only allowed a uniform aragonite film to form on the surfaces. In addition, a biofilm could also act as a nucleus for calcium precipitation by increasing the number of nucleation sites and encouraging the formation of a more homogeneous film.

A slight increase in the current demand was observed for samples with *V. natriegens* biofilms as a possible consequence of local disruptions due to bacterial metabolism. In the presence of a coculture biofilm, X-ray diffraction analyses also indicated the presence of iron sulfides within the calcareous deposits. These deposits were already present on coupons exposed to the coculture medium prior to application of cathodic polarization. Consequently, an increase in the current demand was noted. This may have resulted from the activities of SRB, as a result of favourable conditions created by both the calcareous deposits and the exopolymeric substances from *V. natriegens*.

In each of these experiments it has been shown that cathodic polarization did not significantly influence bacterial attachment. Many parameters, including surface charge (Marshall, 1973), the surface conditioning processes that proceed bacterial attachment (Corpe, 1980), or the electrophoretic mobility of bacteria towards surfaces, can be modified by cathodic polarization. It is apparent from the present results that a cathodic current density of 50 $\mu A.cm^{-2}$ or a cathodic potential of –950 mV/SCE did not affect bacterial attachment to the surfaces. In addition, in the experiments conducted on previously colonized surfaces a cathodic potential of –950 mV/SCE was not sufficient to remove the bacteria from the preexisting biofilm. The high bacterial populations found in both cases suggested that bacteria were buried under the calcareous deposits. However, the cathodic potential or cathodic current might change bacterial metabolism and consequently affect the current demand or the cathodic potential. Maxwell (1986) showed that a cathodic polarization of –950mV/Cu–$CuSO_4$ did not prevent development of an active population on protected surfaces. He also suggested that the lower activity on cathodically protected coupons was not directly caused by inhibiting effects of the cathodic potential. The build-up of calcareous deposits on the surfaces caused an increase in pH, which may have had an inhibitory effect on biofilm development. However, the buffering effects of the seawater and microbial activity within the biofilm could reduce the interfacial pH from theoretical expectations during cathodic polarization (Lewandowski *et al.*, 1989). On some coupons, a large amount of exopolymeric substances was associated with the scale. Exopolymer production might be a response to the local environment, *e.g.* high pH.

CONCLUSIONS

Not enough is known about the relationship between cathodic polarization and biofilm and how the biofilm and bacteria interfere with CP systems. In this study two eubacteria were shown to change the efficiency of cathodic polarization, primarily by modifying the amount of calcium and magnesium deposition and stability on the surfaces. These scales are of great importance for the cathodic current demand, acting as a barrier for the diffusion of oxygen to the surfaces and reducing the cost of cathodic polarization. Organic acids produced by bacterial activities can modify the interfacial pH and hence change the kinetics, the constituents, and the stability of the deposits. In the same manner, a preexisting biofilm can act as a barrier for oxygen diffusion to the surfaces and create considerable changes in the current demand required to maintain a given potential.

These results also indicate the important role of sulphate reducing bacteria in cathodic protection. It has been shown that SRB considerably affect the current demand. Further research efforts are required in order to understand such alterations in natural environments.

Finally, it can be noted that a cathodic potential of –950mV/SCE or cathodic current density of 50 $\mu A.cm^{-2}$ is unable to interfere with bacterial attachment or facilitate removal of bacteria from surfaces. However, this does not mean that cathodic polarization of surfaces does not alter the metabolism of the bacteria. Further investigations are required in this area.

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