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Microbiologically Influenced Corrosion Testing

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Microbiologically Influenced Corrosion (MIC) Accelerated Testing Using a Flow-Through System

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ABSTRACT: Microbes recovered from sediments, slime, tubercles, or corrosion coupons were characterized into functional groups, identified by fatty acid patterns and used in a flow-through test system. Test solutions were prepared as field conditions and supplemented with nutrients for the growth of microbes. Four-sided working electrodes were fabricated to simplify experimental design by combining four steel disks from the same stock into one probe. Concentric electrodes were made to simulate localized corrosion and study the effect of bacteria upon stability of localized corrosion. Electrochemical techniques such as: open circuit potential, electrochemical impedance spectroscopy, and galvanic current measurements were performed to evaluate the corrosion of mild steel in solutions containing different combinations of bacteria. Actual microbial community on the electrode surface was recovered by culture methods for viable counts upon termination of experiments. Preliminary results indicated that this test system provided an accelerated testing to simulate field exposures.

KEYWORDS: microbiologically influenced corrosion (MIC), accelerated testing, multi-electrode, concentric electrode

Biofouling of industrial cooling water systems, marine fouling on offshore structures, and sulfide contamination of oil and gas reservoirs have emphasized the need for a better understanding of microbiologically influenced corrosion (MIC). Recently, there has developed a greater recognition of the complexity of the MIC process. It was proposed [*1–3*] that the mechanisms of MIC can be categorized into three groups: (1) production of differential aeration and concentration cells by biofilm formation resulting from the availability of dissolved oxygen at the metal/solution interface, (2) production of acidic metabolities, that is, organic and inorganic acids, or both, or the end products of fermentation growth by bacteria, and (3) interference in the cathodic process under oxygen-free conditions by obligate anaerobic bacteria and their metabolic sulfide products. However, the use of pure strains of bacteria in experiments has been criticized as being nonrepresentative of actual field situations. Therefore, it is necessary to develop an accelerated test system that can simulate field conditions with known ecological, physiological, and nutritional requirements for bacteria involved in corrosion processes. This paper describes the use of a flow-through system, electrochemical monitoring methods, and bacterial cell counts to evaluate MIC of mild steel in seawater and cooling water systems.

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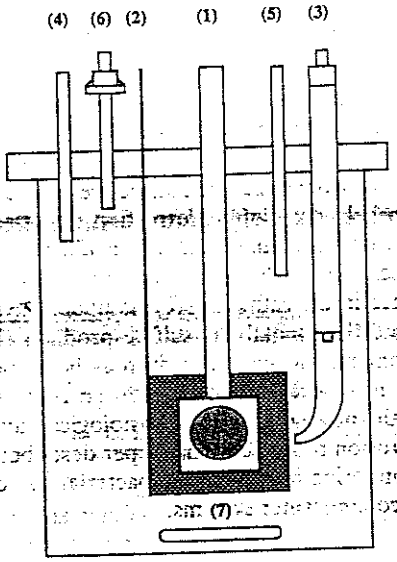
Experimental Procedure

Preparation of Microbial Cultures

Bacteria used in experiments were isolated from field solutions and corrosion coupons. Corrosion products were aseptically transferred to a test tube containing 10 mL of medium consisting of (in g/L): glucose 2, sodium lactate 2, NH_4Cl 0.5, KH_2PO_4 0.1, and $MgSO_4 \cdot 7H_2O$ [4]. This medium was then inoculated into three different types of solutions designed to be enriched for aerobic, fermentative, or sulfate-reducing microbial consortia. Total fatty acid composition of the consortia was measured after saponification in methanol to form the methyl esters. A Microbial Identification System including a Hewlett-Packard 5980A capillary gas chromatograph, autosampler, and computer with microbial identification database (Microbial ID, Inc., Newark, DE) was used to identify the consortia. Prior to starting an experiment, each culture was transferred to, and incubated in, a fresh culture medium for desired periods, centrifuged at 5000 rpm for 20 min and resuspended in a test solution. Test systems were inoculated with bacteria several times during the duration of an experiment to achieve adequate microbial populations and accelerate MIC testing.

Electrochemical Cell

A sterilizable, flow-through electrochemical cell, as shown in Fig. 1, consisting of a 600 mL glass beaker included: (1) a working electrode, (2) a Pt coated Nb mesh counter electrode, (3) a saturated calomel reference electrode, (4) a 0.2 μm sterile filter ventilation port, (5) a magnetically driven, Teflon®-coated stir bar, and (6) a test solution inlet and outlet. A



1. Working electrode
2. Counter electrode
3. Reference electrode
4. Solution intlet
5. Solution outlet
6. 0.2 um air filter
7. Stir bar

FIG. 1—*Electrochemical cell arrangement.*

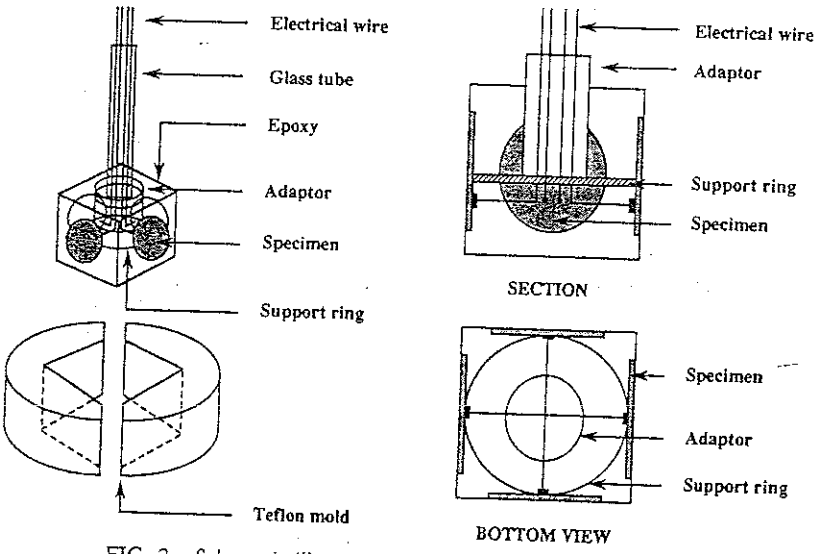


FIG. 2—*Schematic illustration of a four-sided electrode probe.*

four-sided working electrode [5] was fabricated to simplify experimental design by combining four 16-mm diameter AISI 1020 carbon steel disks into one probe, as illustrated in Fig. 2. It was reported that no interferences or crosstalk were observed between electrodes, and the interfacial chemistry at the metal/solution interface was reproducible [5]. In addition, a concentric specimen, as shown in Fig. 3, of AISI 1020 carbon steel was constructed to investigate localized corrosion (pitting) influenced by micro-organisms, where two circumferential steel electrodes were separated by a Teflon insulator ring and embedded in epoxy. The surface area ratio between the large circumferential electrode (25.4-mm diameter) and the small central electrode (2-mm diameter) was about 160:1. Under ambient atmospheric conditions, localized corrosion was simulated by cathodically polarizing the large electrode to −1200 mV Standard Calomel Reference Electrode (SCE) for 3 h, while the small electrode was left at its open circuit potential of −530 mV (SCE) [6]. After this preconditioning phase, the galvanic current between the cathode and the anode was monitored to determine the influence of bacteria upon pitting corrosion.

Test Solutions

Test solutions consisted of both synthetic seawater and cooling water. The preparation of synthetic seawater was based on ASTM Standard Specification for Substitute Ocean Water (ASTM D1141) with several modifications. The solution included (in g/L): NaCl 33.2, $MgCl_2$ 1.11, $CaCl_2 \cdot 2H_2O$ 1.32, Na_2SO_4 4.09, $NaHCO_3$ 0.21, KCl 0.695, KBr 0.101, NaF 0.003, and $SrCl_2 \cdot 6H_2O$ 0.025. Additional nutrients for marine bacteria growth (in g/L): NH_4Cl 0.1, yeast extracts 0.01, sodium lactate 0.05, glucose 0.01, vitamins 1 mL and KH_2PO_4 0.05 were added to accelerate the growth of inoculated bacteria. A simulated cooling water contained (in g/L): NaCl 0.073, NH_4NO_3 0.05, Na_2SO_4 0.12, $MgCl_2 \cdot 6H_2O$ 0.154, KH_2PO_4

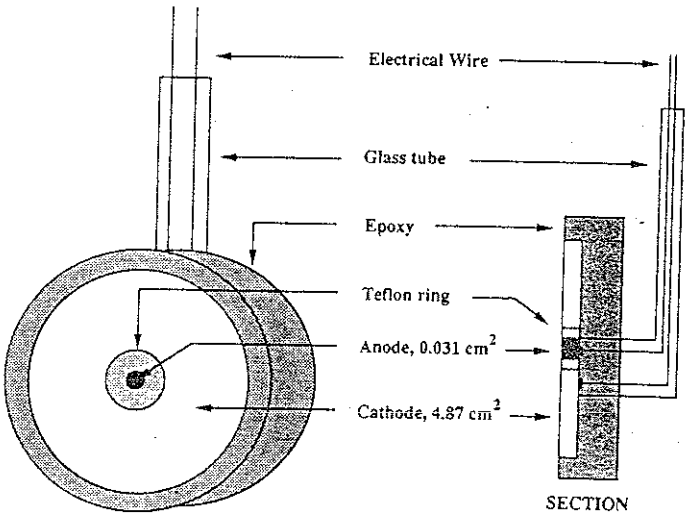


FIG. 3—*Schematic illustration of a concentric electrode.*

0.038, K_2HPO_4 0.124, $FeCl_3 \cdot 6H_2O$ 0.33 mL of a 10 mM solution and Hutner's salt solution 1.0 mL was also prepared. Total organic carbon content of the cooling water was adjusted to about 0.35 g/L by adding sodium lactate 0.8 g/L and sodium succinate 0.5 g/L.

Test Procedures

Prior to the start of each experiment, all specimens were wet polished in sequence with 240, 400, and 600 grit SiC paper, ultrasonically cleaned with distilled water, degreased with acetone, and sterilized with 70% alcohol for 20 min. The electrochemical cell was sterilized with ethylene oxide using the precautions defined previously [*4*]. All inlets and outlets for the cell were autoclaved to achieve sterilization. Test solutions were autoclaved at 121°C for 2 h and solution pH values were adjusted to 7.8 and 7.2 for the synthetic seawater and simulated cooling water, respectively, using either 0.2 M NaOH or HCl. During the test period, the solutions were maintained at ambient temperature, and the flow rate was controlled at 60 ± 5 mL/min by a dual-channel peristaltic pump.

Open-circuit potential (OCP) of test specimens was monitored at intervals of 1 h by a Hewlett-Packard model 3458A multimeter via a Keithley model 706 scanner controlled by a computer. Electrochemical impedance spectroscopy (EIS) analysis was performed by using the Zplot® software (Scribner Associates, Inc.), a Solartron model 1255 frequency response analyzer, and a potentiostat/galvanostat model 273 from EG & G Princeton Applied Research. Sinusoidal potentials of 5 mV were applied between 5 mHz and 10 KHz at 5 steps/decade. For cathodic polarization and galvanic current measurements, a Sycopel model DD10M potentiostat was used.

Total bacterial cell counts from bulk solutions and specimen surfaces were enumerated by acridine orange direct counts (AODC) after fixation in 2.5% glutaraldehyde [*7*]. Additionally, viable plate counts and the most probable number technique (MPN) [*8*] were employed to estimate specific aerobes and sulfate-reducing bacteria, respectively.

Results and Discussion

Microbial Activity Monitored by Open-Circuit Potential Measurement

MIC of mild steel in cooling water systems was studied by placing a four-sided electrode probe in the solution containing *Pseudomonas fluorescens* (*Lux*), hereafter referred to as 5RL, and *Desulfovibrio gigas* (*D. gigas*) for a time up to 200 h. 5RL was selected on the basis of its ability to reduce oxygen concentrations in the lower layers of the biofilm as the biofilm develops [9]. *D. gigas* is an anaerobic, dissimilatory sulfate-reducer. It was detected that 1.0E + 08 cells/mL 5RL and 1.0E + 06 cells/mL *D. gigas* existed in the culture media after 3 days of incubation. It is believed that the established 5RL biofilm on the metal surface may provide prerequisite anaerobic environments for *D. gigas* growth. Hence, inocula of 5 mL of 3-day old 5RL and *D. gigas* cultures into the electrochemical cells were performed at specimen exposure times of 0 and 96 h, respectively.

Typical open-circuit potential (OCP) versus time plots for specimens in the sterile and 5RL + *D. gigas* solutions are given in Fig. 4. For the sterile control, the OCP of specimens remained steady at −230 mV (SCE) for approximately 80 h and then decreased gradually to −350 mV (SCE). While in the solution containing 5RL, a rapid potential drop occurred after about 30 h of exposure and then maintained a −750 mV (SCE) for 10 h, followed by an abrupt increase in potential toward −300 mV (SCE) and kept increasing steadily until

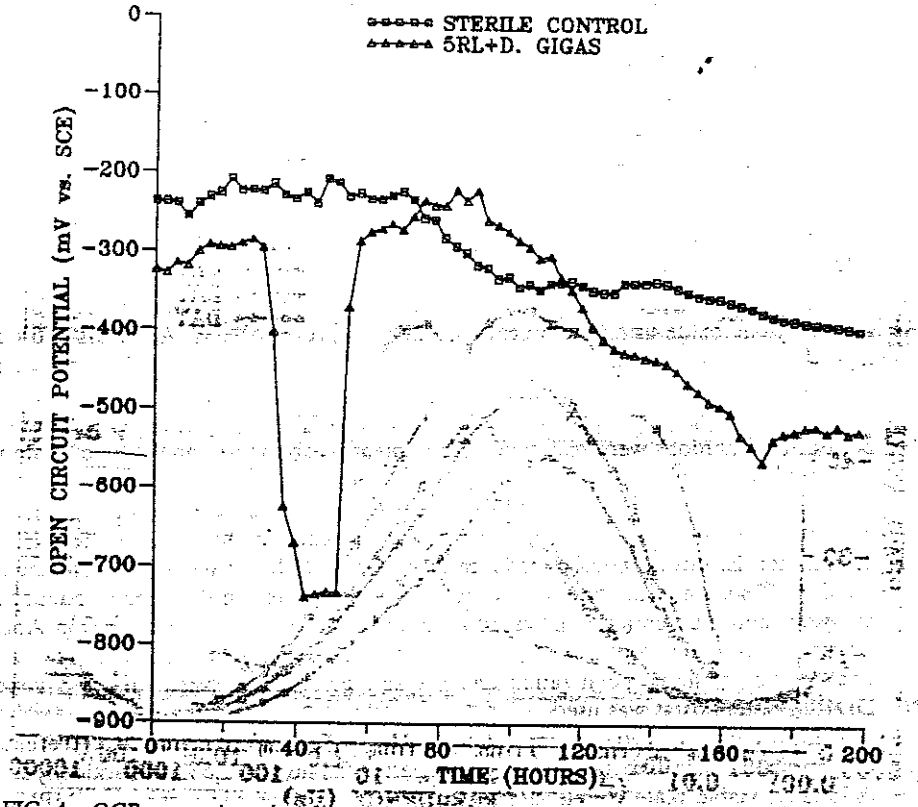


FIG. 4—*OCP versus time plots for specimens in the sterile simulated cooling water with and without inoculation of bacteria.*

D. gigas was added (at specimen exposure time of 96 h). The addition of *D. gigas* caused a gradual decline in potential from approximately −300 to −500 mV (SCE) throughout the rest of the experiment.

It is generally recognized that localized corrosion occurs when environmental effects induce heterogeneities on the metal surface. The physical presence of microbial cells on the surface, in addition to their metabolic activity, modifies electrochemical behaviors of the metal at the metal/solution interface. Adsorbed cells grow, reproduce, and form colonies that are physical anomalies on the metal surface, resulting in local anodes and cathodes. Under aerobic conditions, areas under respiring colonies become anodic and surrounding areas become cathodic [2]. Therefore, it is possible to infer that the colonization of 5RL on the steel surface, followed by nucleation of localized attack, caused a sudden drop in OCP. Subsequent sustained potentials at about −750 mV (SCE) indicated the propagation of the local attack. However, a mature biofilm could prevent the diffusion of corrosive species, such as oxygen, to the metal surface, thereby reducing the metal corrosion [*10*]. Hence, a relatively sharp increase in OCP (Fig. 4) could imply a uniform 5RL biofilm covered on the metal surface. In the presence of *D. gigas*, changes of OCP correlated to both activities of 5RL and *D. gigas* on the metal surface, that is, *D. gigas* modified the biofilm produced by 5RL so as to affect *D. gigas* colonization rates. Since 5RL has been frequently cited as a typical genus of slime forming bacteria, the established 5RL biofilm provided the prerequisite anaerobic habitat for *D. gigas* metabolic activity and growth. This was confirmed by viable counts, which showed 5.0E + 06 cells/cm² 5RL and 1.0E + 03 cells/cm² *D. gigas* on the coupon surface at specimen exposure time of 120 h, while 1.0E + 08 cells/cm² 5RL and 1.0E + 05 cells/cm² *D. gigas* were detected after 200 h of exposure.

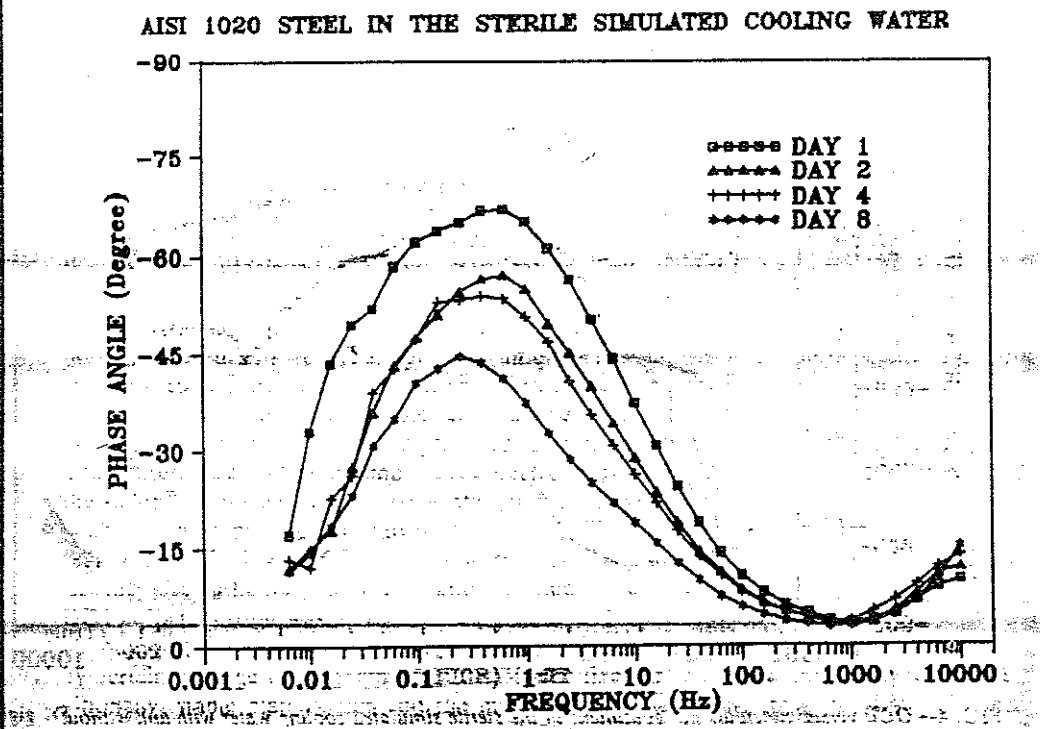


FIG. 5—*Bode plots for a specimen in the sterile simulated cooling water at various immersion times.*

Prediction of Biofilm Formation by EIS Technique

The activity of the biofilm estimated as volatile fatty acid production correlates with the average corrosion rate in terms of charge transfer resistance measured by EIS [*11*]. It was reported [*12*] that EIS can be used to study mechanisms of MIC with little or no damage to the numbers of viable bacteria in a biofilm, or to the activity of the bacteria. However, when corrosion reactions are complicated by diffusion constraints, that is, bacteria strongly adhered to metal surface and introduced diffusion gradients [*13*], Nyquist plots do not permit a reasonably accurate extrapolation of charge transfer resistances. Moreover, it was recognized [*14*] that the combination of microbial films and corrosion products often encountered in MIC causes the impedance to become very high at low frequencies, thus shifting the maximum phase angle to lower frequencies. As a result, an increase of the phase angle at the lowest frequency may reflect the formation of biofilms. Figures 5 and 6 present the phase angle versus frequency plots for four-sided specimens in the sterile cooling water with and without inoculation of 5RL + *D. gigas* at various immersion times. It is apparent that in the sterile control (Fig. 5), no significant changes of phase angle at the lowest frequency (5 mHz) occurred, while in the 5RL + *D. gigas* solution (Fig. 6), a steady increase in the angle (at 5 mHz) was observed. AODC from the coupon surfaces confirmed that the formation and aging of 5RL and *D. gigas* biofilms resulted in an increase of the phase angle at the lowest frequency.

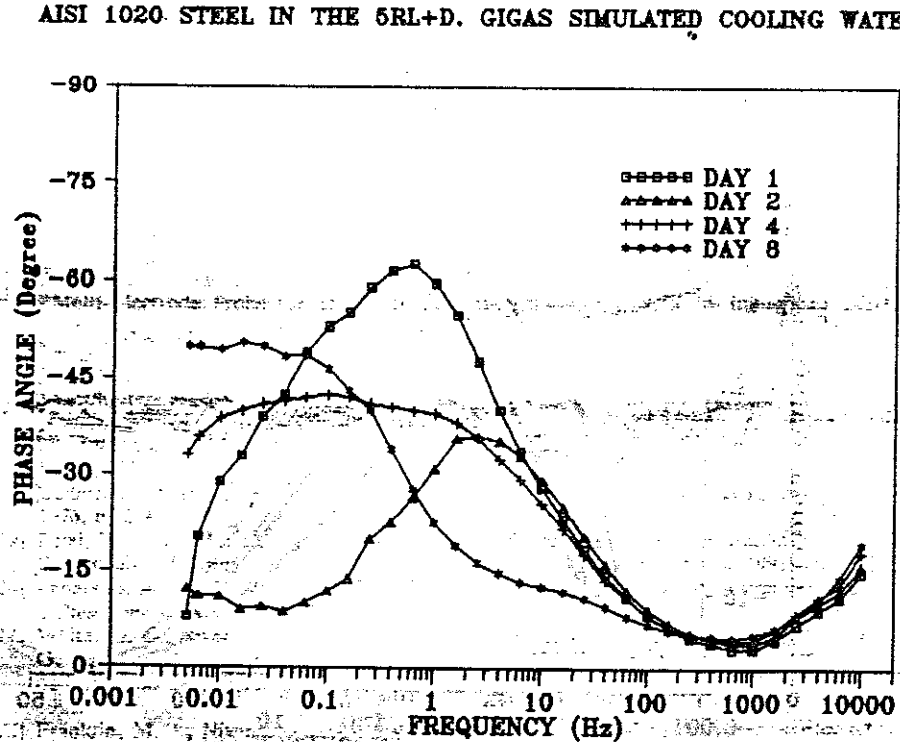


FIG. 6—*Bode plots for a specimen in the simulated cooling water containing 5RL + D. gigas at various immersion times.*

Effect of Sulfate-Reducing Bacteria Upon Pitting Corrosion

It is generally recognized that major field failures due to sulfate-reducing bacteria (SRB) are often in the form of localized corrosion, such as pitting. Pitting corrosion can be described as galvanic cells electrically short-circuited through the body of a metal. In the presence of active pits, galvanic currents between corroded areas and noncorroded sites should be persisted. In order to address these features, the galvanic currents of the preconditioned concentric specimens in the aerobic synthetic seawater containing *Vibrio natriegens, Desulfovibrio vulgaris,* and both were monitored. *V. natriegens* is a slime-forming, acid-producing heterotrophic aerobe, and *D. vulgaris* is a dissimilatory, sulfate-reducing anaerobe, which is ubiquitous in natural seawater. Since hydrogen evolution from cathodic polarization is beneficial to the growth of *D. vulgaris* [*15*], inoculation of bacteria into the flow-through electrochemical cells was performed before cathodically polarizing the large electrode to −1200 mV (SCE).

Figure 7 shows the galvanic current density between the anode and the cathode at open circuit as a function of time for concentric specimens exposed to aerobic synthetic seawater with different bacteria inocula. In all cases, a relatively sharp decrease in current density was followed by a steady final current density. In the presence of *D. vulgaris* alone, a higher

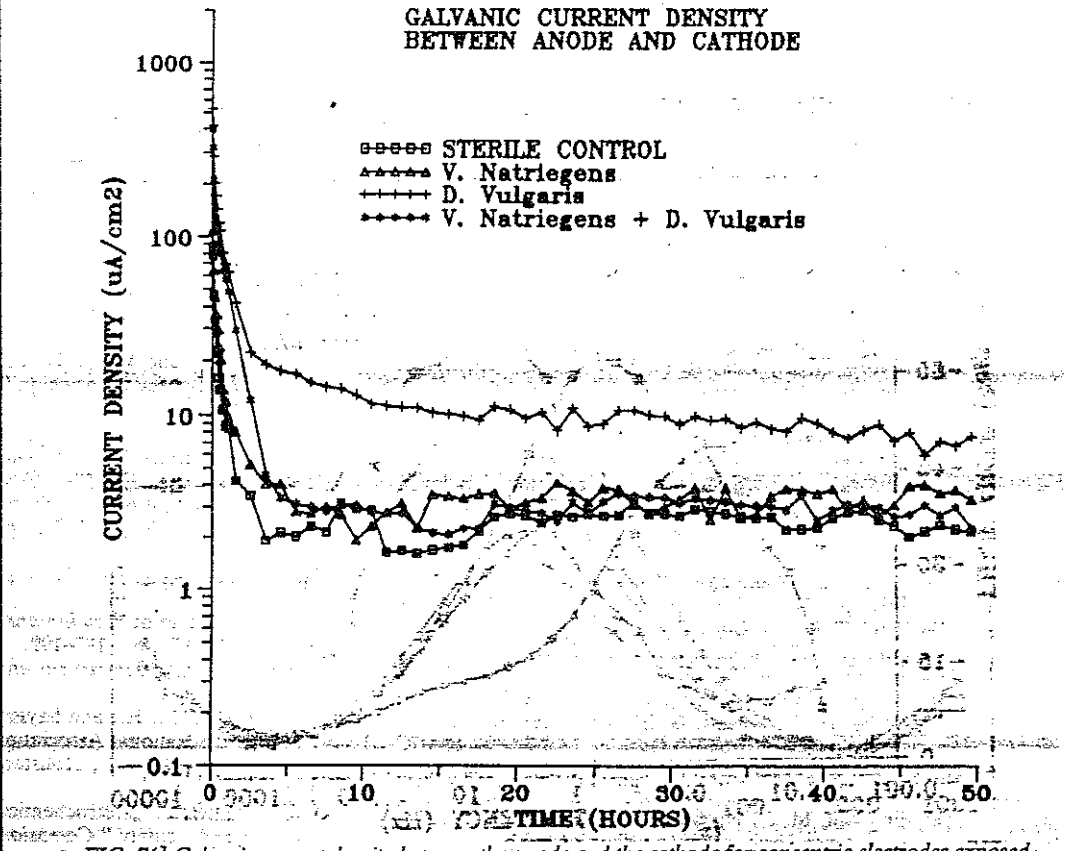


FIG. 7—*Galvanic current density between the anode and the cathode for concentric electrodes exposed to the synthetic seawater containing different types of bacteria.*

final current density (10 $\mu A/cm^2$) was obtained in comparison with that of sterile control or solutions with *V. natriegens*. It is possible to infer that the formation of a *V. natriegens* biofilm could make the anode and the cathode identical and that the galvanic currents between them became infinite. However, biofilms composed only of *D. vulgaris* are able to sustain the localized corrosion created by preconditioning the specimens. It was noticed that AODC of anodic and cathodic surfaces showed equivalent amounts (1.0E + 06 cells/cm^2) for the *V. natriegens* monoculture and the coculture but elevated numbers (2.0E + 07 cells/cm^2) of *D. vulgaris* on the anodic surface in the condition where the coupling current persisted. Consequently, the value of the galvanic current established between the anode and the cathode after the preconditioning phase can be a criterion to evaluate localized corrosion influenced by microorganisms [*16*].

Conclusions

(1) The utilization of the flow-through electrochemical cell associated with inoculation of bacteria accelerated MIC testing.
(2) In the presence of bacteria, changes in specimen open-circuit potential correlated with changes in the Bode plots from EIS measurements, which revealed the development of biofilms.
(3) Galvanic current measurements between the separated anode and cathode of the concentric electrode can be a useful technique to determine localized corrosion influenced by microorganisms.

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