

IMPACT OF BIOFOULING ON THE ELECTROCHEMICAL BEHAVIOUR OF 304 STAINLESS STEEL IN NATURAL SEAWATER

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Biofilm formation on 304 stainless steel (S30400) does not necessarily result in an ennoblement of the corrosion potential. Instead, biofilms composed of aerobic and anaerobic bacteria from Gulf of Mexico water formed an anaerobic biofilm/metal interface and caused the corrosion potential to move in the negative direction. Biofilms from the same source containing photosynthetic diatoms in the presence of light produced aerobic biofilm/metal interfaces and a positive shift (ennoblement of the corrosion potential). Corrosion potentials of stainless steels exposed in natural seawater cannot be predicted without an understanding of the composition of the biofilm and its impact on interfacial chemistry. In this paper, measurements of corrosion potential, interfacial pH and dissolved oxygen have been correlated with SEM/EDAX surface analyses to evaluate the electrochemical behaviour of stainless steels exposed to Gulf of Mexico water. The interfacial chemistries that influence the corrosion potential are also discussed.

KEY WORDS: Microorganisms, seawater, marine, stainless steels, electrochemistry.

INTRODUCTION

It is well documented that the corrosion of stainless steels is more severe in natural seawater than in sterile chloride-containing media and there is agreement among investigators that the increased corrosion is due to the presence of a biofilm (Compton, 1970; Lagutina *et al.*, 1970; LaQue, 1982). Crevice corrosion of stainless steels in marine environments has limited the use and application of these materials. Most investigators have reported that the formation of a biofilm increases the susceptibility of stainless steels to crevice corrosion by shifting the corrosion potential in the positive direction into the range of the pitting potential (Scotto *et al.*, 1985; Johnsen & Bardal, 1985; Dexter & Goa, 1988; Mollica *et al.*, 1989). They have attributed the shift in the positive direction, an ennoblement, to enhanced oxygen reduction in the presence of marine biofilms.

In this paper an attempt has been made to correlate the corrosion potential of S30400, i.e. 304 stainless steel, with interfacial chemistry resulting from the activities of the microorganisms within marine biofilms. It has been further demonstrated that environmental parameters such as light level can alter the interfacial chemistry that determines the rates of electrochemical reactions.

Table 1 Nominal composition of stainless steels

UNS No. ^a	Common Name	C	Cr	Ni	Mo
S30400	304	0.08	18	9	—
S31600	316	0.08	17	12	2.5
N08366	AL6X®	0.03	20	25	6.0

^aUnified Numbering System, American Society for Testing Materials.

MATERIALS AND METHODS

Electrochemical Measurements

Nominal compositions for the stainless steels referred to in this paper are provided in Table 1. Electrodes of these alloys were polished to 600 grit and acetone rinsed prior to mounting in an EG&G PARC® flat electrode holder (EG&G Princeton Applied Research, Princeton, NJ).

Laboratory corrosion measurements were made at the Naval Oceanographic and Atmospheric Research Laboratory (NOARL) using an EG&G PARC 350A Corrosion Measurement Device and a 173 potentiostat/galvanostat programmed through an Apple II Plus computer. Some experiments were performed with reduced illumination, others under continuous illumination. Continuous illumination means that room lights remained on 24 h a day during the course of the experiment (20–50 foot candles depending on time of day). Reduced illumination (dark conditions) means that room lights remained off, windows were covered with black polyethylene and the electrochemical cells were covered with an opaque container covered with black polyethylene (<1 foot candle). Light levels were measured with a Weston Foot Candle Meter (Model 703) (Weston, Mt. Vernon, NY). Each test cell consisted of a 1 l flask with two graphite counter electrodes, a 1 cm² circular working electrode and a reference saturated calomel electrode (SCE) in approximately 750 ml electrolyte. Electrolytes included 1) freshly collected, flowing Gulf of Mexico (15 ml·min⁻¹) natural seawater, 2) sterilised natural seawater (Little *et al.*, 1987), 3) artificial seawater, made from Instant Ocean® (Aquarium Systems, Mentor, Ohio) and 4) NaCl in distilled/demineralised water (21 g·l⁻¹). Gulf of Mexico water was collected in 50 l, acid-washed black carboys, transported to NOARL and flow initiated. The supply was renewed every 10 to 15 days throughout the experiment.

Table 2 summarises the microbiological and chemical composition of all electrolytes. Salinity was measured using a Reichert automatic temperature compensated refractometer (Reichert Sci. Instr., Buffalo, NY). The pH was measured with a Corning digital pH meter, Model 130 (Corning, NY). Numbers of sulfate-reducing bacteria (SRB) were estimated by the Most Probable Number (MPN) method using a medium containing lactate as the electron donor and carbon source made up to the salinity of Gulf of Mexico water (Pfennig *et al.*, 1981). Aerobic heterotrophic bacteria were cultured using Difco 2216 agar. Total organic carbon was determined using the method of Menzel and Vaccaro (1964).

The impact of pH on the open-circuit or corrosion potential (E_{corr}) for S30400 in artificial seawater (3.5% NaCl) was monitored using 10% glacial acetic acid and 10 N NaOH to adjust pH. Solution dissolved oxygen (DO) concentrations were measured using a Yellow Springs Instrument (YSI), Model 51A DO meter (Yellow Springs, Ohio).

Table 2 Microbiological and chemical composition of electrolyte solutions

	Salinity (g·l ⁻¹)	pH	SRB ^a (MPN·ml ⁻¹)	Aerobic Bacteria CFU ^b ·ml ⁻¹ (after 6 days)	DOC ^c mg·l ⁻¹
NaCl solution	21	8.08	0	0	1.96
IO ^d solution	21	7.38	0	0	4.63
Sterile					
Gulf of Mexico water	21	7.64	0	0	15.7
Natural					
Gulf of Mexico water	21	7.95	10 ³	10 ⁴ -10 ⁵	10.0

^aSulfate-reducing bacteria, given as most probable numbers (MPN)·ml⁻¹.

^bColony forming units.

^cDissolved organic carbon.

^dArtificial seawater.

The impact of anodic polarization on interfacial pH was monitored using as S30400 electrode polarised in 3.5% artificial seawater and a 3.5% NaCl solution at sweep rates of 1 mV·sec⁻¹. A microelectrode (Lewandowski *et al.*, 1989) was placed at the metal surface. For protection, the sensing tip of the microelectrode was recessed 2 μm within an insulating glass capillary. The pH microelectrode was calibrated in the range between 1 and 10. The response was linear within this range.

The pitting potential (E_{pit}) for S30400 in unbuffered, deaerated 3.5% NaCl was determined as a function of pH (pH = 2.0, 4.0, 6.0, or 8.0) using a EG&G Model 273 potentiostat/galvanostat (EG&G Princeton Applied Research, Princeton, NJ) controlled through a Zenith (Chicago, Illinois) 386 computer and EG&G software for cyclic polarisation. Potentials were scanned in the anodic direction starting at a potential 100 mV negative to E_{corr} at a rate of 0.2 mV·sec⁻¹ to a maximum current density of 1 mA·cm⁻² at which the scan was reversed. E_{pit} was defined as the potential at which current increased abruptly indicating the onset of localised pitting.

Surface Analyses

The design and use of microelectrodes for interfacial pH and dissolved oxygen measurements at the metal/biofilm interface have been described elsewhere (Lewandowski *et al.*, 1989). Measurements of interfacial chemistry were made after 15 days exposure to Gulf of Mexico water at NOARL laboratory. The electrodes were fixed in 4% sodium cacodylate buffered glutaraldehyde for a minimum of 4 h before dehydration through a series of acetone and xylene washes, and air drying for scanning electron microscope/energy dispersive X-ray analyses (SEM/EDAX) (Marszalek *et al.*, 1979). A Kevex 7000 EDAX spectrometer (San Carlos, California) coupled to an AMRay 1000A® SEM (Bedford, Massachusetts) was used to identify elements heavier than sodium on the electrode surfaces. Electrodes were sputter-coated with gold after EDAX analysis and before photography.

RESULTS

Figure 1 is a plot of E_{corr} vs time for S30400 exposed to a variety of chloride-containing solutions (see Table 2 for chemistry and biology), with reduced lighting (dark con-

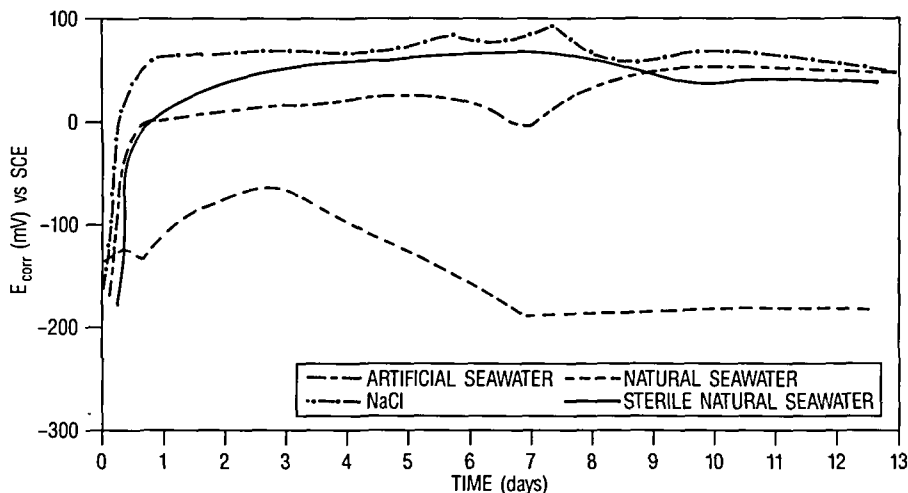


Fig. 1 E_{corr} vs SCE as a function of time for S30400 exposed to chloride-containing media (dark conditions).

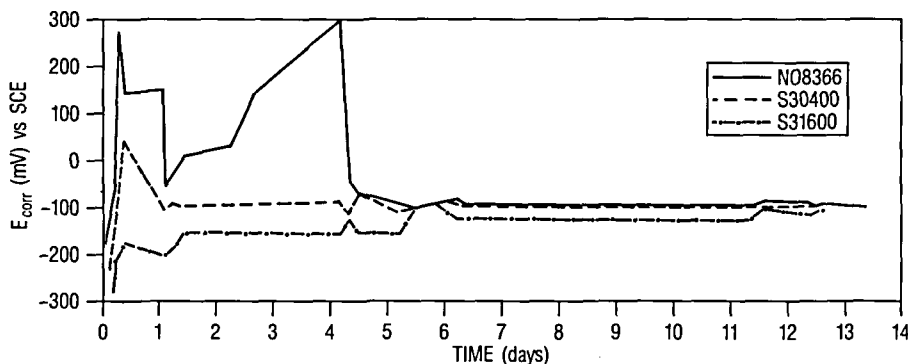


Fig. 2 E_{corr} vs SCE as a function of time for three stainless steels exposed to Gulf of Mexico natural water (dark conditions).

200 mV of the open circuit potential E_{corr} was found in all sterile solutions, but not in natural seawater. Figure 2 is a plot of E_{corr} vs time for a similar experiment in which a variety of stainless steels were exposed to natural seawater. The 3 stainless steels reached a steady E_{corr} of -100 mV, irrespective of alloy composition. Visual observation and SEM examination of the samples confirmed the absence of localised corrosion. Dissolved oxygen measurements with microelectrodes on the S30400 electrodes exposed to natural seawater (dark conditions) indicated that the metal/biofilm interface was anaerobic with a steep oxygen gradient from 8.0 to 0 $\text{mg}\cdot\text{l}^{-1}$ over 250 μm , the thickness of the biofilm (Fig. 3). The interface was probed at 3 locations and all 3 were anaerobic with the same profile. Further measurements could not be made reliably because of the disruption to the biofilm. Microprobe pH measurements indicated highly localised pH values as low as 5.2 in some areas and as high as 9.2 in other areas.

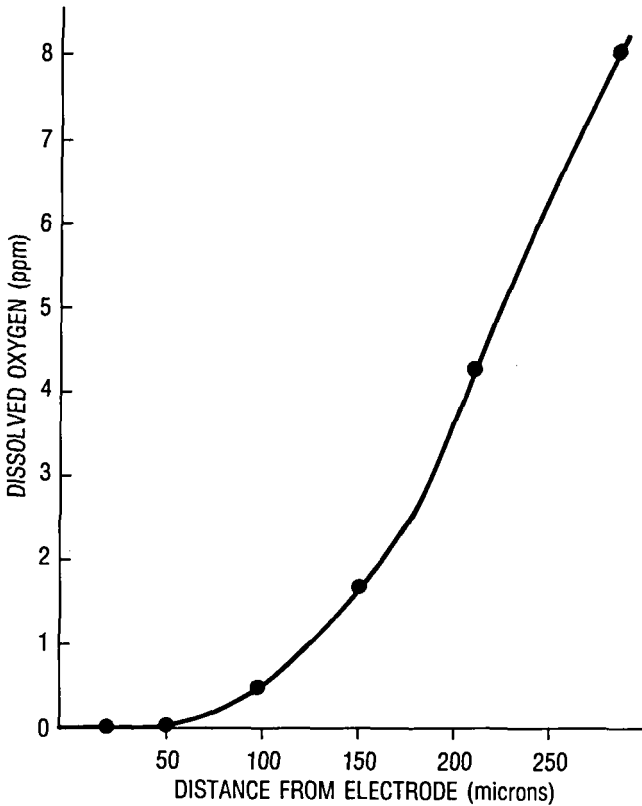


Fig. 3 Dissolved oxygen profile through biofilm from Gulf of Mexico water (dark conditions).

SEM micrographs and EDAX spectra confirmed the presence of a biofilm predominated by bacteria (Fig. 4) and enriched in sulphur (Fig. 5).

Figure 6 is a plot of E_{corr} vs time for two S30400 electrodes exposed to natural Gulf of Mexico water with continuous illumination. With increasing exposure time the corrosion potential shifted in the noble direction by approximately 200 mV. Dissolved oxygen measurements at three locations at the metal/biofilm interface indicate a shallow oxygen gradient from over 8.0 to 2.0–2.4 $\text{mg}\cdot\text{l}^{-1}$ over a 2000–4000 μm depth, the thickness of the biofilm (Fig. 7). Microprobe pH measurements at the metal/biofilm interface suggest that the pH at the metal surface under the biofilm was essentially unchanged from that of the bulk solution. Diatoms were visually obvious in the thick, brown voluminous biofilm. SEM micrographs document a biofilm predominated by diatoms (Fig. 8) and EDAX spectra (Fig. 9) indicate the accumulation of silicon, potassium and aluminium on the surface.

Figure 10 is a plot of E_{corr} vs pH for S30400 in sterile synthetic seawater. Acidic pH forces a shift of potentials to more positive values (an ennoblement) and basic pH has the opposite effect, a shift to negative values.

Figure 11 is a plot of pitting scans for S30400 in an unbuffered, deaerated 3.5% NaCl solution as a function of pH. Potentials were scanned in the positive direction and the resulting currents measured. E_{pit} for S30400 decreased from about +300 mV vs SCE at pH = 8 to about 0 mV at pH = 2.



Fig. 4 SEM micrograph of biofilm from Gulf of Mexico water (dark conditions).

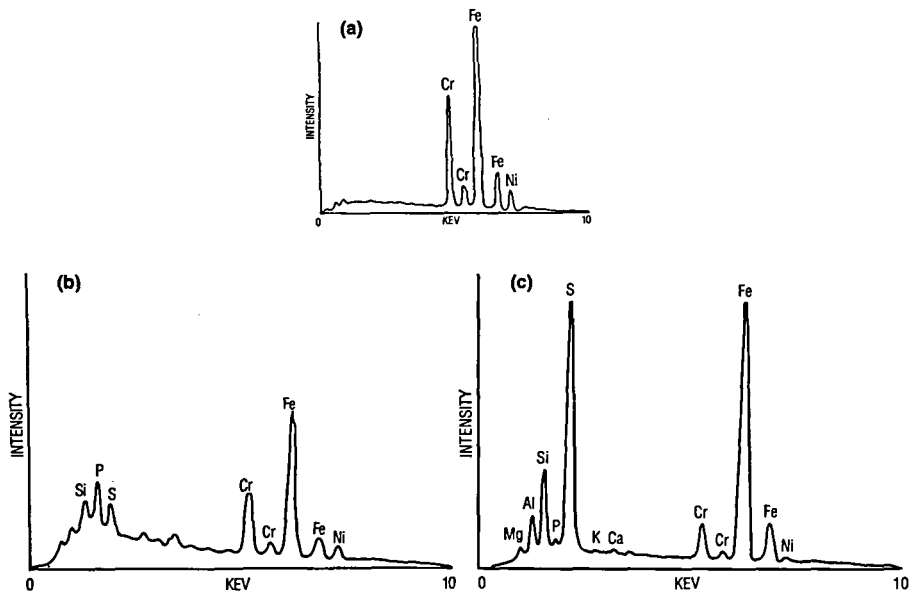


Fig. 5 EDAX spectra: (a) clean S30400, (b) S30400 with biofilm from Gulf of Mexico water formed under dark conditions, (c) crystalline area within biofilm.

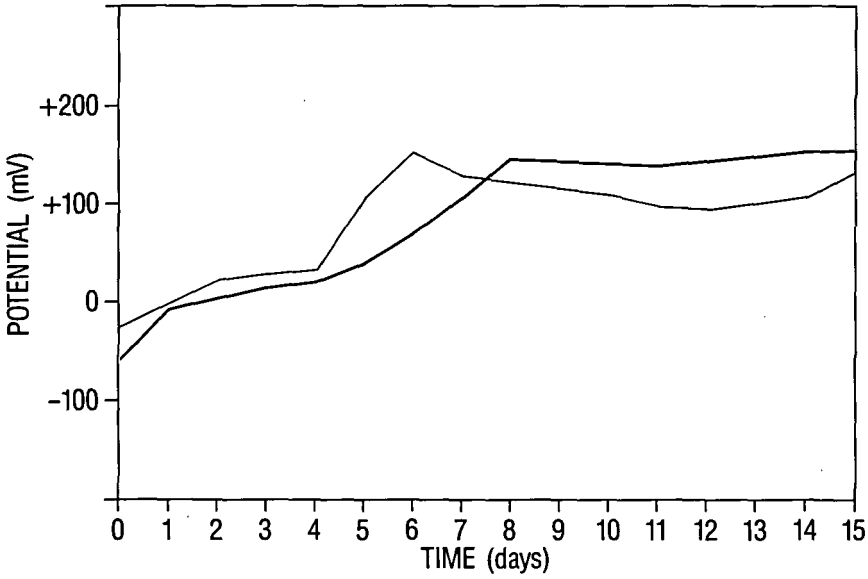


Fig. 6 E_{corr} vs SCE as a function of time for S30400 exposed to Gulf of Mexico water with continuous illumination.

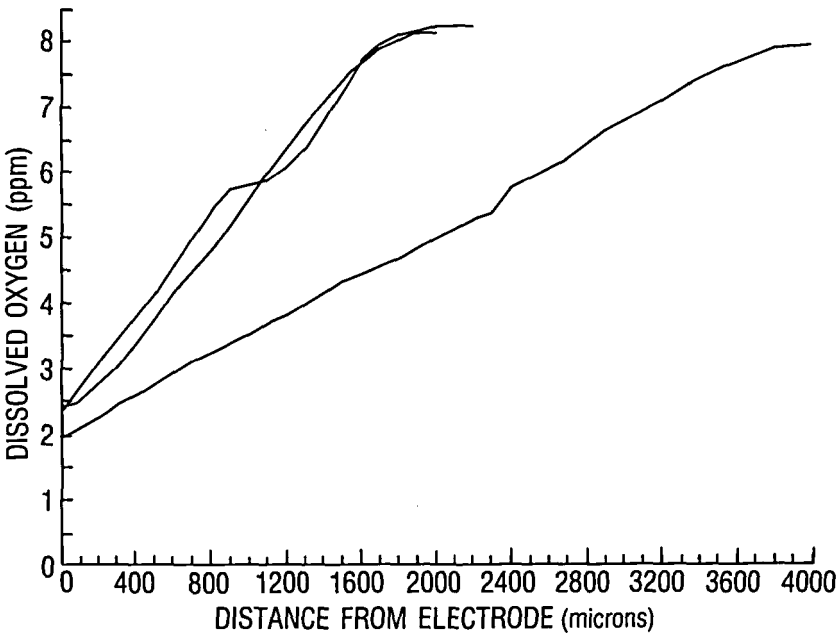


Fig. 7 Dissolved oxygen gradients through biofilm containing oxygen-producing diatoms (continuous illumination).



Fig. 8 SEM micrographs of biofilm from Gulf of Mexico water formed on S30400 during continuous illumination.

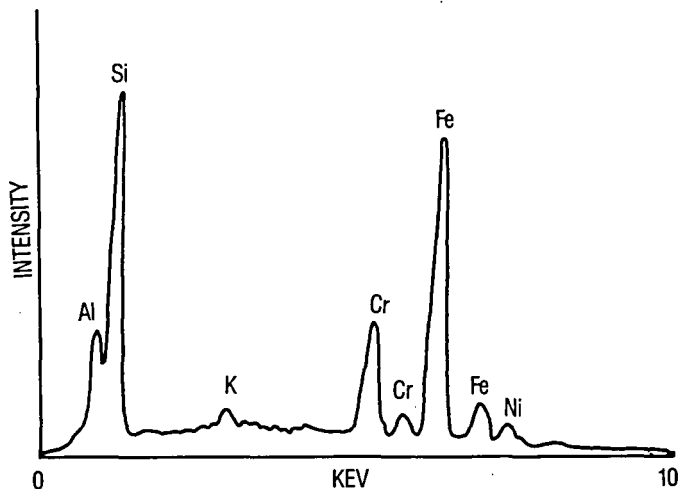


Fig. 9 EDAX spectrum from S30400 surface with biofilm containing oxygen-producing diatoms (continuous illumination).

Figure 12 shows the results of potentiodynamic anodic polarisation experiments for S30400 in 3.5% artificial seawater and 3.5% NaCl, during which surface pH was continuously measured. The impact of imposed potential on interfacial pH varies with the buffering capacity of the electrolyte. Current densities in Figure 12 are expressed in $\text{mA}\cdot\text{cm}^{-2}$. Therefore, the vertical scale can be simultaneously used to represent pH units or current densities. Hydrolysis of the dissolved metal ions from the corroding metal surface caused a marked decrease in the surface pH once the pitting potential had been reached. The lowest measured pH values at the metal surface were 1.57 for artificial seawater (1000 mV) and 0.11 for unbuffered NaCl (860 mV). Since the lowest pH measured in NaCl is below the lowest pH for microelectrode calibration, this value should be considered approximate. Both electrodes were pitted at the conclusion of the test.

DISCUSSION

Numerous observations of an ennoblement of the corrosion potential of corrosion resistant metals during exposure in seawater have been reported over the past decade. Among the metals exhibiting this behaviour are various stainless steels, titanium and platinum (Dexter & Lin, 1988; Holthe *et al.*, 1988; Scotto *et al.*, 1985; Johnsen & Bardal, 1985; Mollica & Trevis, 1976). Videla *et al.* (1989) have recently reported ennoblement for a copper/nickel alloy exposed to seawater.

The ennoblement of E_{corr} is usually explained by the acceleration of the cathodic oxygen reduction in the presence of a biofilm:



Mollica and Trevis (1976) and Scotto *et al.* (1985) have suggested that the observed ennoblement is due to organometallic catalysis of the oxygen reduction reaction. The catalysts are considered to be formed by the reaction of extracellular polymers within

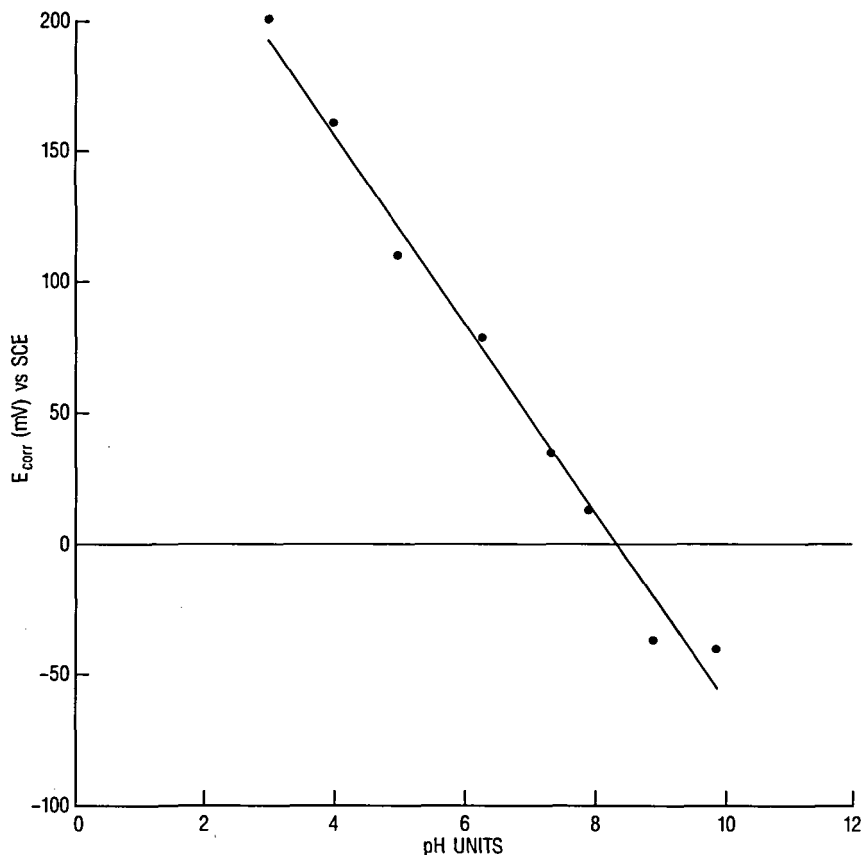


Fig. 10 Impact of pH on E_{corr} vs SCE of S31600. The pH of synthetic seawater was adjusted with 10% acetic acid and 10 N NaOH.

the biofilm and metal species from the corroding metal. This explanation has been widely published and adopted by other investigators to include all marine biofilms (Dexter & Goa, 1988; Cubiociotti & Licinia, 1989). The observation by Johnsen and Bardal (1985) that ennoblement could be observed with non-corroding metals, such as titanium and platinum, limits the applicability of such a proposed mechanism, but does not preclude the possibility of other undefined catalytic reactions.

S30400 was selected for the studies presented in this paper because of its documented susceptibility to crevice corrosion in seawater environments (LaQue, 1975; Vreeland & Bedford, 1970). The susceptibility of stainless steels to localised corrosion, including crevice corrosion and pitting, is often determined in laboratory tests by measurement of the pitting potential (E_{pit}), above which pits can initiate and grow. E_{pit} for stainless steel in NaCl is given by:

$$E_{\text{pit}} = a - b \log a_{\text{Cl}^-}, \quad (2)$$

where a and b are experimentally determined parameters (Uhlig & Revie, 1985). For S30400 the dependence of E_{pit} on the activity of the chloride ion a_{Cl^-} is given by:

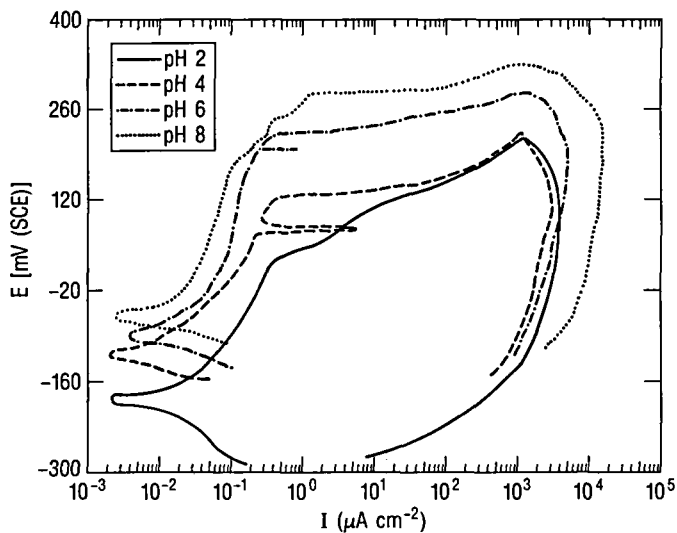


Fig. 11 Pitting scans for S30400 in an unbuffered, deaerated 3.5% NaCl solution as a function of pH.

$$E_{\text{pit}} = -0.074 - 0.088 \log a_{\text{Cl}^-} \quad (\text{V vs SCE}) \quad (3)$$

The closer E_{corr} is to E_{pit} , the greater the probability that pitting will occur.

In the present study it was found that biofilms forming on a variety of stainless steels including S30400 (Figs 1, 2 and 6) did not consistently result in an ennoblement of E_{corr} . Furthermore, localised corrosion was not detected by visual observation and SEM examination on any of the samples after exposure to Gulf of Mexico water.

Under reduced illumination, ennoblement was observed only in the abiotic solutions (Fig. 1). The corrosion potential moved in the more negative direction in the presence of a biofilm. Gulf of Mexico water contained 1×10^3 SRB, and after 15 days the metal/biofilm interface was uniformly anaerobic (Fig. 3), and sulfur had accumulated on the surface (Fig. 5). Ringas and Robinson (1987) and Gouda *et al.* (1991) have demonstrated a similar decrease of E_{corr} for a variety of stainless steels and copper alloys, respectively, in pure and mixed cultures of SRB. Two of the most documented activities of anaerobic bacteria are organic acid production and sulfate reduction. Highly localised areas of pH ranging from 5.2 to 9.2 were found in our study at the anaerobic metal/biofilm interface. The predominance of acidic or basic conditions at the interface directly influences the corrosion potential of stainless steels (Fig. 12).

A shift of the corrosion potential in the positive direction was observed when biofilms from Gulf of Mexico water developed under fully illuminated conditions. Under these conditions the interface remained aerobic and the interfacial pH was unchanged. These biofilms were dominated by diatoms, microscopic algae that have siliceous cell walls, and EDAX spectra from these surfaces indicated the accumulation of silicon. Diatoms can modify interfacial chemistry by influencing the local oxygen concentration, changing the pH and producing aggressive metabolites (Terry & Edyvean, 1986). Motoda *et al.* (1990) have demonstrated an ennoblement of stainless steel of 200–300 mV in the presence of photosynthetic diatoms. Dowling (personal communication) has produced consistent ennoblements of stainless steels using photosynthetic bacteria in the presence of light.

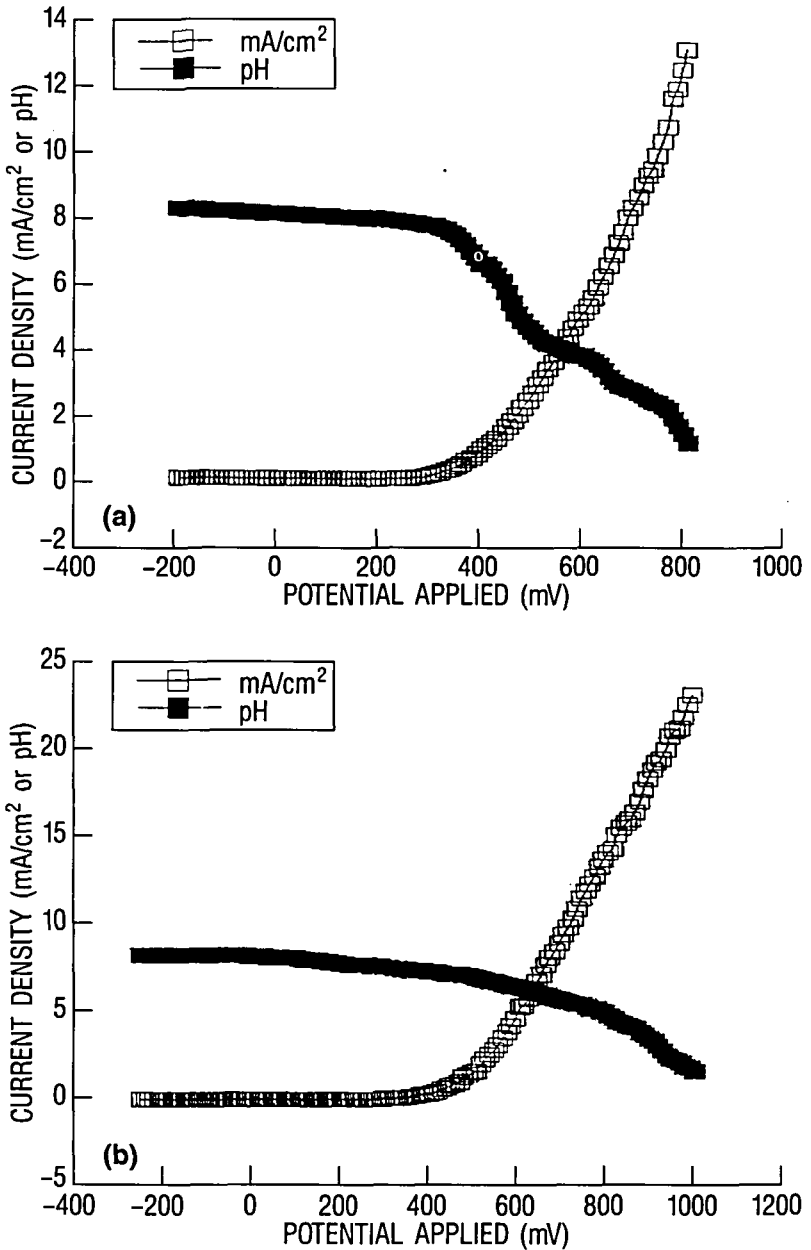
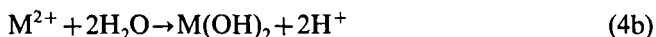


Fig. 12 Impact of imposed anodic potential on interfacial pH for S30400 in (a) 3.5% NaCl solution and (b) 3.5% artificial seawater.

In addition to direct production of acidic metabolites, microorganisms can indirectly influence local pH by accelerating cathodic and anodic reactions. For example, the cathodic reduction of dissolved oxygen results in an increase of pH at the metal/

electrolyte interface (Lewandowski *et al.*, 1989). If microorganisms force dissolution of a metal at anodic sites, metal cations are formed which may be hydrolysed by water with the formation of H^+ ions



causing a decrease in the interfacial pH as demonstrated in Figure 12. The differences between the pH values generated in artificial seawater and NaCl are caused by the higher buffering capacity of the seawater.

Ennoblement of E_{corr} could be due to either thermodynamic or kinetic effects. In terms of thermodynamic effects, localised acidification or an increase of the partial pressure of oxygen pO_2 would shift the reversible potential of the oxygen electrode (Eq. 1) in the positive direction. If all other electrochemical parameters of the system remained constant, an ennoblement of E_{corr} would result for corrosion resistant materials such as stainless steels and titanium. The effect of increasing pO_2 leads to very small increases in E_{corr} . In considering the possible role of localised acidification, account has to be taken of the results in Figure 11, where E_{pit} became more negative with increasing acidity of the electrolyte. This result shows that the parameter a in Equation 2 depends on pH. Since E_{corr} must be more negative than E_{pit} , it can be concluded that localised acidification, which would cause E_{pit} to shift in the negative direction, cannot explain the sometimes marked shift of E_{corr} in the positive direction. Kinetic effects could lead to an increase of the exchange current density, i_0 , for the oxygen reduction/oxidation reaction (Eq. 1) and an increase of the rate of the oxygen reduction at a constant potential. While catalytic effects due to the formation of organometallic compounds under biofilms have been suggested (Mollica & Trevis, 1976; Scotto *et al.*, 1985), no direct experimental evidence for such catalytic effects has yet been presented.

Mansfeld *et al.* (1990) exposed several stainless steels and titanium to flowing Pacific Ocean water for time periods up to 4 months. E_{corr} and the electrochemical impedance were monitored as a function of exposure time. Electrochemical impedance spectroscopy (EIS) was used to monitor the formation of the biofilm and the occurrence of localised corrosion. A significant ennoblement of E_{corr} was not observed under any of the exposure parameters which included light and dark conditions and seasonal variations. The impedance spectra did not show any changes with exposure time due to the formation of biofilms. Observations with SEM/EDAX confirmed the presence of a biofilm and EIS data confirmed the absence of pitting and crevice corrosion.

While it is clear that biofilms from marine waters can exacerbate localised corrosion of stainless steels and other metals, the details of the mechanisms which cause the increased corrosion are determined by the activities of the specific microbial constituents. Environmental factors, including, but not limited to, light level and photosynthetic reactions within the biofilm influence the interfacial chemistry which in turn affects the kinetics of the anodic and cathodic reactions involved in corrosion mechanisms.

CONCLUSION

The electrochemical behaviour of 304 stainless steel exposed to natural seawater is altered as a result of biofilm formation. The kinetics of the electrochemical processes

controlling the type and rate of corrosion reactions vary with the microenvironment at the metal/microbe interface. The direction in which E_{corr} is shifted depends on exposure conditions.

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