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ON-LINE ELECTROCHEMICAL MONITORING OF MICROBIALLY INFLUENCED CORROSION

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ABSTRACT

Newly emerging electrochemical measurement techniques can provide on-line, non-destructive monitoring of the average corrosion rate and indications of localized pitting corrosion together with insight into fundamental electrochemical mechanisms responsible for the corrosion process. This information is relevant to evaluating, monitoring, understanding and controlling microbially influenced corrosion (MIC). MIC of coupons exposed in sidestream devices on site or in laboratory-based experiments where the corrosion response is accelerated by exposure to active consortia of microbes recovered from specific sites can be utilized to evaluate mitigation strategies. The average corrosion rates can be determined by small amplitude cyclic voltametry (SACV), and AC impedance spectroscopy (EIS). EIS can also give insight into the mechanisms of the MIC and indications of localized corrosion. Pitting corrosion can be detected non-destructively with open circuit potential monitoring (OCP). OCP also responds to bacterial biofilm activities such as oxygen depletion and other electrochemical activities. Utilizing these methods, accelerated tests can be designed to direct the selection of materials, surface treatments of materials, and welding filler materials, as well as the optimization of chemical and mechanical countermeasures with the microbial consortia recovered and characterized from the specific sites of interest.

INTRODUCTION

Microbially influenced corrosion (MIC) is being increasingly recognized as a serious problem when metal surfaces are exposed to natural waters[1]. Localized corrosion associated with tubercles, slimes, discolorations, odors of anaerobic metabolism, sludges, and "under deposit corrosion" may indicate microbial involvement in the process. Localization to welds, heat affected

zones (HAZ), areas of stagnation, and dependent areas where water could collect reinforces the contention that MIC is involved.

MIC, once recognized, represents a problem in optimizing prevention and minimizing maintenance and countermeasure efforts. Newly developed non-destructive electrochemical techniques can be utilized to test coupons either in sidestreams of actual plant operations or with accelerated tests wherein microbes isolated from the specific site are stimulated to form corrosive biofilms.

This paper will describe measures that provide average corrosion rates, of materials, surface treatments of specific materials, welds, or biocide treatments can be compared. MIC, however, is often characterized by localized corrosion. Possibly the most damaging MIC related activity is the formation of pits which can readily penetrate container walls. Pitting or localized corrosion can be detected by these non-destructive electrochemical techniques.

AVERAGE CORROSION RATE

The corrosion rate in terms of the corrosion current density, I_{corr}, can be determined from the polarization resistance R_p as defined by the Stern-Geary equation[2]. The traditional way to measure the polarization resistance is to perturb the system over a small range around E_{corr}. The problem is that the linear polarization measurements assume that the system is in steady-state during the test period and ignore capacitive effects [3]. The measurement may also damage the biofilm.

Small Amplitude Cyclic Voltametry (SACV)

The application of a sawtooth (triangular) signal, sufficiently small so the change of electrode potential remains in the linear range, allows accurate determination of R_p[3]. Figure 1 shows the Lissajous' ellipse of AISI 316L stainless steel coupons in seawater. From the tangent to the ellipse illustrated in Figure 1, the polarization resistance, R_p, was 3.7K ohms/cm^2. The sweep involved a maximum of 1 uA over a period of 64 sec. This is slow enough to provide a pseudo-equilibrium that overcomes problems of steady-state. The capacitive component of the system is indicated by the

hysteresis. Both the capacitive contribution to the hysteresis and the problems of non-steady-state decreases at slower sweep rates. The limits on the slowness of the sweep rates are usually related to the stability of the potentiostat. The relationships between the sweep rates and the accuracy of R_p determination have been reported [4,5].

In a laboratory simulation the effects of bacteria in stimulating corrosion can be readily demonstrated by SACV. The SACV of a AISI 316L stainless steel weldment with 308 filler incubated for 2 days in artificial seawater in the presence of a complex consortia of marine bacteria yielded an R_p of 3.7K ohms/cm^2. The uninoculated control yielded an R_p of 18K ohms/cm^2. A low value for R_p corresponds to a high corrosion rate.

AC Impedance Spectrometry (EIS)

A second non-destructive electrochemical technique can be utilized to measure average corrosion rate (R_p). Electrochemical impedance spectroscopy (EIS) differs from SACV in that a small sinusoidal potential is applied to a working electrode at a wide range of frequencies and the phase shift of the resultant sinusoidal current is measured[6]. The results are usually plotted using a Nyquist diagram of Z" (imaginary impedance) versus Z' (real impedance). This analysis gives more information about the system than the SACV measurement. However, the analysis can take several hours and the equipment tends to be more expensive. EIS subjects the MIC biofilm to greater perturbations than SAVC and could lead to artifacts originating in the electrochemical measurement itself. Evidence that EIS does not significantly effect a living microbial biofilm comes from the fact that sequential EIS analyses show no differences. E_{Corr} does not change during the analysis. Both these indicate that the corrosion system including the biofilm is unchanged during the EIS analysis.

Laboratory experiments can be used to readily demonstrate the effectiveness of EIS in the detection of MIC. C_{1020} carbon steel coupons incubated in artificial seawater enriched with diluted nutrients and inoculated with the bacterium Vibrio natriegens showed obvious increases in the corrosion rate (Figure 2)[7]. The corrosion rate R_p is related to the chord distance (charge transfer resistance) formed by the depressed semicircle on the real impedance axis (ordinate). The bacterial biofilm

clearly shortens the chord (open squares) compared to the initial situation (solid squares) or the uninoculated control after 3 days (solid circles). The values of R_p were 250, 550, and 1860 ohms/cm^2 for the inoculated, initial, and sterile control after 4 days. There is a corrosion film of the mild steel surface in the absence of bacteria as indicated by the increase in R_p in the four days of the experiment from 550 to 1860 ohms/cm^{2}h. In these experiments the applied potential was 5 mV RMS at frequencies between 3 mHz and 10 KHz.

Values for R_p obtained by EIS was shown to be equivalent to the corrosion rate determined by DC linear polarization. The corrosion rates from the DC determination were 100 uA/cm^2 and 28 uA/cm^2 for the inoculated and uninoculated control respectively. The anodic Tafel parameter (B_a) was determined in an anodic polarization sweep to be 100mV. This determination destroyed the biofilm. Corrosion current density calculated from R_p (chord distance) (Nyquist plots of Figure 2) as B_a/R_p gave values of 165 uA/cm^2 and 32 uA/cm^2 for the inoculated and uninoculated systems in agreement with the DC linear polarization.

A new application of EIS technology, known as Harmonic impedance spectroscopy (HIS), utilizes a sufficient current or voltage potential application to drive the corrosion electrode into a non-linear response (8). HIS was developed with support of EPRI, requires sinusoidal sweeps of 10-50 mV. If the second and third harmonic responses are examined, direct measurements of the "corrosion resistance" and Tafel coefficients can be performed. This system also allows the electrochemical analysis of cathodically (or anodically) protected systems which cannot be done with the classical EIS analysis. The sweep into non-linear response areas, however, indicates that the metal surface is affected by the measurement. The resulting effects on the living biofilm involved in MIC has yet to be determined.

A second feature of the EIS analysis is both an advantage and a complication in the interpretation of the results. The EIS analysis provides an on-line, non-destructive, time-resolved monitoring of homogeneous corrosion at which the whole surface at least statistically undergoes the same process at the high sweep frequencies. However, it also provides indications of local inhomogeneities in the corrosion process that become

more pronounced as the sweep frequency decreases. The complication results from the influence of the local processes on the average corrosion rates. These complexities are most often detected as deviations from the semicircular response in the Nyquist complex plane analysis at the low sweep frequencies (high values of the ordinate Z'). The deviations can be detected as secondary capacitive loops in the low frequency responses of mild steel corrosion illustrated in Figure 2.

Complexities at low frequency EIS analysis are more pronounced in the responses of stainless steels to MIC than mild steel. Figure 3 illustrates the responses of AISI 316L stainless steel exposed in the laboratory to a diluted seawater medium for 12 days(9). The solid triangles indicate the corrosion response of the surface to the sterile menstrum. The corrosion rate in the sterile system is so slow that it is unobtainable by EIS analysis as there is no satisfactory low frequency response (use SACV). It is obvious that the mixed group of marine microbes induce a significant corrosion rate indicated by the crosses in Figure 3. The estimated charge transfer resistance which is related to R_p as determined by extrapolating the semicircle in the Nyquist plot (indicated by the dotted line) is 1400 ohms/cm^2. The modest deviation from the semicircular response seen with mild steel in Figure 2 is much more pronounced with stainless steel (Figure 3). The complexities are related to inhomogeneities in the corrosion processes as demonstrated by independent experiments. Examination of the metal surface in the experiments shown in Figure 3 showed localized pitting corrosion. These complexities in the EIS analysis response to low frequencies is a disadvantage in the ready estimation of the average corrosion rate in some situations, but can be exploited to provide insight into important localized processes such as pitting corrosion or stress corrosion cracking (10).

The analysis of the deviations from semicircular behavior in EIS interpretation, particularly at the low sweep frequencies, is based on model corrosion circuits in which the localized processes show a frequency dependence (10). In experiments to test the model circuit diagrams, pits were created or stress corrosion cracking induced mechanically in the electrodes and the EIS responses determined. The impedance response behaved as if the pit or crack represents a small conducting area in an insulating plane at low

frequencies whereas the total electrode surface (both the pit or crack and the "passive" surface) is a uniform conductor based on its double layer capacitance at high sweep frequencies(10). The role of bacteria in localized corrosion further complicates the inhomogeneities at the electrode surface as bacteria often attack specific features of the surface. The point is not that the interpretation of EIS analyses are complex and an active area of research into mechanisms of MIC, but that the analysis can give both indications of the microbial influence on the average corrosion rates as well as indications of localized activity that could lead to serious failure problems.

LOCALIZED CORROSION RESPONSE

Possibly the most important on-line monitoring of MIC involves the detection of localized corrosion. EIS measurements, particularly at low sweep frequencies, can indicate pitting corrosion. This localized corrosion is often signaled by changes in the OCP.

Open-Cell Potential (OCP)

Measuring the open-cell potential between the working electrode and the reference electrode allows the detection of complex electrochemical changes when stainless steel weldments are incubated in seawater enriched in diluted nutrient medium in the presence of a consortium of marine bacteria (Figure 4) in the laboratory(8). In this experiment an autogenous weld was created with AISI 316L base metal. The weld was incubated in a nutrient enriched seawater with a consortium of bacteria recovered from a sulfide rich marine mud. Coupons were created from the weld (as welded), polished weld, and the base metal control and incubated in the enriched sea water medium. From the data illustrated in Figure 4 there was a large shift in the OCP within the first day for the as-welded coupons, in the second day for the polished weldments, and a short time later for the AISI 316L base metal when the bacteria were present. No change was detected in the sterile controls. At the time of the potential shifts some of the coupons were removed and examined for pitting corrosion. In the weldments pitting corrosion was obvious in the fused zone. In the un-welded 316L control, the pitting was not localized to

any specific portion of the coupon. A microphotograph of the fused zone pits is illustrated in Figure 4.

The effects of the bacterial metabolic activity in slimes on the OCP have been documented by Scotto and colleagues(11). Their data show that inhibition of the metabolic activity by the bacterial biofilm slimes with respiratory inhibitors such as azide return the OCP to levels near an uninoculated control within a few days. Similar findings in which shifts in the microbial activities in biofilms induce different OCP have been seen by Buchanan et al. (unpublished data). In this work different stainless steels show different OCP responses to bacterial activity with time.

With fast response voltmeters it is possible to monitor electrochemical noise (ECN) between working and reference electrodes. This technique has been shown to correlate with the localization of breaches in iron sulfide films in pipeline steels (12).

TESTING OF MIC ACTIVITY OR MIC COUNTERMEASURES

The development of non-destructive electrochemical procedures that can provide both the average corrosion rate (SACV, EIS, HIS) and evidence for localized pitting corrosion (EIS, OCP, ECN) provides engineers with methods for selection of specific materials, surface treatment of materials, weld filler materials and components. On-going tests in sidestream units at specific locations can provide long-term data on effectiveness of materials and surfaces in minimizing the damage induced by MIC. In the same system the efficacy of types, mixtures, doses and frequency of biocide application to specific waters can be monitored as can the effectiveness of mechanical cleaning. Lay-up procedures and protective protocols for post-hydrotesting treatments can be developed using the electrochemical monitoring systems.

Accelerated testing of MIC potential or MIC countermeasures

Often there is a short time interval during which candidate replacement materials or countermeasure protocol must be developed. The MIC can be accelerated by performing the test in the appropriate water (or surrogate) to which dilute nutrients that mimic the food sources for bacteria are added. The bacteria are then stimulated to form a biofilm on the electrode surface

and the electrochemical responses can be determined.

Accelerated MIC corrosion test cell

A convenient test apparatus for accelerated corrosion analysis is illustrated in Figure 5. This dual (or multiple) cell system as modified from one developed by B. Little and colleagues (13) has a semipermeable membrane separating the chambers. The membrane with 0.2 um maximum pore size allows chemical continuity but restricts the bacteria to specific compartments. The apparatus is stirred and operated as a continuous culture device in which at least 10-15% of the cell volume is replaced/hour. The data illustrated in Figures 1-4 was generated using this type of test cell.

Isolation and characterization of bacteria from specific sites at specific times

Accelerated tests to document candidate materials, surface treatments, weldments or countermeasure protocols become more relevant if the microorganisms from the water, slimes, sediments or corrosion tubercles from the anticipated site can be utilized in the test system. Figure 6 illustrates a technique for characterizing the major microbes found at a specific site. The material (water, slimes, sediments, tubercles) is recovered in sterile containers and shipped on ice (not frozen) to the laboratory for arrival within 24 hours. Once in the laboratory the microorganisms are separated on petri plates containing solidified media of various compositions and individual colonies recovered in a few days. The plates can be incubated aerobically (in air) or anaerobically (in the absence of oxygen and usually in roll tubes) to select various types of bacteria. The bacteria are generally classified by their growth and metabolic properties. Common bacteria that are recovered from corrosion tubercles include organic acid-forming bacteria, slime-forming bacteria, iron-precipitating, oxidizing or reducing bacteria, heterotrophic bacteria that require more complex nutrients, and various anaerobic bacteria such as sulfate-reducing and methane-forming bacteria among many others. Some facultative bacteria are able to grow either aerobically or anaerobically. The isolated bacteria can conveniently be classified by determining the fatty acid patterns derived from the wall and membrane of the bacteria and analyzed

by capillary gas-liquid chromatography. The technique and some of the structures of the fatty acids found in bacteria are diagrammed in Figure 7. Each pattern is compared with a catalogue and the relatedness of the specific strain to the total collection is quantitatively defined. This bacterial identification system is widely used in medical practice and has increasing utility in defining the types of bacteria that are associated with MIC.

There are tests for the presence of specific microbes from sites that depend upon growth, the detection of specific enzymes, or antigens on the surface. Our laboratory has developed methods which define the microbial community structure and biomass based on the phospholipid ester-linked fatty acid patterns which do not depend on quantitative recovery and growth but define the "viable" or "potentially viable" microbial community (14). This methodology can be utilized to test the quantitativeness of the recovery of microbes as diagrammed in Figure 6.

Accelerated Corrosion Testing

With the organisms recovered from the specific site during the specific season, the candidate materials can be tested for accelerated response to MIC. Organisms with known MIC propensities can be recovered from a specific site, their physiology defined, and phenotypic identification determined. These microbes would then be utilized as a consortia to test the relative resistance of specific candidate materials to MIC in a test system like that illustrated in Figure 5 in which the activity of the microbial consortia is increased by incorporation of dilute nutrients. The nutrients are added to water from the specific site, or to a surrogate created in the laboratory that is sterilized and pumped into a sterile system containing the test materials as working electrodes with the standard electrode and the common electrodes. The system is usually operated in the flowing condition with a dilution rate of about 10-20% of the total test system volume removed/hour. This test system can provide an accelerated response of specific materials or surface treatments to MIC. The corrosion rates of the test materials as working electrodes are monitored using non-destructive electrochemical methods described above--SACV, EIS, OCP, ECN.

The test is continued until definative electrochemical differences between the inoculated and sterile controls are observed. To document the

involvement of microbial consortia, there must be distinctive differences in the SACV, EIS, OCP and ECN between the inoculated and test systems and microbes must both be recovered from the working electrode surface and shown to be metabolically active. The test system allows the examination of the activities of specific combinations of microbes against different materials or surface treatments. The effectiveness of chemical or mechanical countermeasures can be tested with this system and the optimization of treatment scheduling established.

EXPERIMENTAL PROCEDURES

Recovery of the microbes

Water samples, slimes, and sediments from MIC damaged components were collected from the site in sterile plastic containers and shipped on ice by air to the laboratory for isolation within 24 hours of sampling. In the laboratory, the microbiota in water samples are recovered from sterile polycarbonate filters using a tangential flow filter apparatus. The tubercles are broken with a sterile scalpel and scraped into sterile test tubes containing glass beads. The contents are agitated with a sterile ultrasonic probe under a stream of nitrogen.

Isolation of the microbes

The microbial suspensions were diluted in sterile medium for direct count epifluorescent microscopic enumeration using cells stained with acridine orange, aerobic spread count plates, and anaerobic most probable number's technique (MPN) isolations. Isolated colonies were recovered from solidified Hutner's medium containing Huntner's salts and trace metals #44 (15). For the MPN tests, 0.25 mg/l $FeSO_4$ was added to the medium to assay for sulfate reducing bacteria (SRB). Bacterial isolates are screened using gram stain, oxidase tests, catalase tests, the Miniaturized Microorganism Differentiation System (Minitek) system, and the phenotype defined using the Microbial ID, Inc. Microbial Identification System.

Coupon Preparation

Coupons of the test metals 16 mm in diameter were prepared to a 600 grit finish, sealed in epoxide, and any edges coated with additional epoxide under microscopic control. Multiple coupon holders were fabricated from epoxy. Counter electrodes of titanium 41mm x 147mm bent in a "U" shape surrounding the working electrode were soldered to a coaxial cable and covered with epoxy so only titanium was exposed. Standard calomel electrodes fashioned into Luggin probes were used for standard electrodes.

Electrochemical Analysis

EIS and SACV analyses were performed using the Solartron 1286 electrochemical interface and model 1250 frequency response analyzer controlled by a microcomputer. Applied potentials and resulting phase shifts were monitored with a dual channel oscilloscope. Electronic switching between working electrodes was achieved with a Keithly scanner. OCP and ECN were measured with a Solartron 7081 precision voltmeter controlled by a microcomputer.

Microbial Metabolic Activity

Post electrochemical analysis to establish the metabolic activity of the biofilm microbes on the working electrode was determined by exposing the biofilm on the coupon to radioactive acetate. The electrode was recovered from the apparatus and immediately covered with a modified 60 ml separatory funnel containing a flanged base with a Viton o-ring. The apparatus was clamped to the disk so only the biofilm was exposed. The o-rings were preextracted to remove any contaminants. One ml of medium containing 1 mCi ^{14}C acetate was exposed to the surface, mixed vigorously and allowed to incubate 12 minutes at room temperature. The reaction is stopped with addition of the one-phase chloroform methanol lipid extraction mixture and the lipids recovered after forming two phases by addition of chloroform and water. The lipids were fractionated on a mini-column of silicic acid and the radioactivity determined by scintillation spectrometry (16).

Determination of the Microbial Community Structure on the Working Electrode

If a consortium of bacteria is utilized in the accelerated test it is important to determine which specific microbes were in the biofilm on the working electrode. The modified separatory funnel with the viton o-ring described above can be used to create a suspension of the bacteria for plate counting or for analysis of the "signature biomarker" phospholipid ester-linked fatty acids (17).

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KEYWORDS
AC impedance
Accelerated MIC analyses
Biofilm community structure
Electrochemical monitoring of MIC
Electrochemical noise (ECN)
Electrochemical impedance spectroscopy (EIS)
Harmonic impedance spectroscopy (HIS)
Isolation and characterization of biofilm microbes
Microbial metabolic activity
MIC
Open-cell potential monitoring
Small amplitude cyclic voltametry

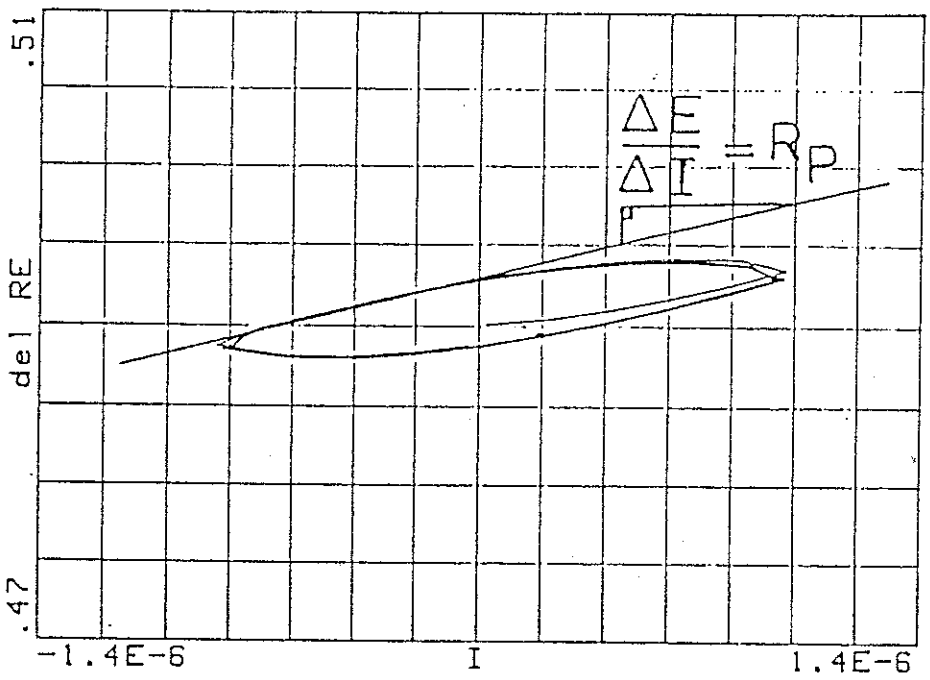


FIGURE 1. Small angle cyclic voltametry of AISI 316L stainless steel incubated in seawater enriched with diluted media. Showing the hysteresis induced in part by the capacitive component and the tangents used to determine R_p.

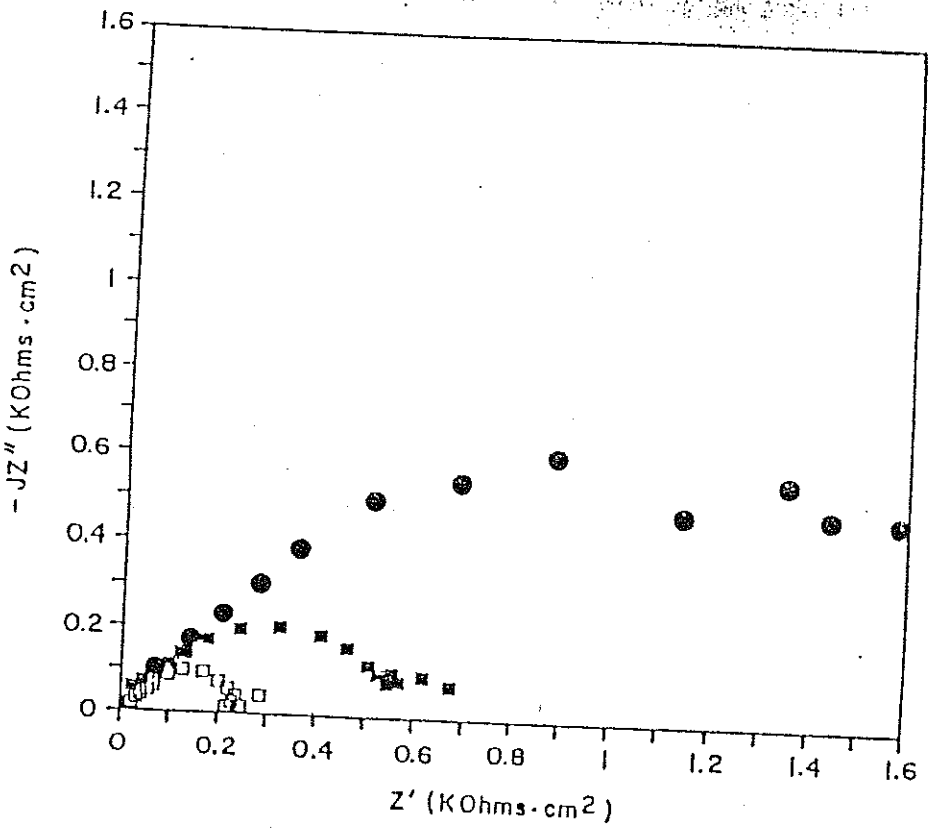


FIGURE 2. Nyquist complex plane plot of electrochemical impedance spectroscopy of mild steel (C1020) coupons incubated in artificial seawater to which nutrients had been added. Middle curve ( ) shows initial response; lower curve ( ) shows response after growth of vibrio natriegens for 4 days and the uninoculated control after 4 days. ( ) showing passivation when compared to the initial value. Note the "second loop" in the low frequency end of the plot. Data from (7).

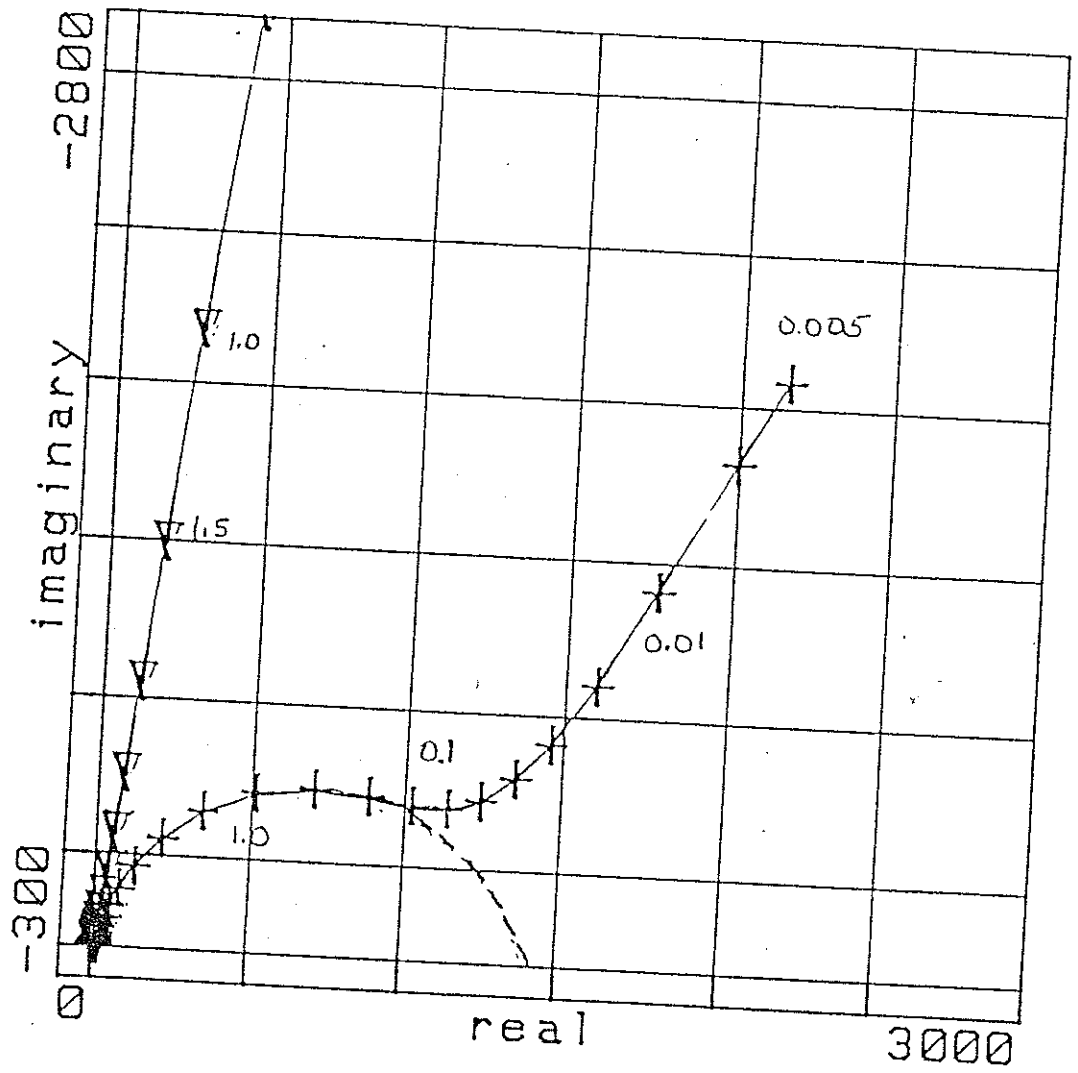
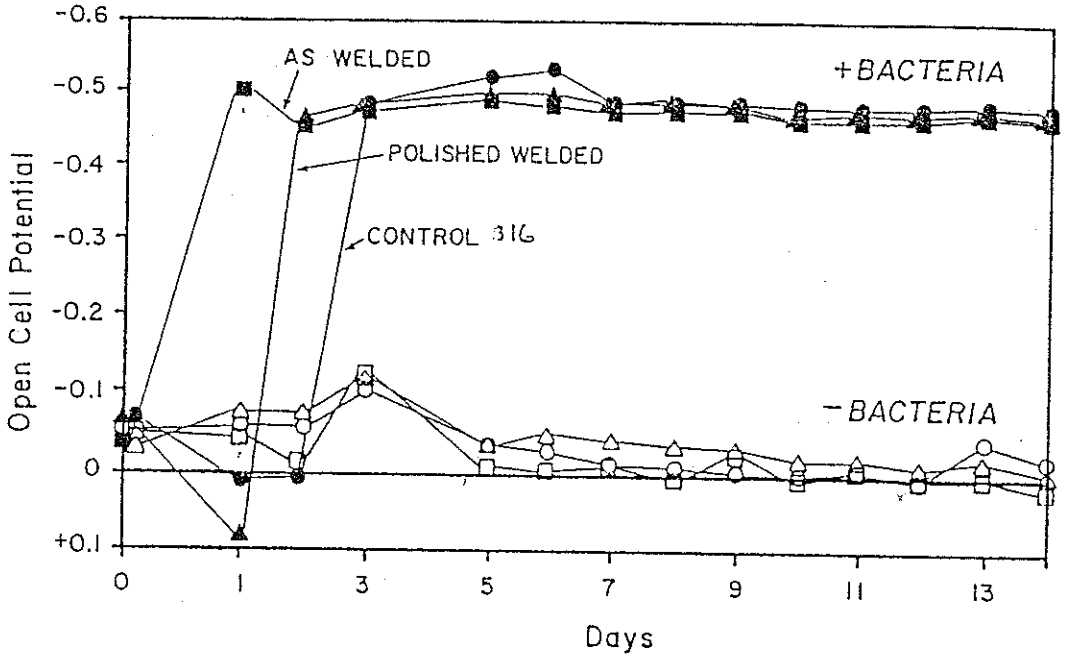


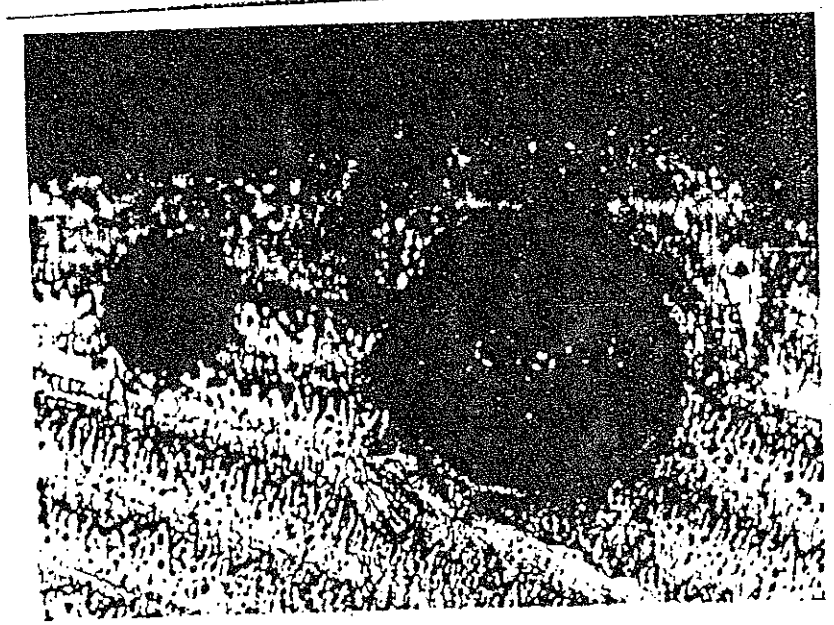
FIGURE 3. Nyquist complex plane plots of electrochemical impedance spectroscopy of AISI 316L stainless steel exposed to diluted nutrient medium in seawater for 12 days. ( ) indicates the sterile control, (+) inoculated with a consortium of microbes from a black marine sediment. Numbers indicate the sinusoidal sweep frequency in KHz. Low sweep frequencies show anomalous behavior related partly to localized corrosion. Data from (9).

FIGURE 4. Open cell potential of AISI 316L autogenous weldments incubated in dilute medium in seawater and inoculated (+ bacteria) with a consortium of bacteria from black marine sediment. A microphotograph of a 0.5mm and 0.1mm pit from the fused zone is illustrated. Data taken from (9).

Open-Cell Potential of Coupons Over Two Weeks

(Indicates pit formation ± biofilm activity)





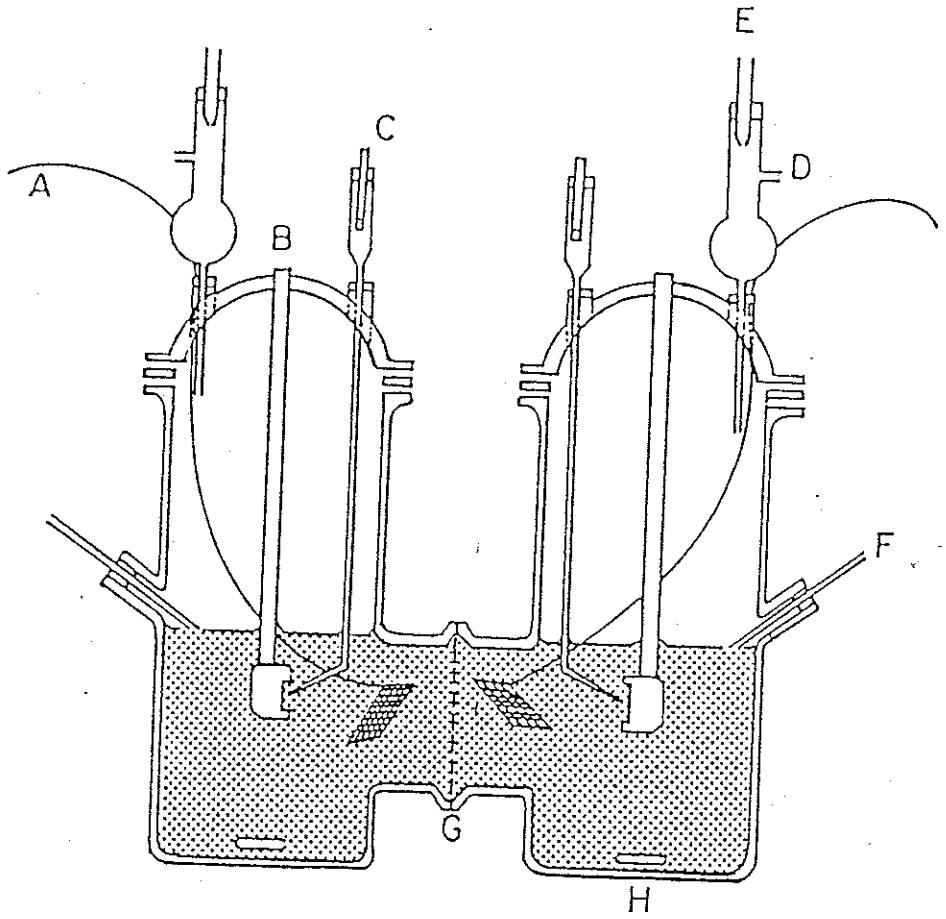
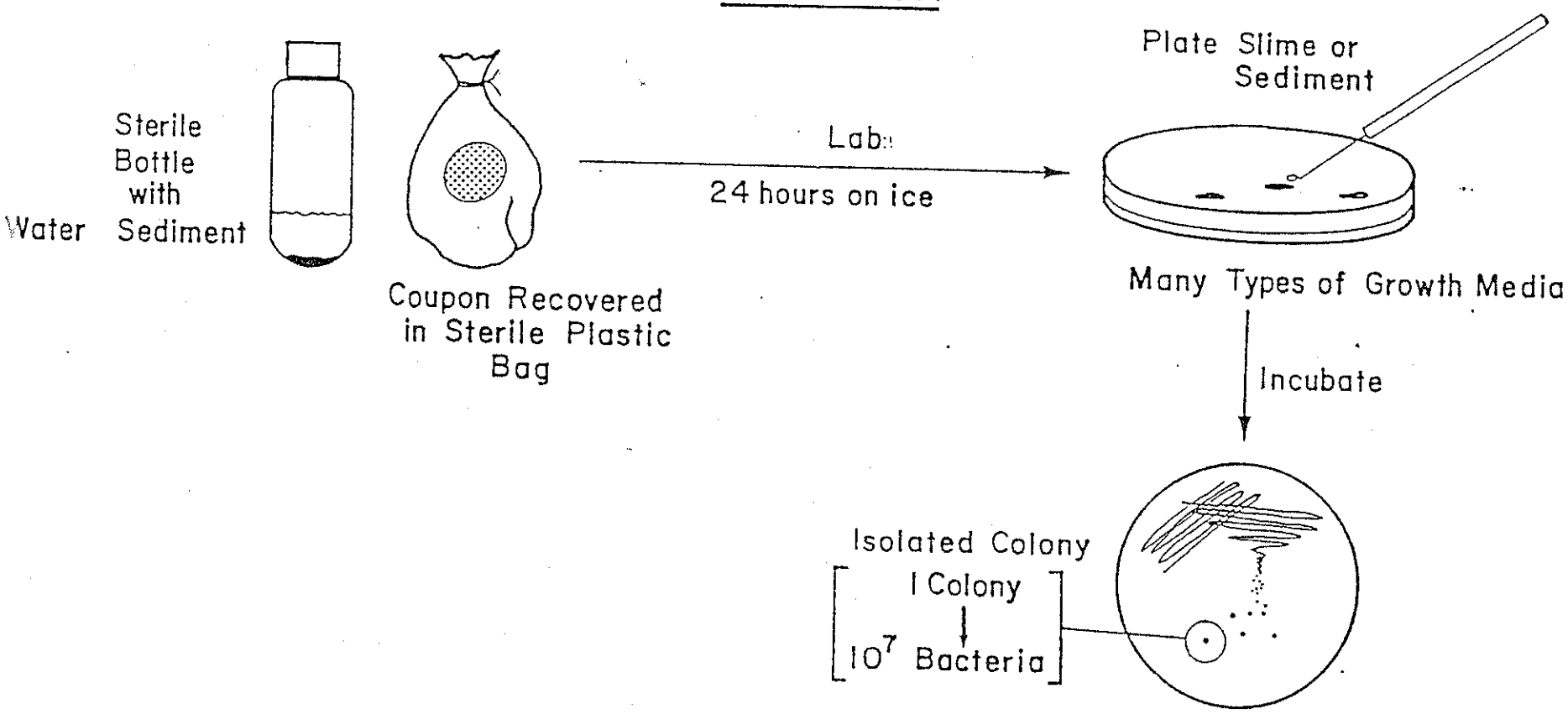


FIGURE 5. Dual chamber, continuous flow apparatus for comparison of MIC and control corrosion parameters. A - common electrode (titanium), B - working electrode, C - Luggin probe, D,E - drip tube for media input, F - media outlet, G - teflon membrane 0.2 μm pore size, H - magnetic stirrer.

FIGURE 6. Recovery and isolation of bacteria from a specific site.

Microbiological Studies

ISOLATION

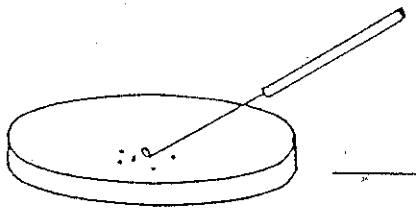


IDENTIFICATION

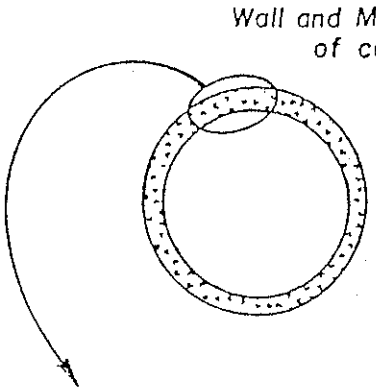
1) Examined with microscope after staining
2) Put on other types of growth media response
3) Various immune or genetic tests
4) Fatty acids determined

FIGURE 7. Characterization of site specific bacteria after growth by the alkaline methanolysis and gas chromatographic analysis of the fatty acid methyl esters.

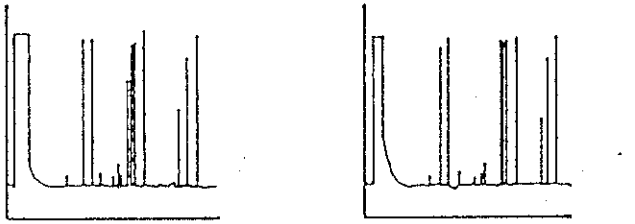
Chemical Identification of Bacteria



Recover Colony → Isolate Fatty Acids → Identify Pattern



PATTERN COMPARISON



IDENTIFICATION

(Likely matches from Library)

STRAIGHT CHAIN SATURATED

$CH_3-(CH_2)_n-\underset{\underset{O}{\|}}{C}-OH$

STRAIGHT CHAIN UNSATURATED

$CH_3-(CH_2)_n-CH=CH-(CH_2)_n-\underset{\underset{O}{\|}}{C}-OH$

BRANCHED CHAIN

ISO

$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH-(CH_2)_n-\underset{\underset{O}{\|}}{C}-OH$

ANTEISO

$\begin{matrix}CH_3CH_2\\CH_3\end{matrix}\!\!>\!CH-(CH_2)_n-\underset{\underset{O}{\|}}{C}-OH$

CYCLIC

$CH_3-(CH_2)_n-\underset{\underset{CH_2}{\diagdown\diagup}}{CH-CH}-(CH_2)_n-\underset{\underset{O}{\|}}{C}-OH$

HYDROXY

alpha (2 OH) $CH_3-(CH_2)_n-\underset{\underset{OH}{|}}{CH}-\underset{\underset{O}{\|}}{C}-OH$

beta (3 OH) $CH_3-(CH_2)_n-\underset{\underset{OH}{|}}{CH}-CH_2-\underset{\underset{O}{\|}}{C}-OH$