Corrosion Control of Mild Steel by Aerobic Bacteria Under Continuous Flow Conditions

Kh.M. Ismail, T. Gehrig,** A. Jayaraman,*** T.K. Wood,**** K. Trandem,***** P.J. Arps, and J.C. Earthman*******

ABSTRACT

*Corrosion of mild steel under aerobic conditions in the presence of a monoculture of aerobic bacteria (*Pseudomonas fragi *K* [P. fragi *K*]*) has been studied in a continuous flow system using electrochemical impedance spectroscopy (EIS).* P. fragi *K grown in Luria-Bertani (LB) medium causes a 10- to 20-fold decrease in the corrosion rate of mild steel after a biofilm becomes visible on the surface of the samples. Live viable bacteria are necessary for the observed corrosion reduction of mild steel, indicating an active role rather than a barrier effect of the biofilm. Flowing nitrogen through the solution was found to be less effective than* P. fragi *K in lowering the corrosion rate of mild steel, suggesting that an effect by bacteria, in addition to scavenging oxygen, is involved. The effect of nutrient flow rate on the ability of the bacteria to control corrosion was also examined. It was found that the corrosion inhibition of mild steel increases somewhat as the medium flow rate decreases below a certain level.*

KEY WORDS: bacteria, electrochemical impedance spectroscopy, nutrient flow rate, polarization curve, Pseudomonas fragi *K, Type 1018 mild steel*

INTRODUCTION

The presence of bacterial biofilms is often shown to be associated with elevated corrosion rates.[1-2] These attached bacterial colonies can cause serious damage to structures exposed to soil, fresh water, and seawater, and they will affect a variety of metals.[3] Controlling the growth of these bacterial colonies with biocides has proven to be very difficult because cells in a biofilm can be up to 500 times more resistant to antibacterial agents than those in fluid suspension. While antibiotics have been designed to kill suspended cells, biofilm bacteria undergo phenotypic changes that make them entirely different from those in suspension.[4-7] Furthermore, since no inherently colonization-resistant material has yet been found, there has been no easy answer to this problem.[8]

Several studies have shown, however, that certain bacterial systems decrease corrosion rates.[9-15] In particular, work performed by Pedersen and Hermansson has shown that substantial corrosion reduction is possible in mild steel exposed to monocultures of *Pseudomonas* sp.S9 and *Serratia marcescens* EF190 compared to a sterile control.[11-12] Pedersen and Hermansson and Jayaraman, et al.,[13-14] suggest that this protective behavior was the result

Submitted for publication October 2000; in revised form, November 2001.

* Department of Chemical and Biochemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575. Present address: Department of Chemistry, University of Cairo, Egypt.

** Department of Chemical and Biochemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575. Present address: Gencorp Aerojet, Sacramento, CA.

*** Department of Chemical and Biochemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575. Present address: Center for Engineering in Medicine, Massachusetts General Hospital, Cambridge, MA.

**** Department of Chemical and Biochemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575. Present address: Department of Chemical Engineering, University of Connecticut, Storrs, CT.

***** Department of Chemical and Biochemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575. Present address: Research and Development, Pall Corp., 2200 Northern Blvd., East Hills, NY 11548.

****** Department of Chemical Engineering and Materials Science, University of California–Irvine, Irvine, CA 92697-2575.

0010-9312/02/000095/$5.00+$0.50/0

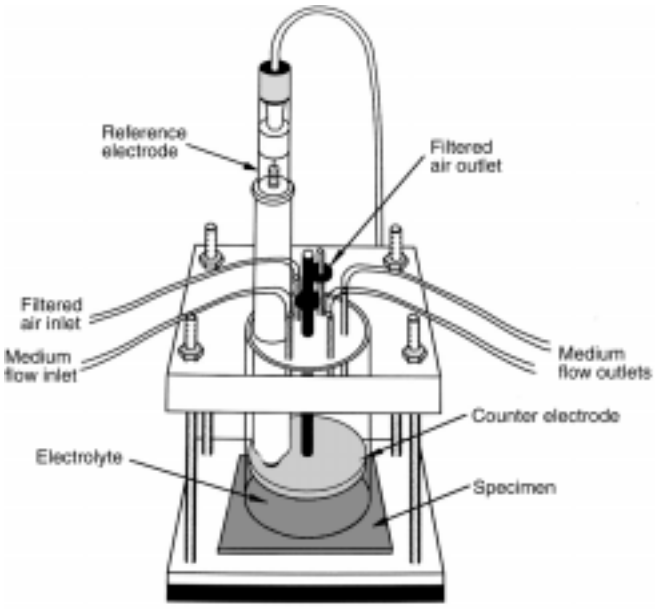

FIGURE 1. *Schematic representation of the continuous-flow bioreactor test cell used for the present electrochemical measurements.*

of oxygen respiration by the biofilm before it reached the metal surface.

The present research was meant to further the understanding of bacterial corrosion reduction by examining the effects of cell viability, oxygen content, and flow rate. Samples of Type 1018 (UNS G10180)[(1)] mild steel were exposed to Luria-Bertani (LB) medium containing a monoculture of *Pseudomonas fragi* K (*P. fragi* K) and to a sterile control in continuous flow reactors. Electrochemical impedance spectroscopy (EIS) was used to investigate the corrosion process and to measure the polarization resistance (R_p) of the systems. The value of R_p is generally considered inversely proportional to the corrosion rate. This technique uses very small alternating current signals to provide an understanding of the corrosion process as it occurs without disturbing the electrode properties and associated microbiology.[16] The first set of experiments was carried out to compare the corrosion occurring in contact with bacteria to that in a sterile control. An additional experiment was conducted to compare control conditions to those in a sterile environment through which nitrogen was bubbled to displace dissolved oxygen. Experiments were also performed to examine the effect of nutrient flow rate on the observed corrosion reduction. Four bacterial test systems were used for these experiments, each corresponding to a different flow rate of the nutrient medium.

[(1)] UNS numbers are listed in *Metals and Alloys in the Unified Numbering System*, published by the Society of Automotive Engineers (SAE) and cosponsored by ASTM.

EXPERIMENTAL PROCEDURES

Test Cells

The bioreactor test cells were designed to support continuous bacterial cultures and facilitate EIS measurements. Figure 1 shows the test cell configuration that allows the continuous flow of pumped nutrient medium and air, and is equipped with a counter electrode and a reference electrode for EIS measurements. Filtered air entered the cell at a point just below the level of the medium to provide oxygen to the fluid. The counter electrode was made of Type 304 (UNS S30400) stainless steel to resist corrosion in the LB environment. An autoclavable silver/silver chloride (Ag/AgCl) electrode was used as a reference electrode. A mild steel specimen was placed at the bottom of the glass cylinder, leaving 26.4 cm^2 to be in contact with the solution, and the plates above and below the cylinder were polytetrafluoroethylene (PTFE) to provide electrical insulation. The entire cell apparatus, including the reference electrode, was autoclavable; the cells were autoclaved at 121°C for 30 min. All experiments included a bioreactor containing 100 mL of medium, inoculated with 1 mL of an overnight culture of *P. fragi* K bacteria, and a sterile control bioreactor. *P. fragi* K is a kanamycin-resistant derivative of *P. fragi*. The experiments were run in batch mode (no flow) for the first 12 h to allow attachment to and colonization of the mild steel surface. The bioreactors were then switched to continuous flow with a nutrient medium flow rate of 2 mL/h. The filtered air was bubbled near the top of the medium at a rate of 50 mL/min to provide an ample supply of oxygen over the duration of the experiments. All measurements were carried out at room temperature, 22°C.

Medium

All tests were conducted using a nutrient-rich LB medium for the overnight growth of the *P. fragi* K and for all of the bioreactor experiments. The medium consists of 10 g tryptone, 5 g yeast extract, and 10 g sodium chloride (NaCl) for each liter of distilled water. Sealed 2-L flasks containing 1.5 L of LB medium were autoclaved for 20 min at 121°C and stored at room temperature. Filter-sterilized kanamycin antibiotic was added to a final concentration of 100 mg/L in the LB medium before use to assure that only the kanamycin-resistant strain, *P. fragi* K, remained.

Specimen Preparation

Sixteen-gauge, Type 1018 mild steel sheet was used for all experiments. Samples (7.64 cm by 7.64 cm) were cut from the stock and polished with 240-grit sandpaper. They were then rinsed in distilled water, cleaned ultrasonically in acetone for 1 min, rinsed again in distilled water, air dried, and stored in a vacuum until use.

Bacterial Culture

P. fragi was obtained from the American Type Culture Collection (ATCC no. 4973). A kanamycin-resistant derivative of the strain was then produced.[13] The bacteria were grown overnight in 250-mL Erlenmeyer flasks containing 10 mL of LB medium in a rotary shaker (250 rpm, 30°C). One milliliter of the cultured bacteria was used to inoculate each of the test cells. The formation of the biofilm produced by *P. fragi* K under the present experimental conditions was quite rapid, as confirmed using confocal laser scanning microscopy.[14,17]

Electrochemical Measurements

Electrochemical impedance measurements were performed using a Solartron-Schlumberger† (SI 1280) electrochemical measurement unit connected to a desktop computer. The program that controls this instrument and records the readings was developed in-house using the LabVIEW graphical programming language. Measurements were made at the open-circuit potential (OCP) using a 10 mV amplitude sinusoidal signal over frequencies ranging from 20,000 Hz to 0.001 Hz. Readings were taken immediately after the addition of medium alone or medium plus bacteria to the bioreactors and subsequently once per day. The duration of a run varied between tests.

Electrochemical direct current (DC) measurements were carried out for mild steel in sterile LB medium and in the presence of *P. fragi* K. The resulting polarization curves were analyzed to determine corrosion current density, i_{corr}, Tafel slopes, b_a and b_c, and the corrosion rate for a sample exposed to bacterial medium and one exposed to sterile LB medium. Polarization curves were measured in the potential region, $E_{corr} \pm 200$ mV, using a scan rate of 0.5 mV/s.

Testing Procedures

One set of experiments consisted of two control bioreactor test cells containing sterile medium and four bioreactor test cells inoculated with *P. fragi* K. The tests were allowed to run until R_p as well as other characteristic features of the spectra remained constant. A light-colored continuous biofilm was clearly visible in each of the samples in the inoculated test cells. The medium also became somewhat turbid from the presence of planktonic bacteria. By contrast, no biofilm was visible and the medium remained clear in the control test cells over the duration of the experiments. A specific bacterial growth rate of 0.64 h^{-1} was determined for the present conditions using a Spectronic 20D† spectrophotometer as reported previously.[13]

Another experiment was performed to determine the effect of living (viable) *P. fragi* K on the corrosion of mild steel. This test consisted of an inoculated bioreactor that was allowed to run for 20 days until R_p held at a constant value. Tetracycline (200 mg/L final concentration) was then added to the nutrient feed of the bioreactor to kill *P. fragi* K, in an attempt to produce a nonliving biofilm. To better interpret the results of this test, experiments were performed to determine the effectiveness of adding tetracycline to kill the biofilm bacteria. For these experiments, *P. fragi* K was added to three continuous flow bioreactors containing LB medium. Tetracycline (200 mg/L final concentration) was then added to the nutrient feed of two of the bioreactors after the corrosion rate stabilized. The bioreactors were carefully dismantled after 14 days, and the metal samples on the bottom were gently rinsed in situ with LB broth (three times with 5 mL for a total of 15 mL) to remove any planktonic bacteria. The entire sample area exposed to the media was then scraped to remove all of the attached bacteria. A sterile plastic spatula was used for each round of scraping. The resulting 6-mL suspension was gently agitated (vortexed) to produce individual cells, and aliquots were then spread onto LB + $kanamycin_{50}$ agar plates to determine the number of viable biofilm bacteria. Three different volumes of suspension were plated for each sample, the plates were incubated at 30°C, and bacterial colonies were counted after several days. The results indicate that the concentration of tetracycline used in the present experiments decreased the number of living *P. fragi* K cells in the biofilm by at least 2 orders of magnitude compared to the untreated bioreactor.

An experiment was also performed to examine the effect of oxygen depletion on the corrosion of mild steel in sterile LB medium. Two sterile control test cells were filled with LB medium and allowed to stabilize for 5 days. The air bubbling into one of the cells was then replaced with high-purity (99.998%) nitrogen bubbling. Readings were taken 2 days later, after allowing the nitrogen to effectively displace the dissolved oxygen in the liquid.

A final series of tests was conducted to investigate the effect of nutrient (LB medium) flow rate on the corrosion of mild steel in the presence of *P. fragi* K. Four bioreactors with bacteria were used in conjunction with four sterile control test cells. During these tests, the flow rates of LB medium into the test cells were held constant at 2, 4, 12, and 20 mL/h.

RESULTS AND DISCUSSION

The corrosion behavior of mild steel under aerobic conditions in the presence of the *P. fragi* K monoculture was studied in a continuous flow system using EIS. Simple inspection of the electrochemical impedance data did not necessarily reveal whether or not the data were valid or had been distorted by some experimental artifact. However, the validity of

† Trade name.

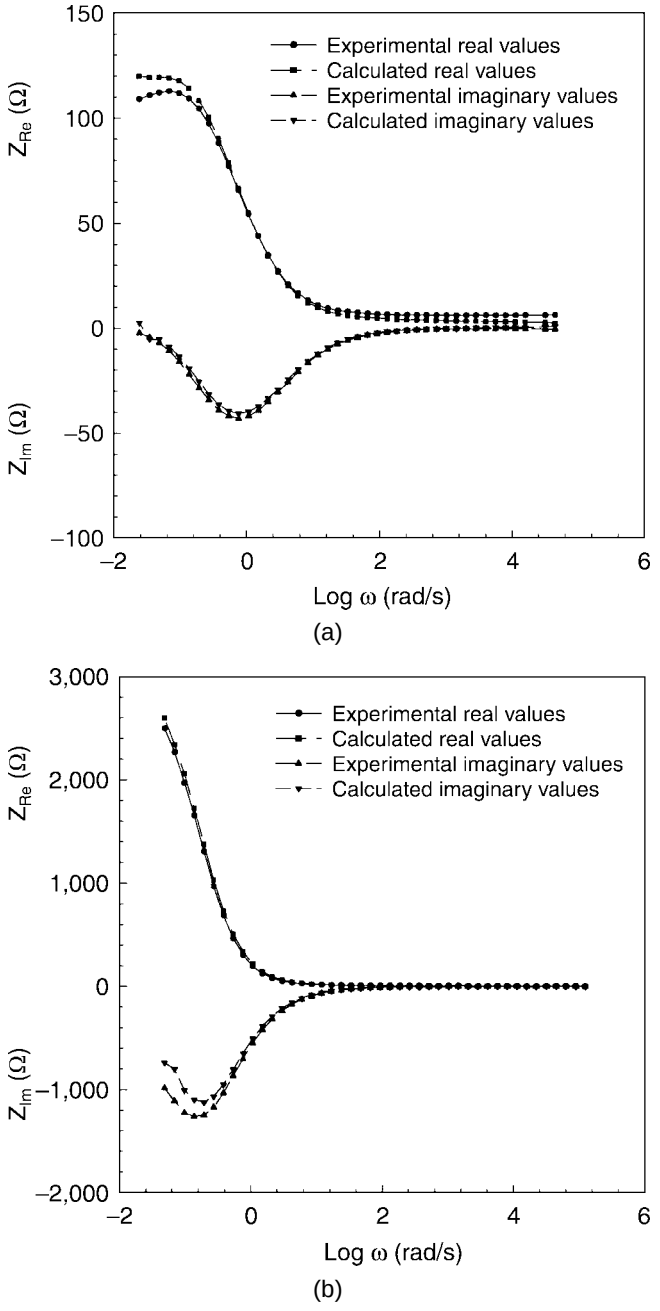

FIGURE 2. *K-K transforms of real and imaginary components of the EIS data for mild steel at OCP after 10 days' exposure in: (a) sterile LB medium and (b) LB medium with* P. fragi *K.*

the data can be assessed using the Kramers-Kronig (K-K) transforms. Any system that satisfies the conditions of linearity, stability, and causality a priori must satisfy the K-K transforms.[18-19]

The K-K technique transforms the real component into the imaginary component and vice-versa, so that the transformed quantities may be compared directly with experimental values for the same parameters. As can be seen from Figure 2, the high fidelity between the experimental and transformed impedance data for both real and imaginary components (Z_{Re} and Z_{Im}, respectively) shows that the system under investigation complies with the linearity, causality, and stability constraints of linear system theory and thereby validates the EIS data.

R_p values obtained after different exposure times in sterile LB medium showed no significant change. By contrast, the presence of *P. fragi* K led to a marked increase in R_p, compared to LB medium without bacteria. After ~10 days of exposure, no further increase in R_p was evident for the mild steel samples in the presence of *P. fragi* K.

Impedance spectra for mild steel obtained after 10 days of exposure to sterile and inoculated LB medium are illustrated in Figure 3. Higher values of R_p for samples exposed to the bacteria compared to those for sterile conditions are demonstrated at the low frequency in Figure 3(a). The phase angle vs frequency (f) plot in Figure 3(b) indicates maximum phase angles of 52° and 68°. Hernandez, et al., observed a similar behavior for sterile conditions and suggested that this phase shift indicates a Warburg-type impedance, and it followed that the corrosion mechanism was controlled by diffusion.[15] However, data on a Nyquist plot for the present study (Figure 3[c]) did not exhibit the linear trend at low frequency that has often been associated with a diffusion-dominated impedance (i.e., Warburg impedance). In the presence of bacteria, the phase angle vs log frequency curve shows a maximum at ~80° for four mild steel specimens.

The Nyquist data curves plotted in Figure 3(c) are characterized by a depressed semicircle at high and medium frequencies and, for the sterile medium, an inductive loop at low frequencies. The presence of a low-frequency inductive loop is typical for iron and mild steel and could be attributed to the relaxation process of adsorbed corrosion intermediate species, such as $Fe(I)_{ads}$ and/or $Fe(II)_{ads}$, on the steel surface as described in previous studies.[20]

Polarization curves measured in a sterile LB medium and LB medium plus bacteria are illustrated in Figure 4. Based on the data in Figure 4, the corrosion rate was 56 µm/y in sterile LB medium and 2 µm/y in the presence of *P. fragi* K. This finding is consistent with the 10- to 20-fold difference in R_p determined from EIS measurements. It is also apparent from Figure 4 that the corrosion-controlling effect of the bacteria was accompanied by an ennoblement of the OCP, as observed in EIS measurements. The data indicate that E_{corr} of mild steel was shifted in the electropositive direction by ~130 mV in the presence of *P. fragi* K. This ennoblement of E_{corr} could possibly have been caused by a decrease in the rate of the anodic reactions as a result of microbiological activity of the aerobic bacteria on the metal surface.

The presence of *P. fragi* K in LB medium led to a significant decrease in the corrosion rate of mild steel: R_p increased by a factor of 10 to 20, compared

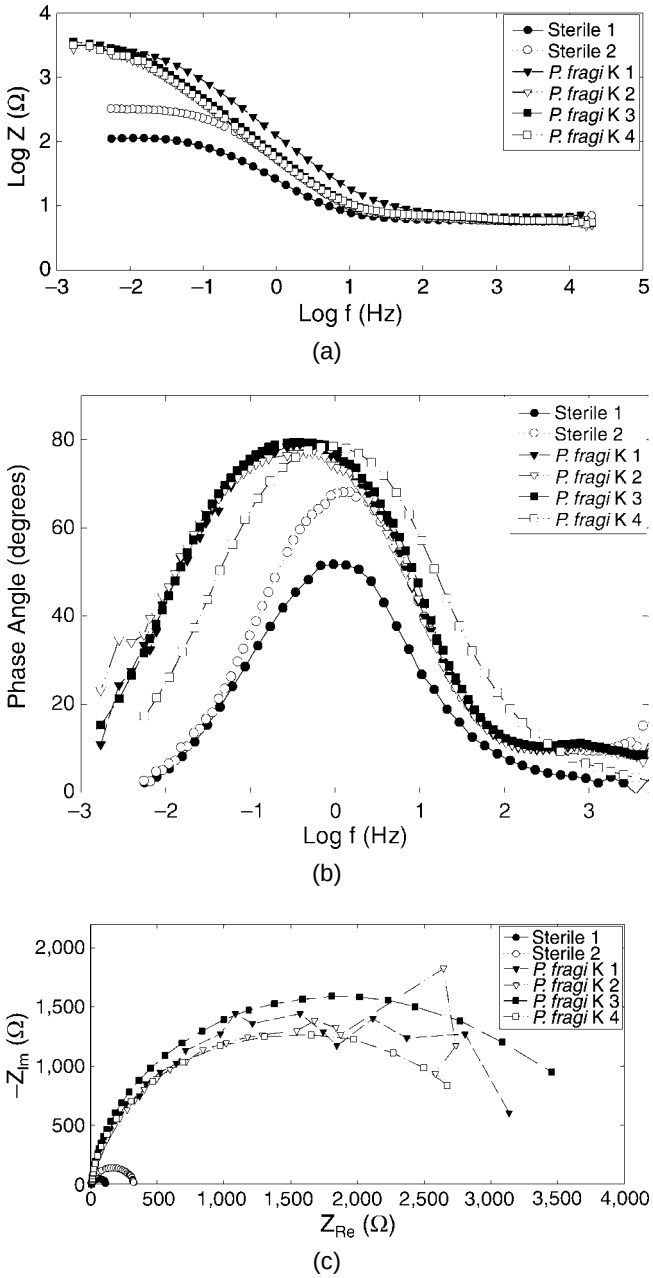

FIGURE 3. *EIS spectra illustrated as (a) Bode, (b) phase angle vs frequency, and (c) Nyquist plots for mild steel after 10 days' exposure in LB medium.*

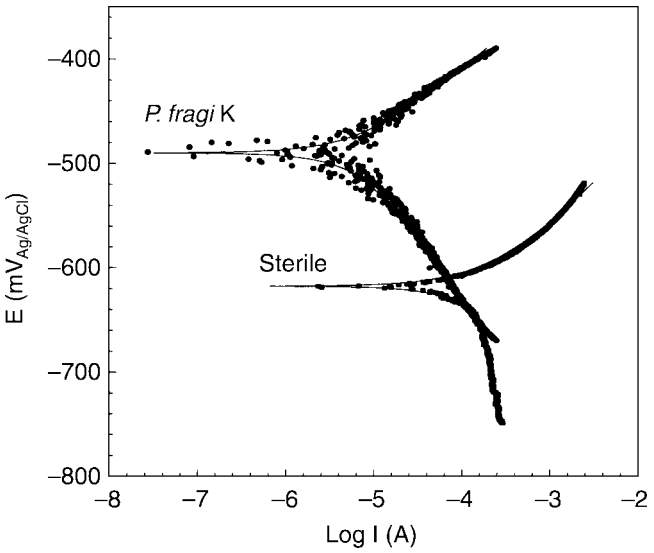

FIGURE 4. *Polarization curves for mild steel in sterile LB medium and in the presence of* P. fragi *K, after 15 days, using a scan rate of 0.5 mV/s.*

to that in sterile LB. This may have been a result of one or more of the following: (a) the presence of a biofilm acting as a barrier to the diffusion of corrosion products and thereby suppressing the process of metal dissolution; (b) diminution of the concentration of oxygen reaching the electrode surface; and/or (c) the bacteria producing a metabolic product that acted as a corrosion inhibitor for mild steel. In an additional experiment, the bacterial cells in supernatant and in the biofilm were killed by adding 200 mg/L of tetracycline after 20 days' exposure. As opposed to the procedure used by others,[15-16] the bacteria in the present work were killed in situ without otherwise disturbing the biofilm. The change in impedance with time after adding tetracycline to the LB medium is shown on a Bode plot in Figure 5. The results indicate that R_p values were higher by at least an order of magnitude when a biofilm containing metabolically active bacteria, rather than tetracycline-treated bacteria, was present on the mild steel surface. Hence, the present experiment indicates that actively respiring bacteria are necessary for the observed corrosion reduction. This result also suggests that the corrosion reduction mechanism is not a result of, in large part, the presence of a physical barrier of dead bacteria and extracellular material since the biofilm remains after the addition of tetracycline. Rather, the observed reduction in corrosion rate is more likely attributable to the diminution of the concentration of oxygen reaching the metal surface and possible production of a protective metabolite by active cells.

Another experiment was carried out to examine whether lowering the oxygen concentration gives rise to the same effect on corrosion of the mild steel samples as the biofilm containing aerobic *P. fragi* K. Lowering the oxygen content should retard corrosion by reducing the driving force of the cathodic reduction reactions. In theory, actively respiring aerobic bacteria can bring about such a decrease of oxygen in their environment. EIS spectra plotted in Figure 6 demonstrate that a lowering of the oxygen content in the sterile medium by bubbling nitrogen in the test cell decreased the corrosion rate, but not as effectively as the aerobic bacteria.

A final set of experiments was designed to investigate the biofilm as a living ecosystem. The amount

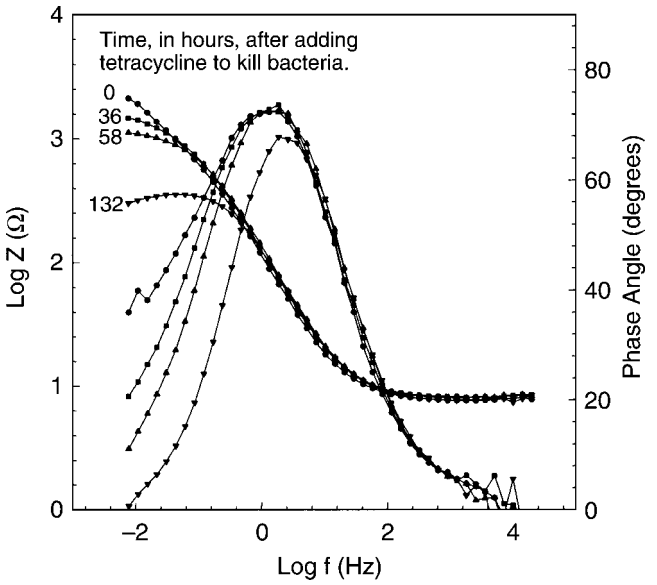

FIGURE 5. *Bode plots for mild steel in LB medium after adding tetracycline to kill bacteria.*

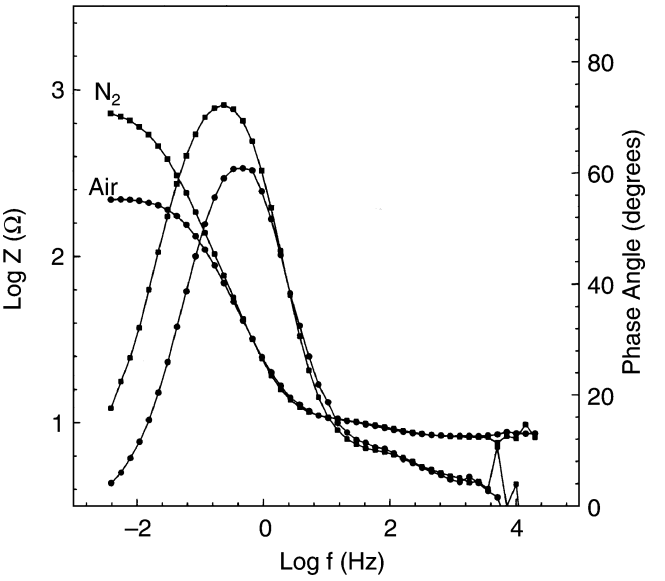

FIGURE 6. *Bode plot for mild steel in aerated and deaerated LB medium, after 7 days.*

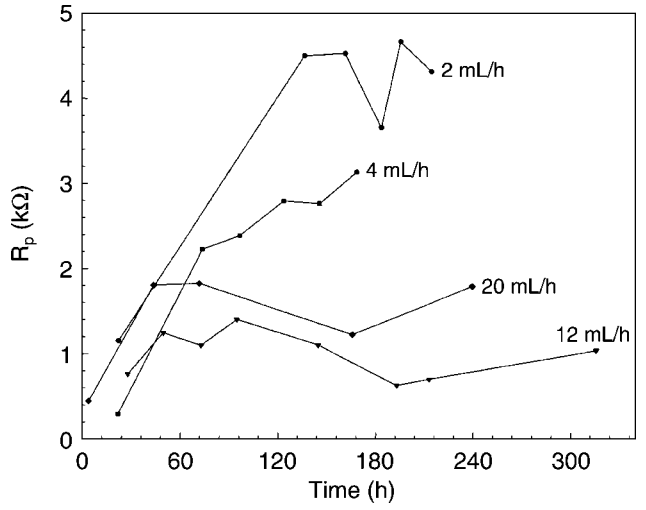

FIGURE 7. *R_p as a function of time for mild steel in the presence of* P. fragi *K for different LB medium flow rates.*

of oxygen that the bacterial cells in solution and biofilm consume and, hence, the amount of corrosion reduction provided should depend on the growth rate and metabolic activity of the bacteria. If this corrosion reduction effect is to be put to any practical use, the bacterial cells in the solution and biofilm must be regulated to provide for maximum oxygen consumption. The amount of nutrients available to the bacteria is one of the major factors controlling their metabolic state.

Impedance spectra for mild steel exposed to *P. fragi* K under four different medium flow rates (2, 4, 12, and 20 mL/h) were obtained. Figure 7 shows that the R_p value decreases about four-fold as the medium flow rate increases from 2 mL/h to 12 mL/h, and then increases slightly at the highest flow rate of 20 mL/h. By contrast, there was no significant difference among the impedance spectra for the corresponding sterile cells tested under different flow rates. It is known that biofilms generally do not form under nutrient-poor conditions. The bacteria tend to stay in suspension and consume less oxygen until enough food is available.[6,15]

Bacteria in suspension can be quite dense depending on the flow conditions.[12-13] Thus, it is reasonable that not only the biofilm bacteria but also planktonic bacterial cells can have an effect on the observed corrosion of mild steel. Bacteria in suspension act hydraulically similar to those in the liquid phase, which, in continuous culture systems, limits the residence time and productivity of the bacterial cells in the bioreactor.[21] Accordingly, the present results suggest that the wash-out of bacterial cells decreased with decreasing flow rate below ~12 mL/h, leading to an increase in bacterial activity in the supernatant. Another possibility to consider is that, as the flow rate increased, the supply of oxygen increased at the biofilm surface. The bacteria then had more oxygen to consume and, if they were already at maximum consumption rate, the corrosion rate would have increased. However, since an ample amount of oxygen was supplied during all experiments regardless of the medium flow rate, it appears that the former explanation is more plausible.

CONCLUSIONS

❖ The current experiments indicate that exposure of mild steel samples to a monoculture of *P. fragi* K bacteria in LB medium results in a 10-fold or greater

decrease in the corrosion rate. The observed corrosion protection of mild steel appears to be caused by actively respiring aerobic bacteria as opposed to a barrier effect by the biofilm. An observed ennoblement of E_{corr} in the presence of *P. fragi* K compared to E_{corr} for sterile conditions suggests that, in addition to scavenging oxygen, the bacteria must secrete a metabolic product that acts as a corrosion inhibitor. The corrosion rate decreased as supernatant flow rates dropped from 12 mL/h to 2 mL/h. This trend suggests that the corrosion reduction was a result of the bacteria present in both the supernatant and biofilm at low flow rates, while the corrosion reduction was primarily caused by bacteria in the biofilm at the higher flow rates.

ACKNOWLEDGMENTS

The authors acknowledge the support of this work by the Electric Power Research Institute (EPRI) under Contract no. RP8044-02. Assistance from L. Chau in performing some of the experiments and B.C. Syrett of EPRI for his helpful comments and suggestions is also acknowledged.

REFERENCES

1. R.F. Jack, D.B. Ringelberg, D.C. White, Corros. Sci. 33, 12 (1992): p. 1,843.
2. G.J. Licina, MP 28, 10 (1989): p. 55.
3. B. Badan, M. Magrini, E. Ramous, J. Mater. Sci. 26, 7 (1991): p. 1,951.
4. K.D. Xu, G.A. McFeters, P.S. Stewart, Microbiology 146 (2000): p. 547.
5. P.S. Stewart, G.A. McFeters, C. Huang, Biofilms II: Process Analysis and Applications, ed. J. Breyers (New York, NY: Wiley-Liss, Inc., 2000), p. 373.
6. J.W. Costerton, Z. Lewandowski, D.E. Caldwell, D.R. Korber, H.M. Lappin-Scott, Ann. Rev. Microbiol. 49 (1995): p. 711.
7. S.A. Blenkinsopp, J.W. Costerton, Trends Biotechnol. 9, 1 (1991): p. 138.
8. J.W. Costerton, J. Ind. Microbiol. 15 (1995): p. 137.
9. R.J. Soracco, L.R. Berger, J.A. Berger, L.A. Mayack, D.H. Pope, E.W. Wilde, "Microbiologically Mediated Reduction in the Pitting of Mild Steel Overlaid with Plywood," CORROSION/84, paper no. 98 (Houston, TX: NACE International, 1984).
10. T. Ford, J. Maki, R. Mitchell, Biodeterioration 7 (Amsterdam, The Netherlands: Elsevier Applied Sciences, 1988), p. 378.
11. A. Pedersen, M. Hermansson, Biofouling 1 (1989): p. 313.
12. A. Pedersen, M. Hermansson, Biofouling 3 (1991): p. 1.
13. A. Jayaraman, J.C. Earthman, T.K. Wood, J. Appl. Microbiol. Biotechnol. 47 (1997): p. 62.
14. A. Jayaraman, E.T. Cheng, J.C. Earthman, T.K. Wood, J. Appl. Microbiol. Biotechnol. 18 (1997): p. 396.
15. G. Hernandez, V. Kucera, D. Thierry, A. Pedersen, M. Hermansson, Corrosion 50, 8 (1994): p. 603.
16. M.J. Franklin, D.E. Nivens, J.B. Guckert, D.C. White, Corrosion 47, 7 (1991): p. 519.
17. A.K. Sun, T.K. Wood, J. Appl. Microbiol. Biotechnol. 84 (1998): p. 485.
18. M. Urquidi-Macdonald, S. Real, D.D. Macdonald, J. Electrochem. Soc. 133 (1986): p. 2,018.
19. M. Urquidi-Macdonald, S. Real, D.D. Macdonald, Electrochim. Acta 35 (1990): p. 1,559.
20. M. Keddam, O.R. Mattos, H. Takenout, J. Electrochem. Soc. 128 (1981): pp. 257, 266.
21. J.D. Bryers, Biotechnol. Prog. 3, 2 (1987): p. 57.