

Analysis of Carbon Steels Affected by Bacteria Using Electrochemical Impedance and Direct Current Techniques[☆]

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ABSTRACT

The failure of metal structures in contact with natural, untreated waters is frequently ascribed to bacterial corrosion.¹ This study compares the corrosive effects of *Vibrio natriegens* (*V.natriegens*) when in batch and continuous flow culture. Evidence is presented for enhanced corrosion of carbon steel resulting from "aerobic" culture of *V.natriegens* with two sulfate-reducing bacteria (SRB). The corrosion processes are quantified and, to some degree, described by nondestructive electrochemical impedance and direct current (DC) polarization techniques. The *V.natriegens*/SRB coculture is shown to induce a faster corrosion rate of carbon steel than *V.natriegens* alone or under sterile conditions. Batch culture permitted a faster corrosion rate than continuous flow systems. When continuous flow conditions were allowed to lapse into stagnation (batch culture), however, the highest corrosion rate was observed. This confirms practical experience in which metal failure caused by bacteria is often correlated with stagnation.

INTRODUCTION

Analysis of bacterial corrosion problems using electrochemical methods have usually been restricted to direct current (DC) potentiodynamic experiments where the various components required for the Stern-Geary equation are determined.²⁻⁵ This method is problematic since the acceptable criteria for Tafel behavior are easily breached. For example, in aqueous systems of low-ionic strength, high overpotentials may induce mass transfer effects. Iron-oxidizing bacteria are frequently found to accelerate corrosion in low-ionic strength fresh or brackish water systems where these effects may be large. The small DC potential changes used to determine the polarization resistance (R_p), for instance, modify surface films. Bacteria on the working electrode surface are particularly susceptible in this respect since large local fluctuations in potential are likely to disrupt internal processes.

Impedance techniques have been applied to corrosion problems in natural-water-containing systems from electrochemical⁶⁻⁹

and microbiological^{10,11} standpoints. The advantage of the electrochemical impedance technique lies in its relatively nondisturbing analysis of the surface effects of bacterial films. This results from the application of low-amplitude oscillating voltages or currents. As a result of these low amplitudes and zero net current flow, the problems of mass transfer are reduced considerably, and the technique might be suited for bacterial corrosion analysis in fresh water as well as in other electrolytes.

This study is concerned with analyzing the corrosive properties of *Vibrio natriegens* (*V.natriegens*) using the alternating current (AC) impedance technique to examine microbial influenced corrosion and determine the usefulness of the technique with respect to bacterial systems. This bacterium is characterized by production of organic acids at low partial pressures of oxygen; e.g., close to a metal surface, the bacterium produces short chain organic acids.¹² This bacterium was previously examined by Nivens,⁵ et al who showed that corrosion was facilitated by the secretion of extracellular material, probably polysaccharide and deposition of calcium hydroxide. The corrosion rate of monocultures of *V.natriegens* is also compared with that of a consortium of *V.natriegens* and sulfate-reducing bacteria (SRB) in the so-called aerobic water column. Examination of the metal surfaces for fatty acid biomarkers (molecules particular to specific bacteria) was also used to detect the bacteria. Such biomarkers have been used successfully to characterize bacteria involved in attachment to metal surfaces in seawater.¹³

MATERIALS AND METHODS

Media

Static batch culture media for Experiment I contained (g/L-filtered aged seawater) glucose 1.98, peptone 0.1, yeast extract 0.5, and Fe(III)phosphate 0.012. Continuous culture media for Experiments II and III contained (g/L) glucose 0.6, peptone 0.02, yeast extract 0.1, Fe(III)phosphate 0.1. Continuous culture media for Experiments IV through VI contained (g/L distilled water) 3 Na₂SO₄, 0.2 KH₂PO₄, 0.25 NH₄Cl, 20 NaCl, 4 MgCl₂, 0.5 KCl, 0.15 CaCl₂, 0.003 yeast extract, and 0.06 glucose. Complex vitamins, trace elements, and selenate solution were added to these experiments, as was described by Widdel and Pfennig.¹⁴ Phosphate buffering was provided by addition of K₂HPO₄ to a final concentration of 3.4 mM. Media pH was adjusted with Na₂CO₃.

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Bacteria

V.natriegens strain 4115 was obtained by R. Colwell and P. Brayton (University of Maryland). The *Desulfovibrio* and *Desulfobacter* species used in these studies were isolated from black sulfide-rich marine sediments off of Turkey Point (Appalachicola Bay, Florida). Inocula of 10 mL of fast growing cells were used routinely. Both SRB were inoculated into the dual cell in aerobic tris buffer (pH 7.0) after Cypionka,¹⁵ et al. Experiments involving the addition of SRB to one side of the dual cell in tris buffer were controlled by the addition of an equal quantity of sterile tris buffer to the other side.

Dual Cell System

All experiments were conducted in a modified dual cell^{16,17} (see Figure 1). The cell apparatus is a two-compartment chamber, with a working volume on each side of 385 mL, which is divided by a 0.2- μm , pore-size Teflon[†] filter. The filter allows for the passage of bacterial metabolites, but not the bacterial cells. During the experiments, a small aquarium pump constantly blew air through sterile filters (0.2 μm) into the head spaces while the medium was stirred magnetically. Constant flow experiments delivered the carbon-poor media at a constant rate of 0.1 h^{-1} to each side using a dual-channel peristaltic pump. In both batch and continuous flow experiments, media was delivered through line breakers which prevented the aerosol back contamination of the sterile media vessel. All lines to and from the dual cell were autoclaved to achieve sterility, except the cell itself. The working electrode coupon holder⁽¹⁾ and the cell were sterilized with 2% glutaraldehyde or 7% formaldehyde for 1 h. Subsequently, the cell was rinsed with 2 L of sterile distilled water, all in a laminar flow fume cabinet.

Waste media was removed by the slight head pressure provided by the air pump. All experiments were conducted at $\sim 20^\circ\text{C}$.

The dual cell allowed a three-electrode arrangement, including a flat coupon holder which exposed 1 cm^2 to the electrolyte. A SCE was introduced into both sides of the cell via an extruded tubular salt bridge with a glass frit in the end. The salt bridge was filled with potassium chloride solution. A counter electrode of titanium mesh, approximately twice the surface area of the working electrode, was also introduced into both sides of the cell. The tips of the salt bridges were placed no more than 5 mm from the working electrode surface.

Electrochemical Analyses

AC impedance analyses for Experiments I through III were performed using a Solartron[†] 1170 frequency response analyzer through a Solartron 1186 electrical interface using the stand-alone capability. Sine wave voltages and currents were monitored with a dual-channel 5110 Tektronix[†] oscilloscope. The applied voltage amplitude was 5 mV at frequencies between 2 and 5 mHz, and 10 kHz. Five frequencies were examined per decade. Output from the Solartron was via a strip chart printer through an IEEE 488-1978 (GPIB) board. These data were manually entered into a Tektronix microcomputer and analyzed using programs written by P. Blanchard and D. Choqueuse of IFREMER (Brest, France). AC and DC data for Experiments IV through VI were generated with an EG & G Model 368 impedance system controlled by an IBM[†] XT microcomputer. The impressed AC voltage amplitude was 5 mV at frequencies between 3 mHz and 10 kHz. This system allowed both control and data handling within the same program. DC determinations of the anodic Tafel slope and the current density for Experiments I through III were conducted via a Solartron 1286 electrochemical interface controlled by the microcomputer. The programs used were written by D. Choqueuse.

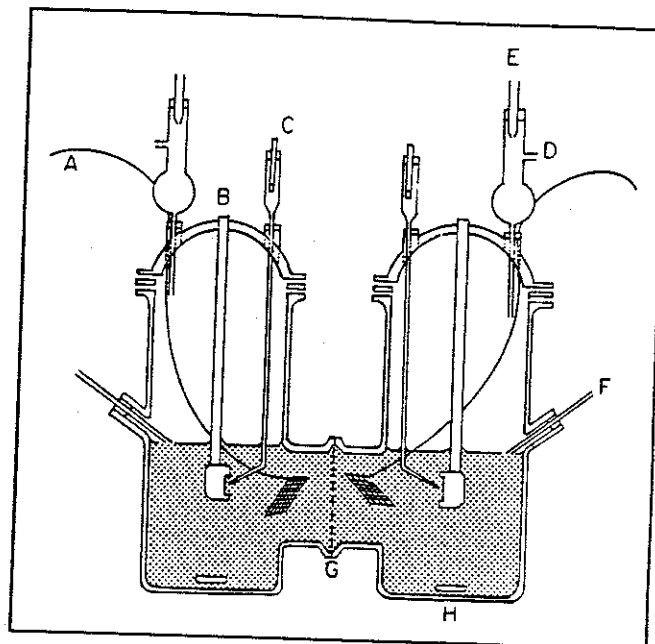


FIGURE 1. Dual cell apparatus adapted from Reference 6; (A) counter electrode, (B) working electrode, (C) reference electrode, (D) air in, (E) medium in, (F) medium and air out, (G) 0.2- μm pore filter, and (H) stir bar.

Metal Samples

Two similar carbon steels, E24 and C1020, were examined. Experiments I through III used E24 and Experiments IV through VI used C1020. The choice of metals was based upon construction use and availability in France and the USA respectively. The E24 flat coupons were polished with 1000-mesh aluminum oxide and then 0.25- μm mesh diamond. The C1020 flat coupons were given a 600-grit finish.

Analysis for Fatty Acid Biomarkers

Lipids were extracted from lyophilized metal coupons by addition of a one-phase chloroform:methanol:water mix (2:1:0.8 mL) mixture. The extracts were transferred to test tubes, and the single phase was broken by adding 1 mL water and 1 mL chloroform. The fatty acids ester-linked to phospholipids were recovered and analyzed by gas chromatography, as previously described.^{18,19}

RESULTS

The majority of the impedance diagrams exhibited single depressed capacitive loops with centers below the real axis [Figure 2(a)]. The results are tabulated as A which is the chord distance formed by the depressed semicircle on the real axis (Tables 1 through 4) and, for the purposes of this study, is assumed to be a good approximation of the polarization resistance (R_p).

A comparison of the corrosive effects of *V.natriegens* in batch culture (Experiment I) with that of a sterile chamber over a three-day period showed that the presence of the bacteria considerably increased the corrosion rate. The value for A for the sterile side was considerably larger (4.2 $\text{K}\Omega\text{-cm}^2$), with respect to the inoculated side (0.32 $\text{K}\Omega\text{-cm}^2$) and the initial value (1.2 $\text{K}\Omega\text{-cm}^2$) at 4 h. If the assumption that A is a good approximation of R_p proves true and the anodic Tafel slope (β_a) is assumed to be constant, then:

$$i_{\text{corr}} = \frac{\beta_a}{2.3A} \quad (1)$$

[†]Registered trade name.

^{††}Model K105. EG & G Applied Research, Co., Princeton, New Jersey.

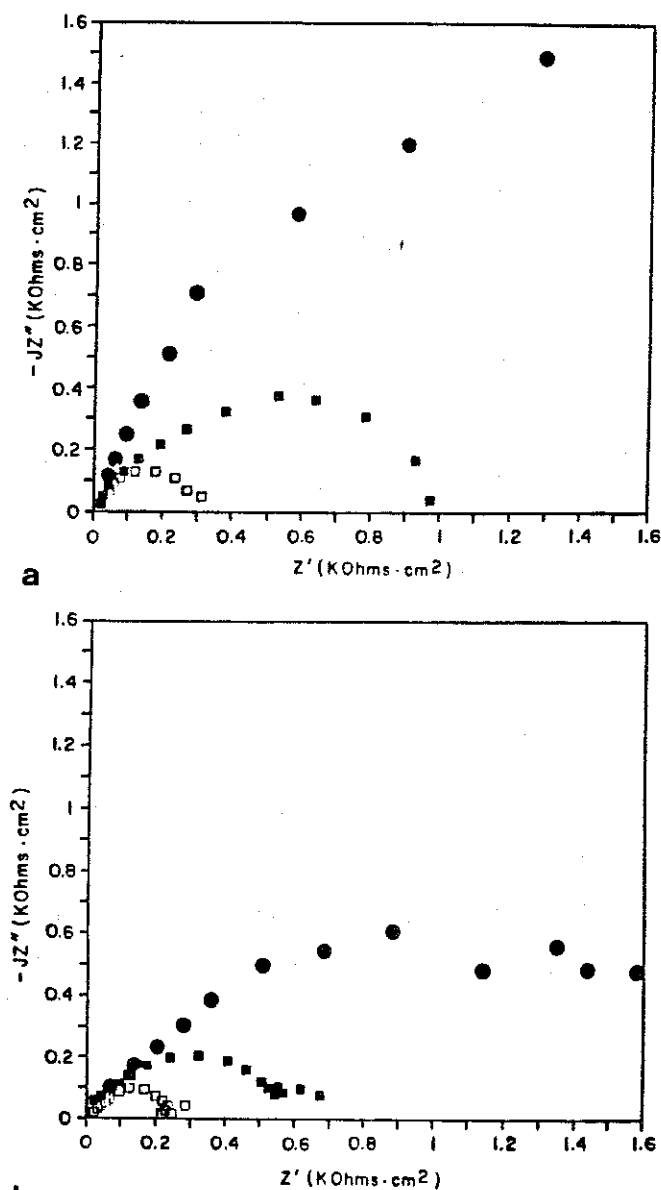


FIGURE 2. Impedance data obtained at (a) initial time (■) and after three days with (□) and without (●) *V.natriegens* in batch culture (Experiment I), and (b) after three days with the vibrio in continuous flow followed by 24 h in batch culture (Experiment II).

Thus, the presence of *V.natriegens* in batch culture accelerated the corrosion rate compared with the sterile side over the same period and also the initial rate before passivation.

The system was then set up, allowing the flow of media through both sides of the dual cell (Experiment II). The concentration of the carbon source was decreased to encourage the formation of a biofilm. Impedance analysis of the system after three days showed primary capacitive loops with and without bacteria, which had large values for A (2.0 and 1.98 K Ω \cdot cm 2 respectively in Table 1). The peristaltic pump was then halted, and the system was allowed to lapse into batch culture conditions. After 24 h more, the impedance behavior of both sides was examined and the value for A in the inoculated side was shown to have decreased to 0.25 K Ω \cdot cm 2 , indicating a high current density (Table 1). Subsequent DC polarization analysis showed that the current density i_{corr} and β_a were 100 μ A/cm 2 , 95 mV/decade, and 28 μ A/cm 2 , 137 mV/decade.

TABLE 1
Impedance Analysis of Carbon Steels
Affected by *V.natriegens* (Experiments I and II)

Experiment	Initial	With <i>V.Natriegens</i>	Sterile
I (3 days)	1.2	0.32	4.20
II (3 days (+ 24 h))	0.5a	2.00	1.98
	—	0.25	1.86

⁽¹⁾Values are given for A (K \cdot cm 2), which is the chord distance formed by the depressed semicircle of the complex plane plot.

TABLE 2
Impedance Analysis of Carbon Steel Coupons
Affected by Corrosion Products from a Large Steel Surface
and *V.natriegens* (Experiment III)

Day	Side A	Side B
0	—	1.4
1	1.9	1.3
2	5.2	3.0
3	8.8	10.2

For comparison with the impedance data, insertion of obtained values for A into Equation (1) gives corrosion rates of 165 μ A/cm 2 with the bacteria and 32 μ A/cm 2 sterile.

To determine if corrosion products could affect bacterial metal attack, large surface areas (190 cm 2) of E24 steel were placed in each side of the cell (Experiment III). Both the extra metal pieces and the electrodes were allowed to corrode for 15 h before inoculation of *V.natriegens* into both sides. The electrodes were analyzed for impedance behavior after 4 h and every day for three days (Table 2). Finally, DC anodic polarizations gave β_a and i_{corr} values for each side of 47 mV/decade, μ A/cm 2 , and 67 mV/decade, 5 μ A/cm 2 . Calculated final current densities [Equation (1)] from the impedance data, which compared well with the DC data, were 2.3 and 2.8 μ A/cm 2 .

The remainder of the experiments were conducted in a different medium to facilitate SRB. A *V.natriegens*/SRB consortium corroded slightly faster than the vibrio alone in continuous flow culture (Experiment VI, Table 3, and Figure 3). The SRB included a *Desulfotomobacter* species (which oxidize hydrogen) and a *Desulfobacter* species (which oxidize acetate). Both hydrogen and acetate were produced by the vibrio. The evolution of the impedance distribution was followed over eight days in continuous flow and then a further 24 h in batch culture. Finally, DC values for the polarization resistance (R_p) were obtained. These were 585 Ω \cdot cm 2 for *V.natriegens* alone; however, with the SRB, this was 540 Ω \cdot cm 2 . Final Impedance data showed the presence of a low-frequency capacitive loop with the monocultured vibrio, but not with the SRB consortium. However, Table 3 only gives a value for the primary capacitive loop A. Figure 3 (Day 9), however, shows the presence of a low-frequency secondary capacitive loop which was not included in the estimation for A in Table 3. After the medium pump was switched off and the system was allowed to lapse into stagnant conditions, the A values decreased in both sides of the cell, indicating an increased corrosion rate.

To determine the impedance behavior of the system under artificially decreased pH, an experiment (V) was performed where *V.natriegens* was run on one side of the dual cell and the coculture with SRB on the other side. Initially, the pH was stabilized at 7.0 with sodium carbonate and buffered with phosphate for 4 days; however, on Day 4, the medium was changed to one without buffer and a pH of 5.5 to 6.0. The impedance was monitored for 4 more days (Table 4) and then DC values for R_p were determined as 537 Ω \cdot cm 2 for *V.natriegens* alone and 561 Ω \cdot cm 2 for the coculture.

An investigation was conducted to observe the behavior of mild steel without contact with bacteria or their metabolites over a

TABLE 3
Impedance Analysis of Carbon Steel Coupons
Affected by the Combination of V.natriegens and Sulfate-Reducing Bacteria
or by V.natriegens Alone (Experiment IV)

Day	V.natriegens + Desulfovibrio species + Desulfobacter species	V.natriegens Alone	Condition
0	0.4	—	continuous flow
2	2.3	2.5	continuous flow
4	2.7	4.1	continuous flow
6	3.3	3.6	continuous flow
8	3.2	3.8	continuous flow
9	2.8	2.0	batch/static

⁽¹⁾Media buffered at pH = 7.0. The dual cell was in continuous flow for 8 days and then in batch culture for 1 day.

TABLE 4
Impedance Analysis of Carbon Steel Analysis
Affected by the Combination of V.natriegens and
Sulfate-Reducing Bacteria or by V.natriegens Alone

Day	V.natriegens + Desulfovibrio species + Desulfobacter species	V.natriegens Alone	Condition
0	0.75	0.75	7.0
2	1.85	2.65	7.0
4	1.50	2.30	7.0
6	0.70	0.80	5.5 to 6.0
8	0.80	0.75	5.5 to 6.0

⁽¹⁾Continuous flow system with buffered media was adjusted to pH 5.5 to 6.0 on Day 4.

four-day period (Experiment VI). The medium was identical to that used in Experiment IV at pH 7.0 with buffer. The results (Figure 4) show a rapidly increasing value for A with time.

Fatty acid analysis of the coupon surfaces in Experiments IV and V showed that the characteristic fatty acids of *V.natriegens* were present. These included cis-octadecen-11,12-oic acid (18:1w7c) and cis-hexadecen-10,11-oic acid (16:1w7c). No biomarkers for *Desulfovibrio* species or *Desulfobacter* species were detected.

DISCUSSION

The arguments for electrochemical impedance analysis as applied to corroding systems are similar to that for imperfect dielectrics²⁰ in that a surface corroding in a particular electrolyte has a resistive (real- Z'), as well as capacitive (imaginary- Z''), response to a sinusoidal perturbation. These elements may be represented in an equivalent circuit where values for the transfer charge resistance (R_t), and polarization resistance (R_p) may be included. In this article, the authors have elected to present the data as complex plane plots to avoid the smoothing effect of logarithmic functions. This allows for a simple diagram where imaginary impedances are plotted vs their real equivalents for a wide range of frequencies. Simple corroding systems where there is one rate-limiting step tend to produce a single capacitive semicircle where the chord is located on the real axis (see Figure 5). In fact, experimental evidence shows that the expected semicircle is depressed with the theoretical center located below the real axis. Wenger, et al²¹ provide a formal description of the depressed semicircle after Cole and Cole²² as:

$$Z = R + A/1 + (j\tau_c w)^{1-\alpha} \quad (2)$$

where Z is the total impedance, A is the chord distance actually located on the real axis, R is the uncompensated resistance, τ_c is the relaxation time constant, w is the frequency in radians, $j^2 = -1$, and α is a character describing the degree of flatness of the semicircle, which may have values between 0 and 1.0. In Figure

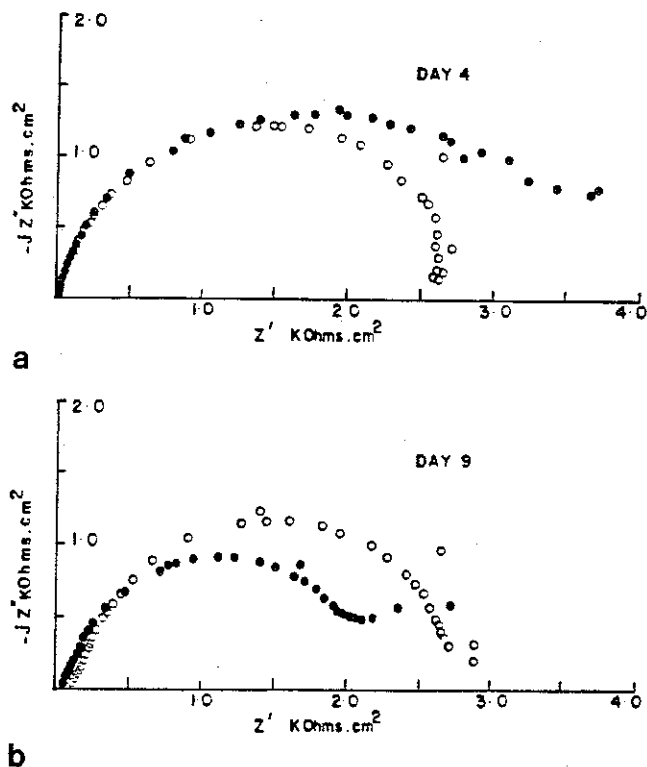


FIGURE 3. Impedance data of a monoculture of *V.natriegens* (closed circles) was compared with a coculture of the vibrio and two SRB (o) in continuous flow (Day 4) and batch culture (Day 9) (Experiment IV).

5, the depressed semicircle is compared to the idealized one located on the real axis after Debye.²³

$$Z = R + A/1 + (j \cdot Cdl \cdot R_p \cdot w) \quad (3)$$

where Cdl is the double layer capacitance. In certain situations, α is very small and the actual low-frequency intercept on the real axis may be an acceptable approximation of the idealized value R_p . The high-frequency intercept on the real axis provides R_t . The A values tabulated are the actual or extrapolated best-fit chord distances located on the real axis (or R_p for the idealized plot). R_p is related to the current density i_{corr} via the Stern-Geary equation:

$$i_{corr} = \frac{\beta_a \beta_c}{2.3 (\beta_a + \beta_c) \cdot R_p} \quad (4)$$

However, if the rate-limiting step is the diffusion of oxygen to the electrode surface the equation reduces to Equation (1).²⁴

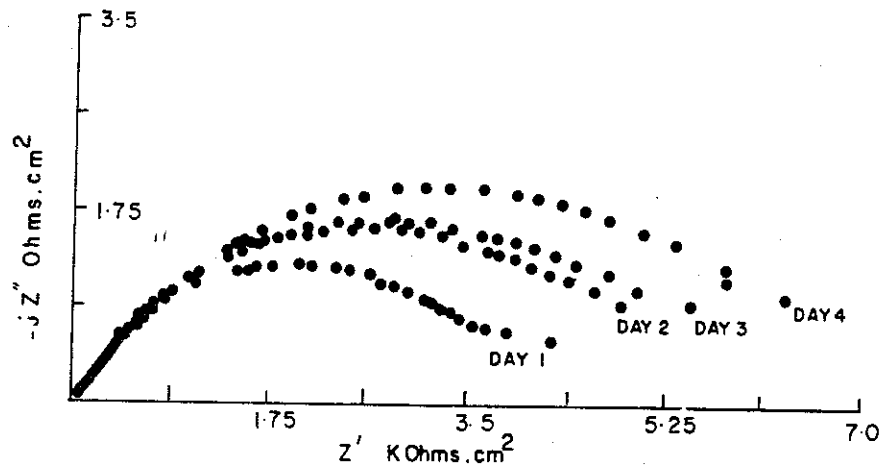


FIGURE 4. Impedance data showing the increasing values for A over four days as the passivation layer forms on a carbon steel electrode uninfluenced by bacteria or their metabolites (Experiment VI).

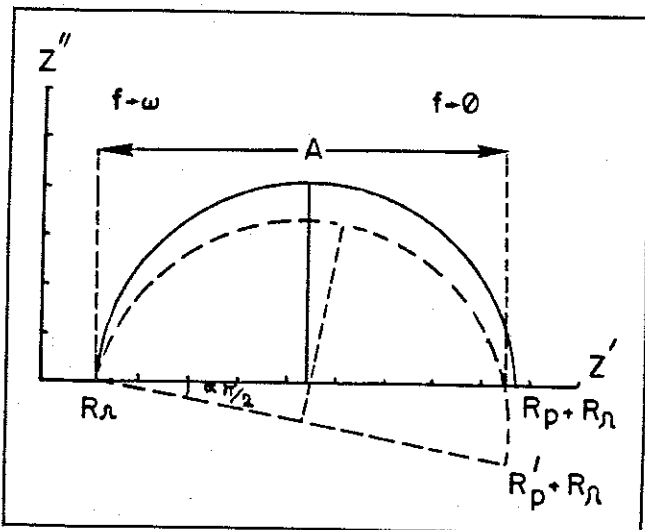


FIGURE 5. A comparison of expected complex plane impedance plots with (broken lines) and without (solid lines) dispersion of capacitance with frequency (R_p): theoretical polarization resistance (R_p'): actual polarization resistance obtained by data extrapolation (R_L): uncompensated resistance (A): the actual chord distance formed on the real axis.

A comparison of the corrosive effect of *V.natriegens* in batch culture [Experiment I, Figure 2(a), and Table 2] with that of a sterile chamber over a three-day period showed that the bacteria considerably increased the corrosion rate. A red oxide film was visible on the sterile side, but not on the inoculated side. This passivation layer apparently inhibited the dissolution of metal.

Generally, experiments designed to analyze the corrosive properties of bacteria have used static batch culture media with high concentrations of bioavailable carbon. Such rich media are environmentally unrealistic, except in the most polluted sites. Batch culture also introduces problems with the excreted metabolic products of the bacteria under testing. In the natural environment, these either diffuse away or are exploited by other bacteria of a different metabolic type. Both of these problems may be partially solved by continuous flow systems with low levels of carbon.

Examination of the impedance behavior of a continuous flow system with low-carbon levels showed that the corrosion rate was comparable between sides with and without bacteria. However, within 24 h of switching to batch culture (zero flow rate), the corrosion rate of the bacteria side increased with respect to the sterile

electrode. Anodic DC polarizations confirmed the impedance data. Thus, the corrosive effect of the bacteria was apparently minimized in continuous culture, but when the peristaltic pump was switched off and stagnating conditions were achieved, corrosion increased rapidly. In industry, condenser tubes and other ferrous metal structures in contact with untreated water have been shown to be at risk, particularly when normal operation is halted and stagnating conditions prevail.^{1,25,26}

Data from Experiment III showed that corrosion products have a significant effect on bacterial corrosion. Large metal surface areas were introduced into both sides of the dual cell. The working volume of the cell was adjusted to allow for the same dilution rate as in Experiments I and II. Large quantities of particulate corrosion products apparently adhered to the working electrode surfaces and prevented the attachment of the bacteria at the bare metal. This was shown by the rapidly increasing A value (Table 2) and DC data. This indicates that the passivation layer in E24 carbon steel may successfully prevent accelerated corrosion caused by fermentative bacteria in the short term. However, the situation where a metal surface is allowed to corrode in raw, untreated water before exposure to bacteria is unlikely.

While the failure of buried pipes partially caused by the redox and pH interfaces encouraged by bacteria is well-documented,^{27,28} the corrosion of other metal structures in environments where the bulk phase is oxygen saturated and by bacteria which are anaerobic is less understood. Hamilton²⁹ envisioned a biofilm of sufficient thickness and containing large numbers of oxygen-scavenging organisms such that the partial pressure of oxygen at the metal substratum is near to or at zero. Thus, at the metal/biofilm interface, anaerobic bacteria may grow upon the reduced products provided by fermentative bacteria. An example of those terminal carbon users are the SRB which respire at the expense of sulfate instead of oxygen.

Experiments with SRB cocultures used different media. A *V.natriegens*/SRB coculture corroded slightly faster than *V.natriegens* alone in continuous flow culture (Experiment IV). Also, upon halting the inflow of media and changing to batch culture conditions a low-frequency capacitive loop was observed only in the vibrio monoculture side (Figure 3, Day 9). Since a similar low-frequency capacitive loop appears in Experiment II under similar conditions (continuous flow culture lapsing into batch culture), it was concluded that the SRB modified the biofilm so as to slightly change the nature of the overall corrosion mechanism and thus change the impedance diagram. Duprat,⁶ et al ascribe the appearance of a similar low-frequency capacitive loop to molecular diffusion of oxygen though corrosion products which obscure the metal surface. In which case, the present data imply that the SRB may

remove or modify the particular corrosion product colloid that produces the secondary capacitive loop.

The data for the first four experiments is consistent with corrosion resulting primarily from pH changes by volatile fatty acid production by the vibrios. All media used in these experiments had some, though limited, buffering capacity. This prevents, to some extent, the dissolution of metal caused by a low pH. However, when the continuous flow of media was interrupted, it would be expected that the increasing concentration of organic acid produced by the bacteria would increase the corrosion rate. The corrosion rate did increase, but so did the pH (7.3) of the bulk phase.

To determine the effect of a lowered pH on the impedance behavior of the system the bulk phase pH was artificially decreased to 5.5 to 6.0 with HCL and without buffering capacity (Experiment V) midexperiment. The results (Table 4) show that the corrosion rate of both sides rapidly increased because of the lower pH. These results mimic the corrosion effect seen in Experiment IV when that system became static in batch culture and provides further evidence that most of the corrosion rate increase resulted from localized acidic conditions created by *V.natriegens*.

Fatty acid biomarker analysis of the metal coupon surfaces in Experiments IV and V showed that characteristic fatty acids of *V.natriegens* were present. Fatty acids characteristic of *Desulfobacter* species and *Desulfovibrio* species could not be detected because the biomass of SRB was very small. The difficulty of introducing anaerobic SRB into what is nominally aerobic media would certainly allow for only a small biomass in an 8-day period. In two separate experiments, over 8- and 9-day periods, the sides inoculated with SRB/*V.natriegens* cocultures consistently exhibited slightly higher corrosion rates than those with just *V.natriegens* (Tables 3 and 4).

All the above experiments show that two types of carbon steels in contact with the above bacteria or their metabolites tend to corrode rapidly. Experiment VI showed that C1020 steel not in contact with bacteria or their metabolites developed a thick passivation layer which produced an increasing value for A with time (Figure 4). Clearly, carbon steels in aggressive saline electrolytes with neutral pH and the absence of bacteria tend to generate oxide layers which retard the dissolution rate of metal.

CONCLUSIONS

- ▶ Two common types of mild steel (C1020 and E24) were shown to be susceptible to bacterial attack.
- ▶ The mechanism(s) of attack are unclear; however, organic acid production at the metal surface may be a major contributor.
- ▶ Stagnation or zero flow rate increases the corrosion rate substantially.
- ▶ The absence of the bacteria permits the formation of a passivation
- ▶ The presence of anaerobic bacteria may change the corrosion mechanism(s).

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