MICROBIALLY INFLUENCED CORROSION OF CARBON STEELS

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ABSTRACT

Microbially influenced corrosion of pipeline steels is an economically important problem. Microbes form tubercles which block fluid flow and can facilitate localized corrosion leading to through-wall penetrations. Microbes of diverse physiological types and metabolic potentialities have been recovered from fresh tubercles or under-deposit corrosion and characterized. In tests utilizing sterilizable flow-through systems containing pipeline steel coupons, corrosion rates have been determined by non-destructive electrochemical means. These tests have indicated that increasing numbers of physiological types of microbes are inoculated into the system, the severity of the microbially influenced corrosion (MIC) generally increases. This study reports the MIC of monocultures and combinations of monocultures in an aerobic fresh water system with low sulfate as well as an anaerobic saline system. In both the aerobic and anaerobic systems, the combination of microbes induced greater MIC responses than did the monocultures. In tests involving a combination of microbes in both systems, in which one member was a sulfate-reducing bacteria (SRB), the corrosion mechanism was different for the control and the monocultures. This difference was indicated by the phase shift in the electrochemical impedance spectra (EIS). The localization of corrosion, that in many cases is the hallmark of MIC, may be initiated by the inhomogeneities of supposedly smooth metal surfaces. The scanning vibrating electrode technique (SVET) demonstrated non-uniform current densities over carbon steel electrodes polished to a 600 grit finish suggesting pitting and repassivation of pits in sterile medium.

INTRODUCTION

Mild steel pipes are an integral component in the transport of solutions and gases. Unfortunately, mild steel is susceptible to microbially influenced corrosion (MIC). MIC is being increasingly recognized as a serious problem when metal surfaces are exposed to natural waters[1]. MIC can often be recognized as corrosion associated with tubercles, slimes, discolorations, the odors of anaerobic metabolism, or sludges. The microbially mediated "under deposit corrosion" is often associated with a localized pitting process. Specific attack at weldments, heat affected zones (HAZ) and areas of stagnation (dependent areas where water collects) are also characteristics of MIC. Certain metallographic "signatures" can suggest MIC. Specific attacks on the austenitic or ferritic components of welds[2] or "tunnel" pitting of pipe line steel[3] suggest bacterial involvement.

It has been the general experience of this[4,5] and many other laboratories, that consortia of microbes with widely different physiological functions induce more serious corrosion than when isolated monocultures are utilized. In this paper, microorganisms with known MIC propensities were isolated, their physiology defined and phenotypic identification determined. These microbes were inoculated as monocultures and in various combinations in systems containing nutrient concentrations that stimulate biofilm formation. The resulting biofilm formation was monitored by open circuit potential (OCP) and the corrosion rate by electrochemical impedance spectroscopy (EIS).

The scanning vibrating electrode technique (SVET) allows non-destructive mapping of the localized anodic and cathodic sites on the working electrode. The potential for correlations between localized corrosion and the activity of MIC microbial consortia should now be possible.

EXPERIMENTAL PROCEDURES

Recovery of the Microbes

Organisms were isolated from tubercles recovered from utility service water systems. Intact tubercles were transported to the laboratory on ice in a plastic bag with sufficient water to maintain a saturated atmosphere. The isolations were initiated within 24 hours of pipe recovery. The tubercles were broken with a sterile scalpel and scraped into sterile test tubes containing glass beads. The contents of the test tubes were agitated with a sterile ultrasonic probe under a stream of nitrogen.

Isolation of the Microbes

Selective media of various types were utilized to recover individual bacterial colonies. Anaerobic bacteria were isolated from most probable number's technique (MPN) dilutions[6]. Isolated colonies were recovered from solidified Huntner's medium containing Huntner's salts and trace metals #44 [6]. A pH indicator was utilized to identify acid production and $FeSO_4$ was added to detect iron precipitation. For the MPN tests, 0.25 mg/l $FeSO_4$ was added to the medium to assay for sulfate reducing bacteria (SRB). Individual bacterial colonies were then screened using gram stain, oxidase, catalase, and the Miniaturized Microorganism Differentiation System (Minitek) assays.

Characterization of the Bacterial Isolates

Isolated bacteria were grown using solidified Huntner's medium with salts and trace metals [6]. Their total fatty acid composition was measured after saponification in methanol to form the methyl esters. The Microbial Identification System consisting of a Hewlett-Packard 5980A capillary gas chromatograph, autosampler, and computer with the microbial identification and library generation system (Microbial ID, Inc, Newark DE) was utilized to identify the isolates.

Coupon Preparation

Coupons (16 mm diameter) of AISI C1020 carbon steel were supplied by Metals Samples (Mumford, AL). They were finished to a 600 grit finish, sealed in epoxy, and any exposed edges were coated with additional epoxy under microscopic control to avoid any micro air bubbles. Multiple coupon holders were fabricated as illustrated in Figure 1. Counter electrodes of titanium (41mm x 147mm) were bent in a "U" shape and surrounded the working electrode. The counter electrodes were then soldered to a coaxial cable and covered with epoxy so only the titanium was exposed. Saturated calomel electrodes were fashioned into Luggin probes with glass fiber tips and used as reference electrodes. This design allowed the working electrode, that was being electrochemically analyzed, to be placed close to the tip of the Luggin probe. This system allowed replication of the electrochemical analysis to be done in the same flask. The surfaces of the working electrodes were polished with 600 grit and then lightly polished with 9 micron diamond paste. All polished surfaces were then cleaned with ethyl alcohol.

Test Apparatus

The electrochemical test cell was made from a 500 ml glass kettle with a viton o-ring seal at the top (Figure 1). The

kettle was covered with a polypropylene disk 1 cm thick through which leads for the multiple working electrode unit, the Luggin probe, the counter electrode, the inoculation port, the media entrance drip tube and the exit ports were placed. The inlet from the sterile media system was maintained under positive pressure to prevent back contamination. Ventilation ports for the drip tube and the kettle were connected to filter holders containing 0.2 um pore diameter filters. All ports were protected from air contamination when anaerobic experiments were performed. The vessels were mixed with a magnetically driven, teflon-coated stir bar.

The apparatus was assembled with double coverings of aluminum foil over each port then sterilized by exposure to an atmosphere of ethylene oxide/carbon dioxide 80/20 (relative humidity 60%) for 4 hours at 50° C. The test systems were immediately placed in drying ovens following sterilization to eliminate accumulated moisture. Wet steam autoclaving will induce corrosion of mild steel coupons. The apparatus was then connected to masterflex peristaltic pumps using silicon tubing. The medium utilized for aerobic experiments consisted of (in mg/l) glucose, 50; lactate, 50; ammonium chloride, 15; potassium dihydrogen phosphate, 5; hepta hydrated magnesium sulfate, 80. The medium was prepared in 45 liter glass carboys and autoclaved at 121 C for 5 hours. Filter sterilized glucose was added after the medium was autoclaved and cooled. The medium was allowed to cool with air equilibration using a 0.2 um microbiological filter vent. The medium was delivered through the drip tubes to the vessels at a rate of 60 ml/hr (15% replacement of the vessel volume/hr). The medium was removed from the vessels using masterflex pumps calibrated to deliver 100 ml/hour into sealed waste glass vessels. The vessels were aerated with sterile air at a rate of 20 l/min. All experiments were performed under aseptic conditions in a laminar flow hood.

For anaerobic experiments the media was purged with hydrogen:carbon dioxide:nitrogen 5:10:85 (v%) and pumped into the vessels which had been previously purged with the same mix of anaerobic gases. Traces of oxygen were removed from the gases by passage through hot copper filings. The medium contained (g/l) sodium sulfate, 3; potassium dihydrogen phosphate, 0.2; ammonium chloride, 0.25; sodium chloride, 20; hydrated magnesium chloride, 4; potassium chloride, 0.5, hydrated calcium chloride, 0.15; and one ml of 0.01% (w/v) resazurin. After autoclaving at 121° C for 5 hrs, the carboy was purged with the deoxygenated gas phase and Trace elements[7], selenate[7], vitamins[7], sodium bicarbonate (1.26 g/l) and sodium sulfide (36mg/l) were added. These items were added as filter sterilized solutions to the cooling carboy under the gas purge. The final pH was adjusted to 7.5.

Electrochemical Analysis

EIS analysis was performed using the Solartron 1286 (Schlumberger Technologies, Burlington, MA) electrochemical interface and 1250 frequency response analyzer controlled by a microcomputer. Sinusoidal potentials of 5 mV (rms) were applied between 3 mHz and 10 KHz. Results were plotted as the imaginary impedance versus the real impedance in a Nyquist diagram or as the log of real impedance versus the log of the frequency in a Bode plot. The system was monitored with a dual channel oscilloscope to verify the waveform of the perturbations. Open circuit potential (OCP) was measured with a Solartron 7081 precision voltmeter controlled by a computer. Measurements were made every 10 minutes throughout the experiments.

The scanning vibrating electrode measurements were made with an apparatus described by Franklin et al.[8].

RESULTS

DIFFERENTIAL EFFECTS OF MICROBES IN AN AEROBIC FRESH WATER SYSTEM

The medium used in these experiments contained 300 uM of sulfate, which is in the range of freshwater lakes and streams[9]. Three physiological types of bacteria were used in these experiments. The first organism used was a gram positive, spore-forming, Bacillus sp., which produced only traces of acid when grown on glucose or lactate and was an obligate aerobe. It was isolated from a tubercle recovered from a mild steel pipe in a utility. The same tubercle also yielded a gram-negative facultative anaerobe which produced large amounts of acetic acid and hydrogen when grown anaerobically on glucose. The anaerobe also grew on lactate. The organism was identified as Hafnia alvei when it was grown on standard trypticase soy medium and analyzed with the MIS-Hewlett Packard Microbial Identification System. The Bacillus sp. most closely matched the fatty acid pattern of Bacillus thuringiensis. Desulfovibrio gigas, culture #19364, was procured from the American Type Culture collection for the co-culture experiments. This organism was a hydrogenase positive, lactate-utilizing SRB which was an obligate anaerobe.

Biofilm Formation

Medium flow was cut off at inoculation to prevent the bacteria from being washed out of the reaction vessel and also to allow time for the bacteria to adhere to the surface of the working electrodes. The stirring rate was kept constant over the course of this experiment. When the vessels were inoculated with any of the monocultures, with the three bicultures, or with the

triculture, a visible biofilm formed on the working electrodes in a period of about 15-25 hours. The formation of the biofilm resulted in a drop in the OCP (Figure 2). As colonization progressed on the working electrode surface, the OCP declined rapidly and during the 24-28 hour period (see the arrows in Figure 2) the OCP dropped from -250 mV to -650 mV vs/Sce. The control flask OCP remained at approximately -250 mV. At about 30 hours, the system was manipulated by changing the flow rate to 5 ml/min. This resulted in a large rise in the OCP for the inoculated flask and only a small perturbation in the control flask. At 35 hours, there was a power failure and both the pump and the stirring ceased for about 6 hours. The OCP computer has a backup power supply so data was still being collected during the power failure. Surprisingly, no drop in OCP was observed for the sterile control during the absence of stirring and medium flow. When the power came on (arrow in Figure 2) there was a second period of OCP increase in the system containing the biofilm (but not in the control system). The OCP stabilized after 57 hours and then continued on a gradual decline throughout the rest of the experiment.

Corrosion Rate

The charge transfer resistance (determined by EIS) for the sterile control, the Bacillus sp. monoculture, the Bacillus sp. + H. alvei and the Bacillus sp. + D. gigas from the 4th to and 16th day of exposure are illustrated in Figure 3. The corrosion rate was the lowest for the sterile control (R_{ct} > 2000 ohms cm^2) over the course of this experiment. The Bacillus sp. monoculture corroded slightly faster. The biculture of the Bacillus sp. + H. alvei showed still greater corrosion. Maximal corrosion was observed with a biculture of Bacillus sp. and D. gigas (600 ohms cm^2). The biculture containing H. alvei and D. gigas or the triculture showed a corrosion rate that was similar to the Bacillus sp. + D. gigas biculture (data not shown in Figure 3). Charge transfer resistance values were measured in triplicate with different working electrodes in the same vessel. The differences in the corrosion rates were found to be statistically significant.

Corrosion Mechanism

Figure 4 shows differences between the phase angle on mild steel with biofilms and sterile controls. These measurements were made by EIS. The presence of the SRB in the biofilm consortia caused the most pronounced phase shift. The Bode plots showed a maximum phase shift at a frequency of 4.65 Hz in the sterile system. The Bacillus sp. + H. alvei biculture showed a maximal phase shift at 1.32 Hz and the triculture at 0.782 Hz.

DIFFERENTIAL EFFECTS OF MICROBES IN AN ANAEROBIC SALINE SYSTEM

The effects of monocultures of an acetogen and an SRB in an anaerobic system were examined. The acetogen Eubacterium limosum (American Type Culture Collection # 8486) is a gram-negative bacterium that forms acetate from hydrogen and carbon dioxide. This organism was used in combination with a Desulfovibrio sp., which was isolated from a tubercle recovered from a marine ship hulk. This SRB was identified from its phospholipid ester-linked fatty acid pattern as described by Edlund et al. [10] and its pattern of total fatty acids using the MIS-Hewlett Packard Microbial Identification System. The SRB grew on lactate and was hydrogenase positive. It did not grow as a monoculture in the medium used in this experiment.

Corrosion Rate

A biofilm formed after several days in the inoculated anaerobic systems and this resulted in a slight (~20 mV) decrease in the OCP. The average corrosion rate measured as the charge transfer resistance by EIS showed a gradual slowing over a period of 5 days to a steady rate in the uninoculated sterile control (Figure 5). In the system inoculated with the acetogen E. limosum, there was a slightly greater corrosion rate. The biculture of E. limosum + Desulfovibrio sp. showed the greatest corrosion rate.

Corrosion Mechanism

The Bode plots from the EIS analysis of the corroding anaerobic system are shown in Figure 6. The sterile control and the biofilm of E. limosum have a similar phase shift maximum at around 10 Hz. The diculture of E. limosum + Desulfovibrio sp. had a maximal phase angle at about 0.9 Hz.

A TECHNIQUE FOR THE NON-DESTRUCTIVE LOCALIZATION OF CORROSION ACTIVITY

Figure 7 illustrates the current density map over a mild steel coupon exposed to sterile aerobic medium. This map was obtained with the scanning vibrating electrode technique (SVET). The exposed area was 18.4 mm^2. Figure 7a indicates the presence of a local anodic site which formed after 0.5 hours of exposure to the sterile medium. Figure 7b shows the same sample after 3 hours of exposure. The anodic activity seen at 0.5 hours became inactive and a new anodic site was observed at the 3 hour mark. Pitting and repassivation occurred over the course of the 29 hour experiment (data not shown). The addition of bacteria resulted in the propagation of the anodic site[8].

DISCUSSION

EFFECTS OF MICROBIAL CONSORTIA WITH DIFFERENT PHYSIOLOGICAL CAPABILITIES ON THE CORROSION RATES AND MECHANISMS OF PIPE LINE STEEL

In the experiments illustrated in this paper, we showed that multiple physiological types of active bacteria in a sessile biofilm enhanced corrosion to a greater extent than did monocultures. In the flow-through aerobic system, the presence of acid forming facultatively anaerobic bacteria potentiated the growth and corrosiveness of a biofilm of the strictly aerobic Bacillus organism. The addition of the SRB, Desulfovibrio gigas, to an aerobic system with typical freshwater concentrations of sulfate, did not result in colonization unless an aerobic bacteria was also present in the biofilm. The combination of an aerobe (or an aerobe + a facultative anaerobe), generated an anaerobic niche adjacent to the metal surface. In experiments not reported herein, the characteristic "signature" phospholipid ester-linked fatty acids of each species have been recovered directly from the biofilms on the working electrodes used in these experiments (R.F. Jack, unpublished experiments). The anaerobic niche allowed the SRB to colonize the biofilm, grow and subsequently increase the corrosion rate (Figure 3).

The corrosion mechanism, as indicated by the maximal phase shift in EIS of the biofilm, was clearly different in the biofilms containing the SRB. This is illustrated with the triculture (Figure 4) and was true with bicultures of the SRB and either aerobe (data not shown).

In an anaerobic system with only gaseous carbon and electron donors, the biculture of the acetogen (E. limosum) and the SRB (Desulfovibrio sp.) resulted in an accelerated corrosion rate (Figure 5) as compared with the sterile control or the monoculture of E. limosum. The SRB could not colonize the mild steel electrodes in this medium unless the E. limosum was also present. E. limosum generates acetate and the SRB can use this acetate as a source of electrons for its metabolism.

The EIS analysis of the maximal phase shift angle for the anaerobic system (Figure 6), were quite similar to the aerobic system (Figure 4). The activity of the SRB in the biofilm induced a shift in the frequency of the maximal phase angle to lower frequencies of the EIS analysis (Figure 6). Oltra and Keddam [11] suggested that these shifts of maximal phase shift angle to lower frequencies may be associated with more localized corrosion.

NONDESTRUCTIVE MONITORING OF MIC

The OCP provides a convenient, non-destructive tool for following the maturation and status of biofilms on the working electrodes (Figure 2). The electrochemical signal perturbations corresponded with the extractable lipid phosphate and acridine orange direct cell counts (Unpublished data).

The EIS offers a means to follow the average corrosion rate as well as give indications of localized corrosion. Non-destructive measurements using electrochemical techniques such as EIS, OCP, and small amplitude cyclic voltametry (SACV), have been shown to work effectively in the on-line monitoring of MIC [12]. The EIS analysis allows determination of both the solution resistance and the polarization resistance[12]. When the polarization resistance or charge transfer resistance are known, the average corrosion rate can be determined. Comparison of I_{corr} from the anodic Tafel slope and R_p (determined with EIS), and DC polarization analyses showed equivalence between the two measures [13]. The DC polarization measurements destroy the biofilm. Repeated measurements of R_p by EIS, on noble electrodes with biofilms show no evidence of damage to the biofilms.

One advantage of EIS measurements is that response to effects of surface inhomogeneities in resistance and capacitance is accentuated at lower scan frequencies. This has been interpreted as evidence for more localized activity [11].

LOCALIZED CORROSION

One of the central problems in monitoring MIC is the detection of localized corrosion. A low average corrosion rate can nevertheless create problems if the corrosion is highly localized and a through-wall lesion develops. In each of the systems which contained the SRB's, there was a pronounced increase in MIC activity and a shift of the maximal phase angle of the EIS to slower frequencies (Figures 4 and 6). The utilization of the scanning vibrating electrode showed reversible anodic sites over the surface of carbon steel coupons (Figure 7). SVET analyses, in combination with consortia such as those utilized in this study, should be useful in further characterizing the relationship between local activity of biofilms and localized corrosion.

CONCLUSIONS

1) The formation of a biofilm on mild steel coupons resulted in a lowering of the open circuit potential (OCP). Changes in the bulk phase conditions such as changes in the rates of stirring or the flow rate, were reflected in shifts in the OCP.

2) The activity of a consortia of microbes of different physiological groups intensified the average corrosion rate. Some combinations of organisms exhibited greater MIC activities than did others. In these experiments the biofilms which contained sulfate-reducing bacteria, were also the most corrosive biofilms.

3) The inclusion of sulfate-reducing bacteria in the biofilm consortia increased the average rate of corrosion in both the aerobic freshwater system and the anaerobic marine system. EIS showed shifts in maximum phase angle to lower frequencies in systems containing biofilms. This shift was most pronounced when SRB's were present in the biofilms. The shift towards lower frequencies for the maximum phase angle has been interpreted as indicating more intensive localized corrosion.

4) SVET can be utilized to map localized anodic sites over carbon steel coupons.

5) The techniques demonstrated in this paper should allow the dissection of complex microbial communities that produce MIC in nature.

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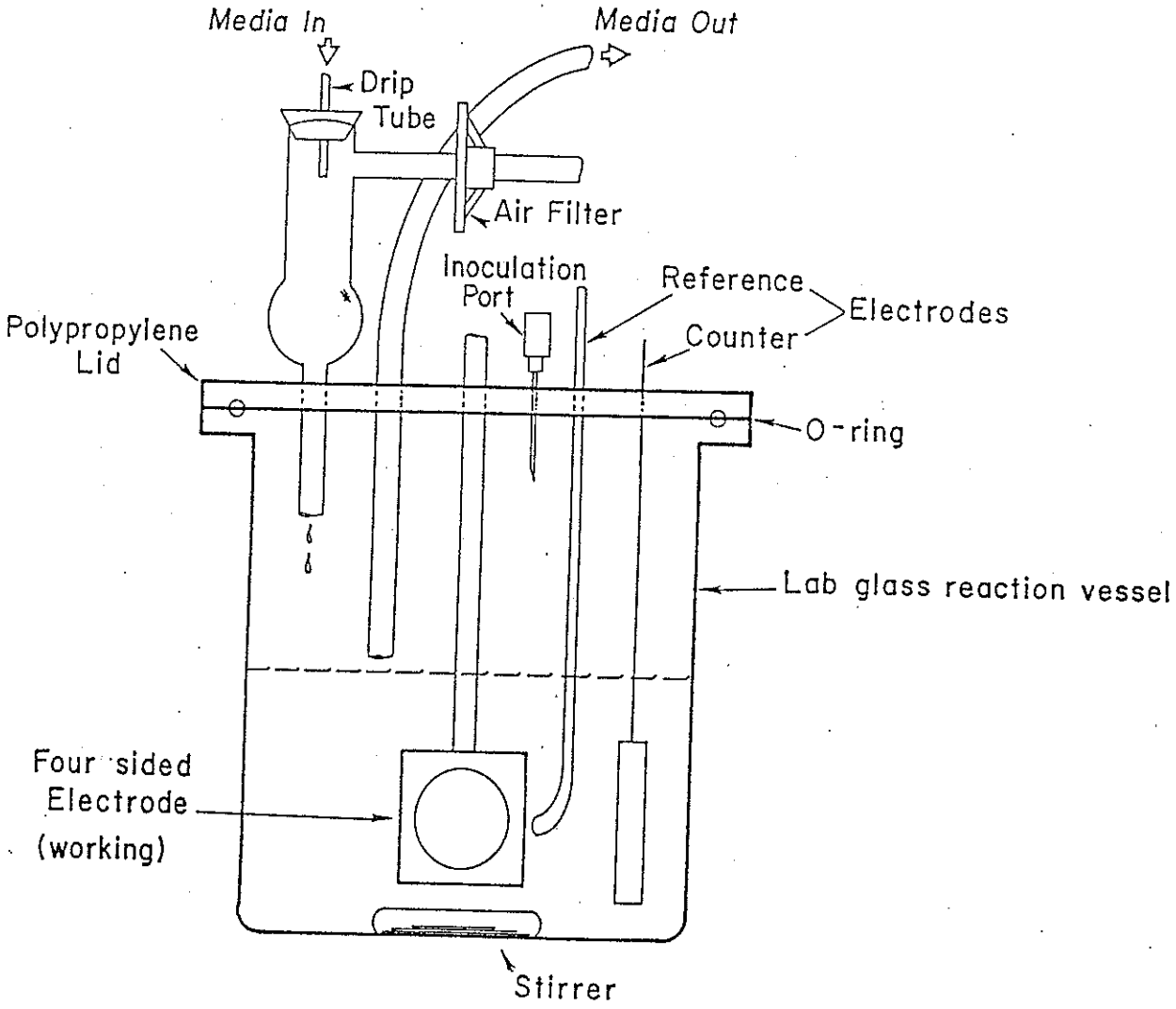

Figure 1. Experimental test vessel for the electrochemical analysis of corrosion by various combinations of monocultures. Both the media in and media out are connected to reservoirs with masterflex pumps and silastic tubing. The four-sided electrode can be rotated so each face of the working electrode can be placed adjacent to the reference electrode during the electrochemical analysis.

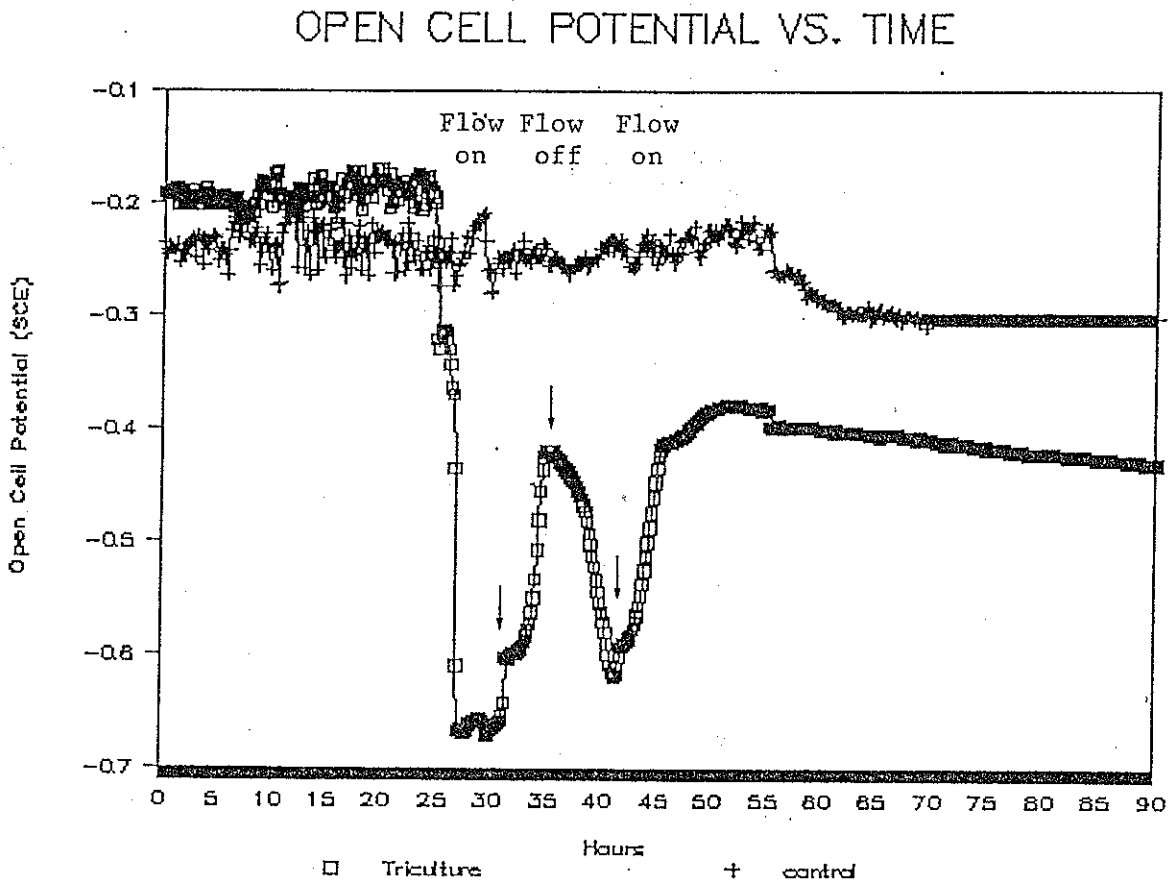

Figure 2. Open cell potential trace of the control (without microbes) (crosses) and the triculture consisting off the Bacillus sp. + H. alvei + D. gigas (open squares) against the calomel electrode. The effects of the microbial biofilm (initial drop) and manipulations in stirring and flow rate are indicated in this aerobic freshwater system.

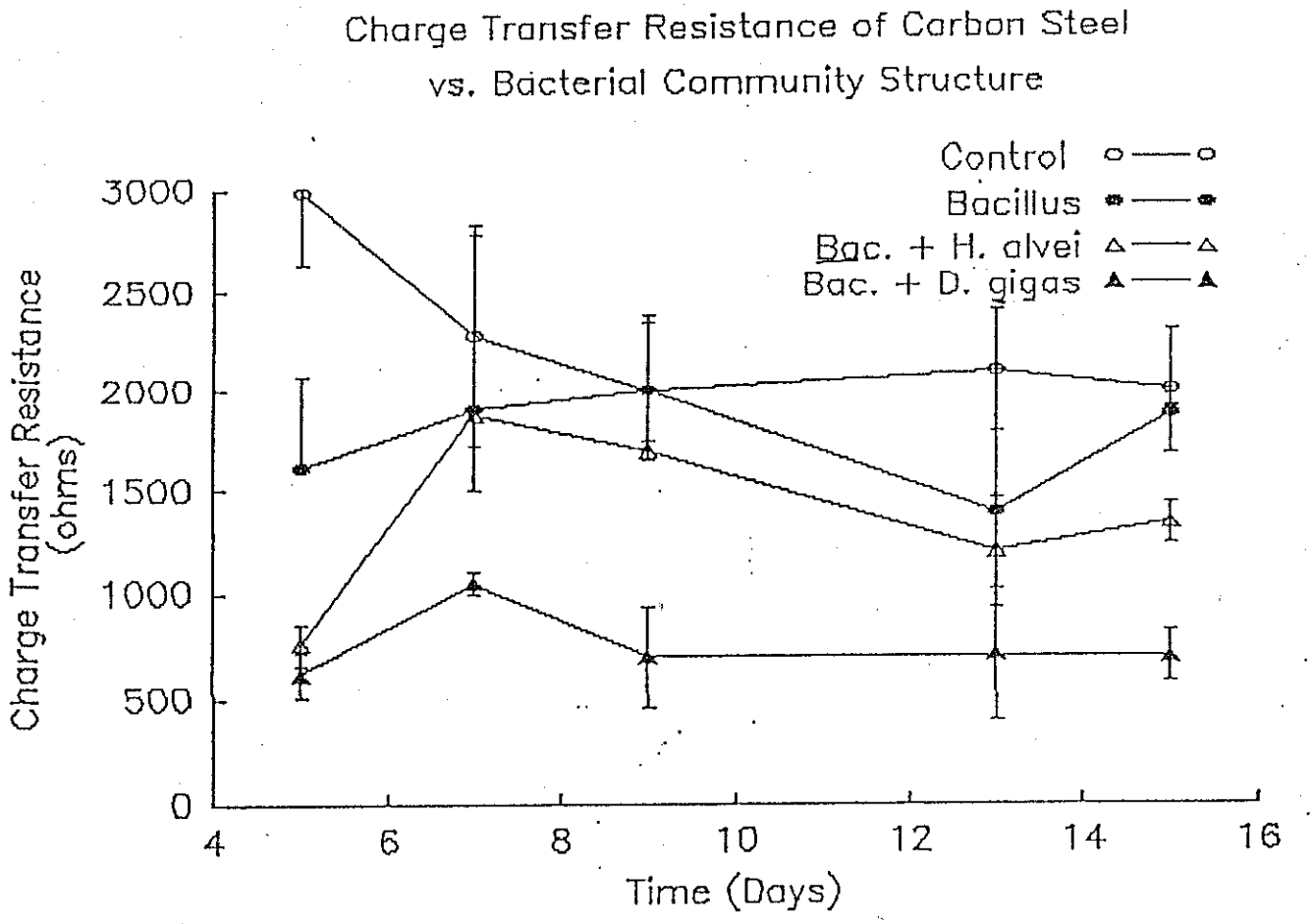

Figure 3. Average corrosion rate measured as charge transfer resistance (= polarization resistance) by EIS on mild steel coupons by the sterile control (open circles), biofilm of Bacillus sp. (closed circles), bicultures of Bacillus + H. alvei (open triangles), and Bacillus sp. + D. gigas) (closed triangles). The lower the resistance the greater the average corrosion rate in this aerobic freshwater system. The means of three determinations in each system were plotted with standard deviations indicated by error bars.

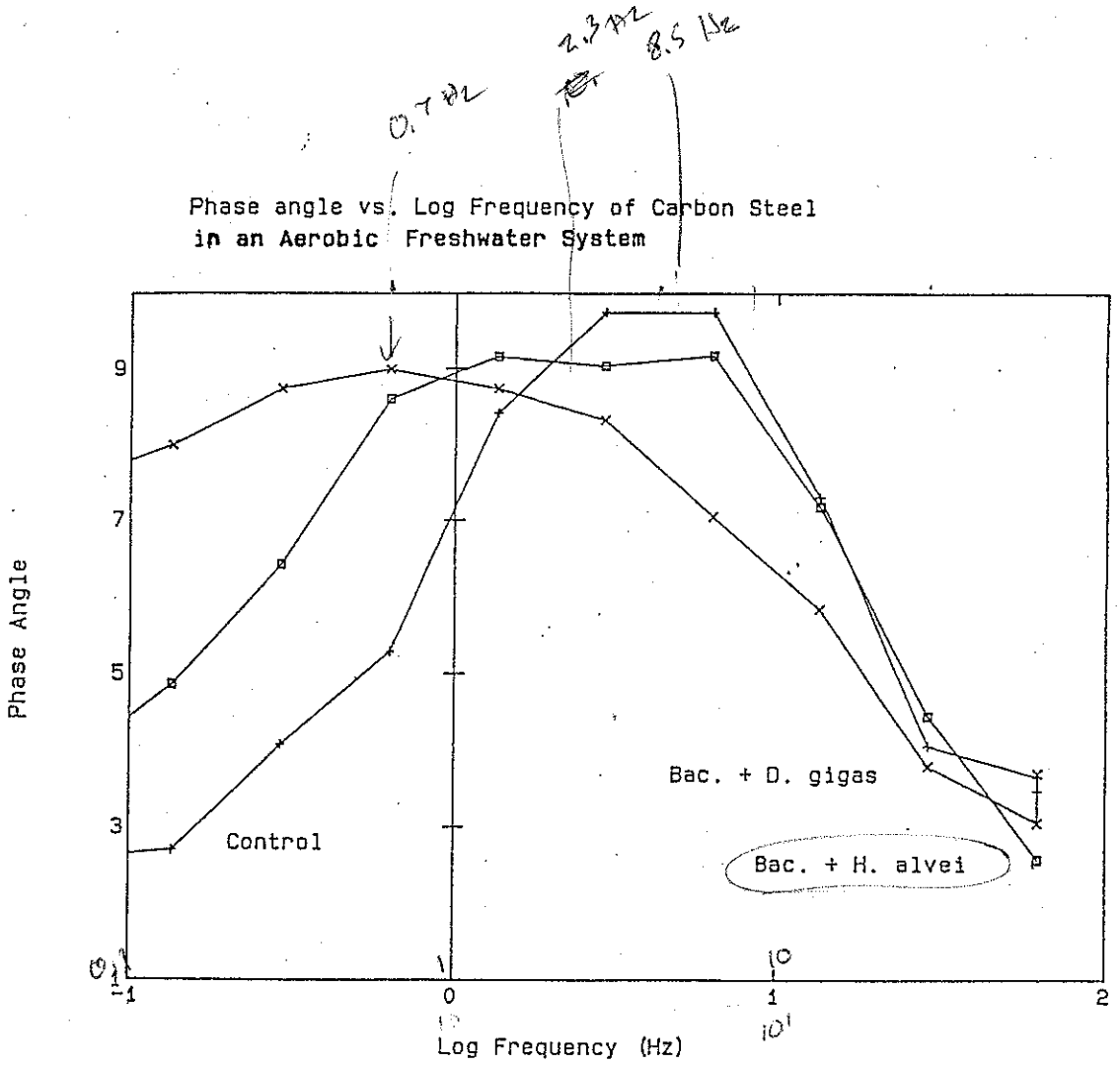

Figure 4. Phase angle versus the log frequency in the EIS analysis of the systems used in Figure 3. The triculture of Bacillus sp. + H. alvei + D. gigas (crosses), biculture of Bacillus sp. + H. alvei (open squares), and sterile control (x) are indicated. The shift to lower frequencies (to the left) may indicate possibly greater localized activity.

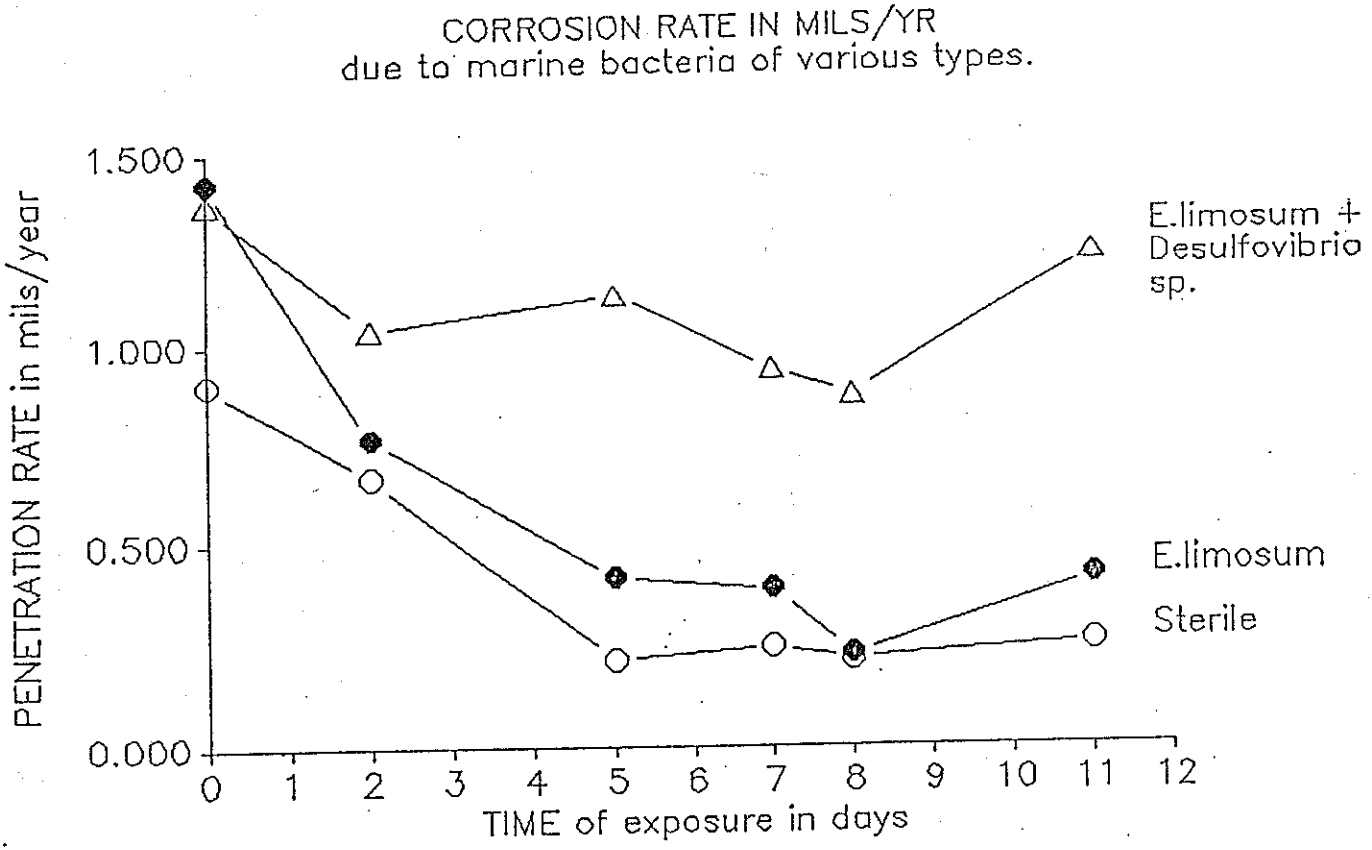

Figure 5. Penetration rate (reciprocal of the polarization resistance) of an anaerobic system supplied with hydrogen and carbon dioxide for the sterile control (open circles), a monoculture of E. limosum (closed circles), and a biculture of E. limosum + D. desulfuricans (open triangles). The maximum average corrosion rate resulted in the highest penetration rate. At 0 time unpassivated mild steel coupons were exposed to the system.

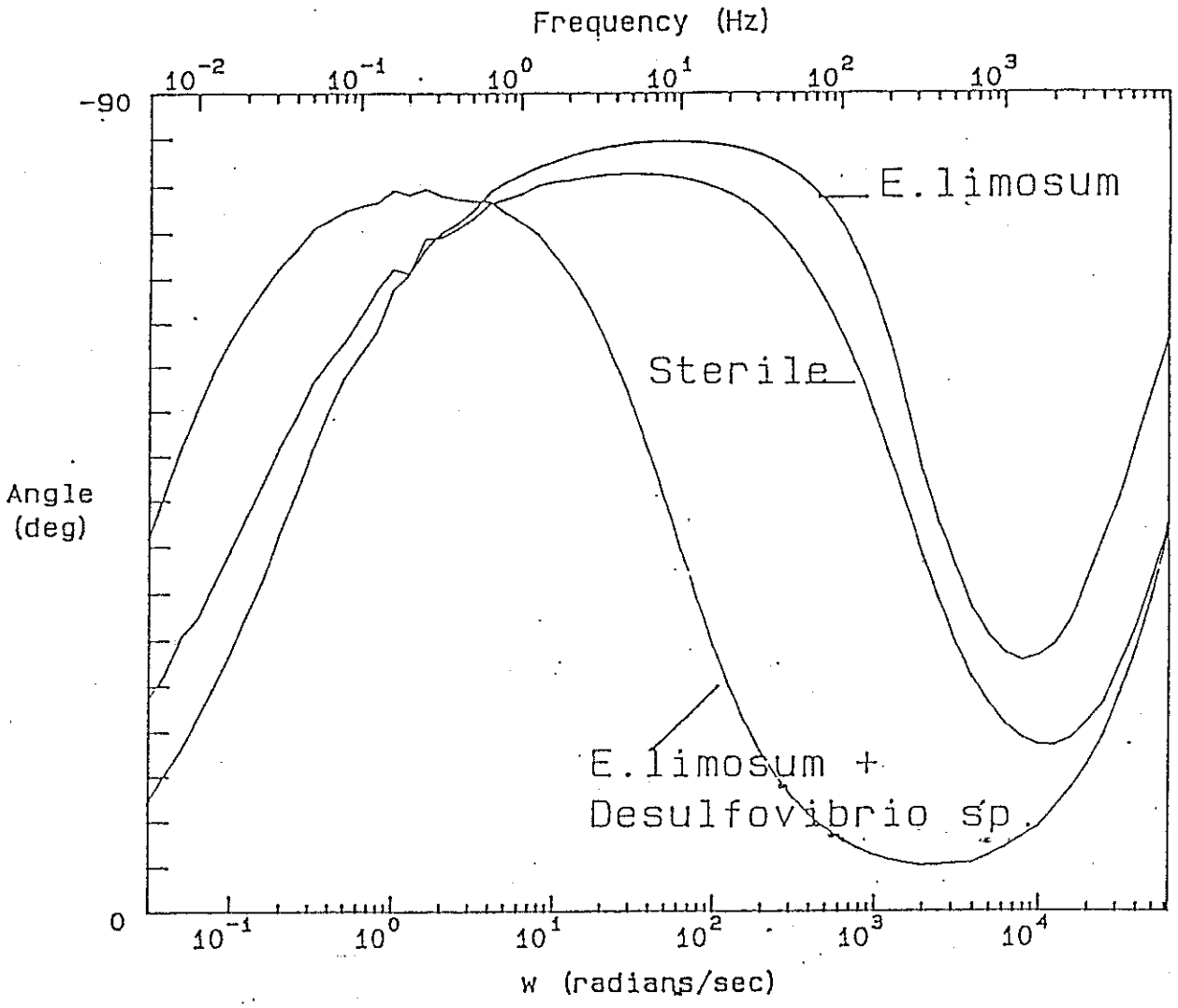

Figure 6. Phase angle versus the log frequency in the EIS analysis of the experiment illustrated in Figure 5. The biculture of E. limosum + D. desulfuricans showed the most pronounced shift in maximum phase angle at lower frequencies (left) which may indicate more localization of the MIC activities.

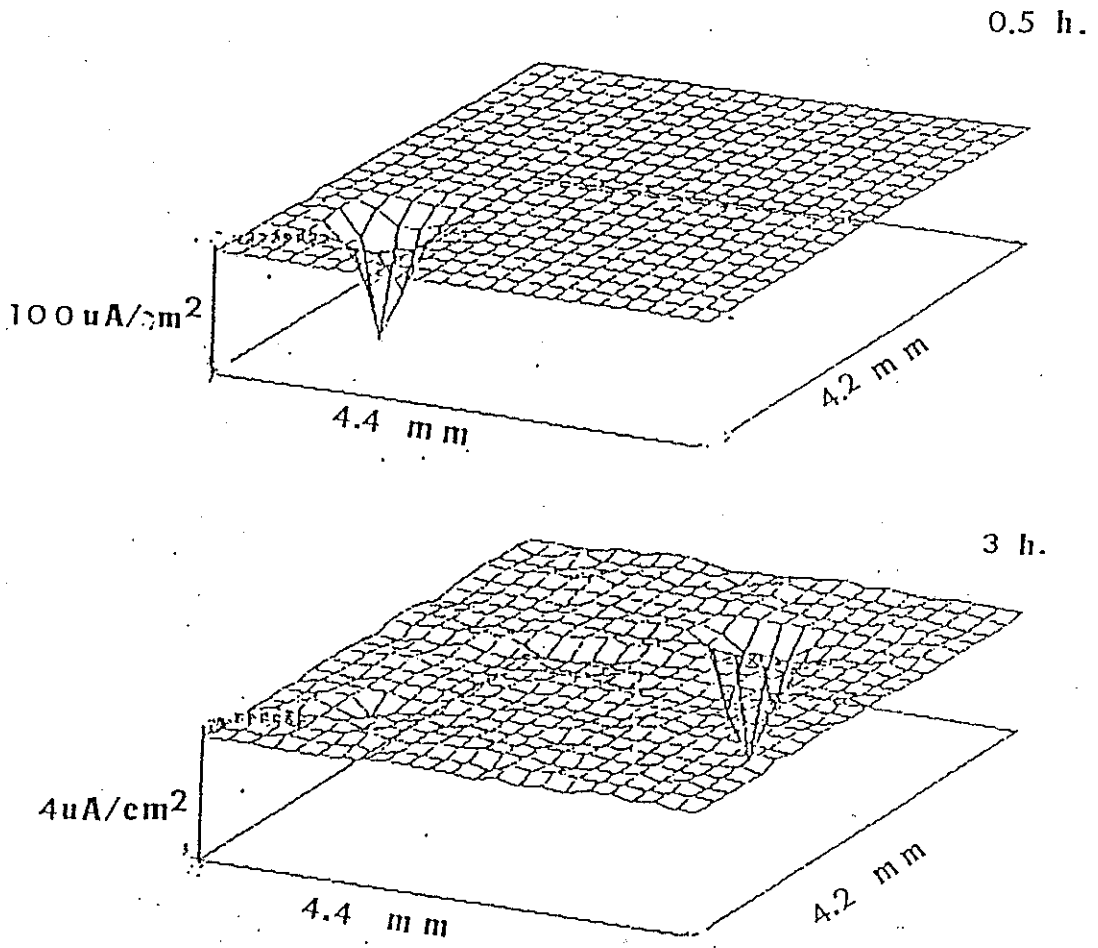

Figure 7. Scanning vibrating electrode analysis of the surface of a sterile mild steel coupon showing non-uniform current densities in a sterile aerobic freshwater medium. A localized anodic site is indicated by the inverted cone in the lower left corner the upper figure (a) after 0.5 hrs. After three hours of exposure (b) the local anodic site seen in (a) became inactive. A new anodic site is seen in the upper right of the coupon. The addition of bacteria to the system resulted in spreading of the anodic area[8].