Pitting Corrosion Control of Aluminum 2024 Using Protective Biofilms That Secrete Corrosion Inhibitors

D. Örnek, T.K. Wood, C.H. Hsu, Z. Sun, and F. Mansfeld***

ABSTRACT

The corrosion behavior of Al 2024-T3 (UNS A92024) exposed to artificial seawater (AS) and Luria Bertani (LB) medium has been studied using electrochemical impedance spectroscopy (EIS). Tests were performed in sterile media and in the presence of three different biofilm-forming bacteria: a Bacillus subtilis *biofilm that was genetically engineered to produce either polyaspartate or polyglutamate, a* Bacillus licheniformis *bacterial biofilm that naturally produces* γ*-polyglutamate, and an* Escherichia coli *biofilm that was genetically engineered to produce polyphosphate. A significant reduction in active pit growth rates and an ennoblement of the corrosion potential (E_{corr}) were observed in both media in the presence of these biofilms, which produce corrosion inhibitors. The lowest corrosion rates of Al 2024 exposed to the LB medium were observed in the presence of the* B. subtilis *bacterial biofilm-producing polyaspartate and the* E. coli *bacterial biofilm that produced polyphosphate. In the presence of the latter biofilm, E_{corr} was more positive by ~400 mV than in the sterile solution.*

KEY WORDS: Al 2024, artificial seawater, biofilms, corrosion potential, electrochemical impedance spectroscopy, inhibitors

INTRODUCTION

The general concept of microbiologically influenced corrosion (MIC) is based on the assumption that microorganisms accelerate the kinetics of electrochemical reactions, thereby producing increased corrosion rates without—in most cases—changing the corrosion mechanism.[1-2] Eashwar, et al.,[3] have suggested that the often-discussed ennoblement of stainless steels in seawater is caused by the production of inhibitors by bacteria retained in the biofilm matrix. Further evidence that bacteria may be beneficial has been presented by Pedersen and Hermansson,[4-5] Obuekwe, et al.,[6] Potekhina,[7] as well as by Jayaraman, et al., who have shown that protective biofilms decreased the corrosion rate of mild steel by reducing the oxygen concentration at the metal surface.[8-10] The beneficial biofilms have also been shown to protect other metals such as brass and copper.[11-12] This and other observations have led to a new approach of corrosion protection based on corrosion control using regenerative biofilms (CCURB). Obviously, successful implementation of CCURB could produce large savings in expenditures for biocides and corrosion inhibitors in many practical applications.

Protective biofilms have also been genetically engineered to secrete small antimicrobial proteins (e.g., indolicidin, bactenecin) active against sulfate-reducing bacteria (SRB) and thereby have reduced the corrosive attack of stainless steel by these deleterious bacteria.[13-14] Following this success, it was in-

Submitted for publication September 2001; in revised form, April 2002.

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TABLE 1
Composition of VNSS and LB Media

Compound	Concentration (g/L)	
	VNSS	LB Medium
NaCl	17.6	10
$NaHCO_3$	0.08	–
KBr	0.04	–
$CaCl_2 \cdot 2H_2O$	0.41	–
$SrCl_2 \cdot 6H_2O$	0.008	–
Na_2SO_4	1.47	–
KCl	0.25	–
$MgCl_2 \cdot 6H_2O$	1.87	–
H_3BO_3	0.008	–
$FeSO_4 \cdot 7H_2O$	0.01	–
Na_2HPO_4	0.01	–
Peptone	1.0	–
Starch	0.5	–
Glucose	0.5	–
Yeast extract	0.5	5
Tryptone	–	10

vestigated whether bacteria could be successfully engineered to secrete general corrosion inhibitors such as the polyanionic polymers polyglutamate and polyphosphate to protect Al 2024-T3 (UNS A92024)[(1)] from pitting attack. These compounds hold promise since purified anionic organic compounds have been shown to reduce the corrosion rate of steel, copper, and aluminum.[15-17] Previous analyses of EIS data obtained for Al 2024 exposed to artificial seawater (AS) have shown that in the presence of biofilms active pit growth stopped after ~2 days.[18] The additional protection afforded by the secreted general corrosion inhibitors will be demonstrated in this paper. A more detailed analysis of the data obtained in Luria Bertani (LB) medium as well as a discussion of the experimental data for Al 2024 exposed to both media will also be presented.

EXPERIMENTAL PROCEDURES

Al 2024-T3 plates (10-cm-by-10-cm squares, 2-mm thick) were cut from sheet stock and polished with 240-grit paper. One of the test solutions was AS prepared from Vätänen nine salts solution (VNSS, pH 7.0).[19] The other test solution—LB medium—is a rich growth medium.[20] Table 1 gives the chemical compositions of these two solutions. These test environments were used either in a sterile condition or after inoculation with bacteria such as *B. subtilis* WB600,[21] a protease-deficient strain obtained from S.-L. Wong of the University of Calgary. Another strain, *E. coli* XLI (blue), was purchased from Stratagene. Plasmid pBE92 containing the alkaline protease (*apr*) promoter, constitutive *apr* signal sequence, and the alkaline phosphatase reporter gene were obtained from E.I. du Pont de Nemours Inc.

B. subtilis WB600 was engineered to secrete either polyaspartate or polyglutamate as 20 amino-acid polymers by using recombinant DNA methods as described previously.[18] *E. coli* XLI (blue) transformants containing the correct insert (pBE92-Asp or pBE92-Glu) were screened as white colonies on LB agar plates containing 100 μg/mL ampicillin ($C_{16}H_{19}N_3O_4S$) and 40 μg/mL 5-bromo 4-chloro-3-indolyl phosphate ($C_8H_4BrC_2NO_4P$) (transformants with the correct insert produced white colonies while the reclosed vector resulted in blue colonies). The plasmids containing inserts were further characterized through restriction digests with *Nhe* I, *Hin*d III, and *Eco* RI. *E. coli* XLI (blue) cells were made electrocompetent according to the method of Smith and Iglewski[22] and electroporated using a gene pulser/pulse controller (Bio-Rad Laboratories). *B. subtilis* WB600 strains were transformed with the two plasmids according to the two-step method of Cutting and Vander Horn[23] and plated on LB agar plates containing 50 μg/mL kanamycin ($C_{18}H_{36}O_{11}N_4$).

Biofilms on metal surfaces were developed in glass/polytetrafluoroethylene (PTFE) cylindrical continuous reactors (30°C, liquid nutrient flow rate: 0.2 mL/min, air flow: 200 mL/min to headspace, working volume: 100 mL, exposed surface area of test electrode: 28.3 cm^2 or 45.4 cm^2). A titanium counter electrode and autoclavable silver/silver chloride (Ag/AgCl) reference electrode (E^o = 0.208 V vs standard hydrogen electrode [SHE] at 25°C) were also used. The continuous reactors (sterile and inoculated) were inoculated with 100 μg/mL kanamycin to ensure sterility or the presence of the engineered kanamycin-resistant strain. A 1-vol% bacterial inoculum from a turbid, 16-h culture was used for all continuous experiments.

Electrochemical impedance data were obtained at the open-circuit potential, E_{corr}, in the frequency range of 20 kHz to 0.5 mHz using an IM6[†] electrochemical impedance analyzer with a 16-channel cell multiplexer (Bioanalytical Systems-Zahner). Impedance spectra were collected once a day during exposure to AS and the LB medium. The impedance spectra were analyzed using the Pitfit and Basics modules of the ANALEIS software developed by Mansfeld, et al.[24-25]

RESULTS AND DISCUSSION

It has been shown previously using continuous fermentors that the corrosion inhibition of aluminum as well as that of mild steel is caused by the biofilm on the metal surface and not a result of the suspended cells.[8] Furthermore, it was shown using confocal scanning laser microscopy that bacteria cultivated under similar conditions produced exopoly-

[(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.

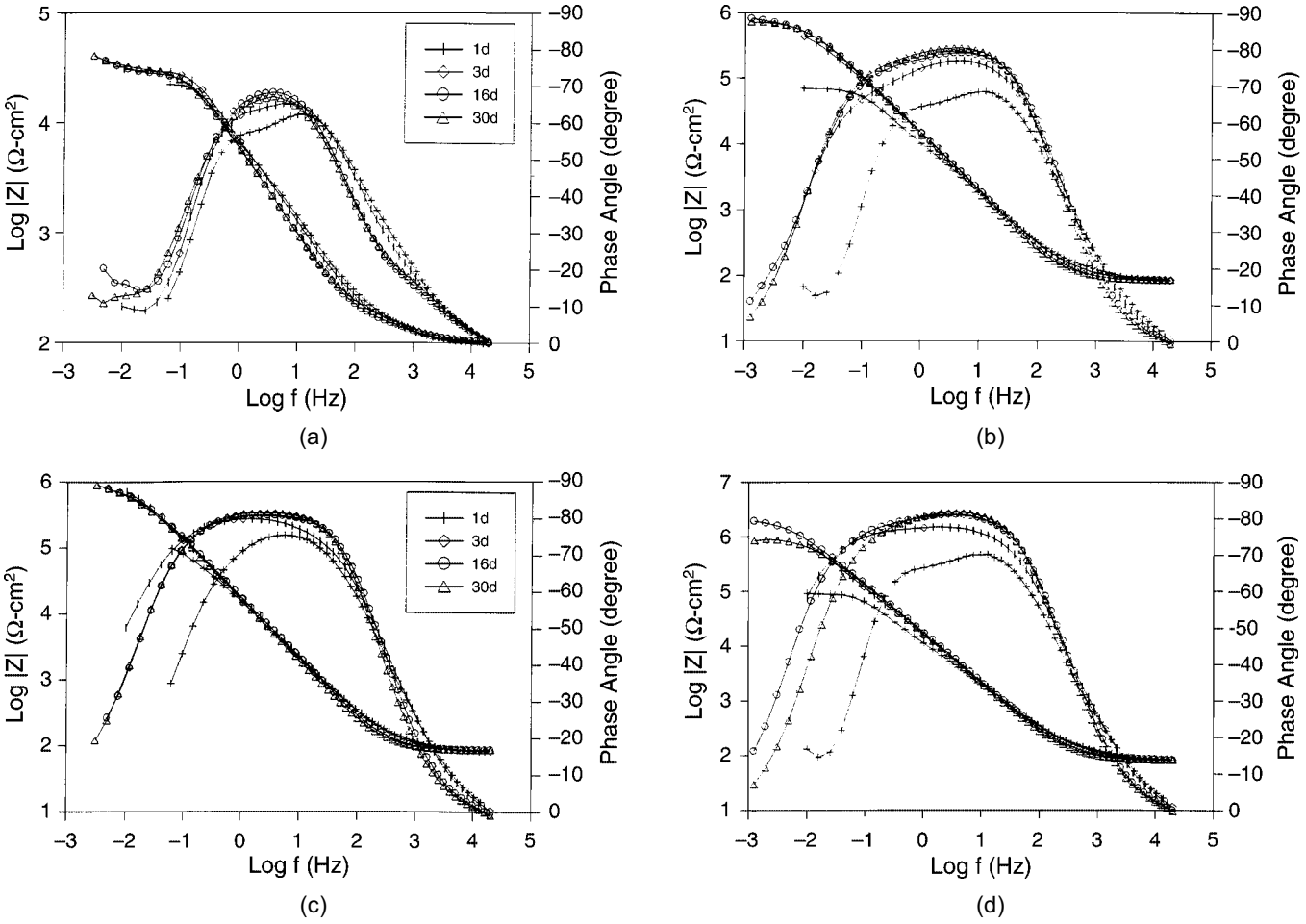

FIGURE 1. *Bode plots for Al 2024 exposed to VNSS for different time periods: (a) sterile solution (Test 45), (b) VNSS containing* B. subtilis *WB600 (Test 42), (c) VNSS containing* B. subtilis *WB600/pBE92 producing polyglutamate (Test 43), and (d) VNSS containing* B. subtilis *WB600/pBE92 producing polyaspartate (Test 44).*

saccharide and formed biofilms of ~15 μm attached to these metal surfaces.[9] Using 15 different aerobic bacteria, it was also demonstrated that the degree of corrosion inhibition was related to the robustness of the biofilm in terms of metal coverage and population of respiring cells.[9]

Figure 1(a) shows experimental impedance spectra obtained for Al 2024 during exposure to AS for 30 days. Only four of the spectra collected during this time are plotted in the Bode plots of Figure 1(a). The spectra suggest that pitting occurred during the entire test period as evidenced by the typical low-frequency minimum of the phase angle, Φ, which is partially masked by the scatter of the data points below 0.01 Hz. Nevertheless, the spectra in Figure 1(a) are in agreement with the pitting model proposed by Mansfeld, et al.[24-25] Qualitatively, it can be observed that the polarization resistance of active pits, R_{pit}, which is close to the impedance value, $|Z|$, at the frequency minimum at low frequencies, increased with increasing exposure time as the pit growth rate decreased.[24-25]

In the presence of *B. subtilis*, pitting also occurred in the first 2 days of exposure; however, after 3 days, the spectra agreed with those for a passive surface (i.e., a simple, one-time constant model in which the polarization resistance, R_p, is in parallel with the electrode capacitance, C).[24-25] The fairly high values of R_p, which approach the MΩ range, suggest that pits formed in the initial stages of exposure have become passivated (Figure 1[b]). Very similar results were obtained in the presence of *B. subtilis*-producing polyglutamate (Figure 1[c]) or polyaspartate (Figure 1[d]). The increased R_p values suggest that the inhibitors produced by the bacteria provided additional corrosion protection.

Figure 2 illustrates the time dependence of the relative corrosion rates expressed as $1/R^o_{pit}$ for the tests in the absence of bacteria and $1/R^o_p$ for the tests in the presence of *B. subtilis.* These values have been obtained by normalizing the experimental R_p values with the total exposed area and the R_{pit} values with the time-dependent values of the pitted area, A_{pit}, determined by analysis of the impedance spectra as

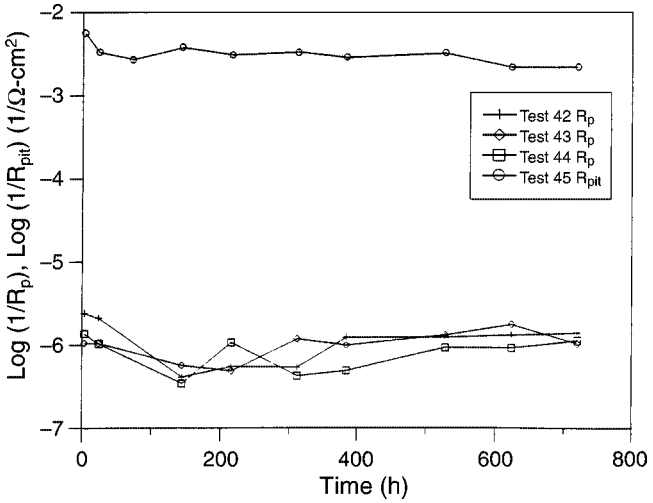

FIGURE 2. *Time dependence of R_{pit}^{o} and R_{p}^{o} in sterile VNSS (Test 45) and in VNSS containing* B. subtilis *WB600/pBE92 (Tests 42 through 44).*

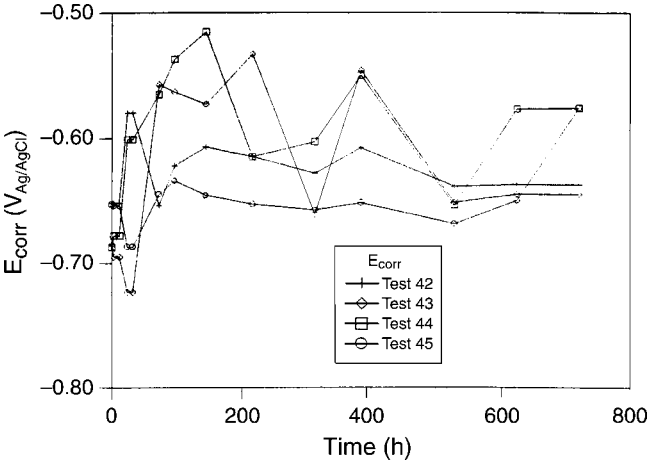

FIGURE 3. *Time dependence of E_{corr} in sterile VNSS (Test 45) and in the presence of* B. subtilis *WB600/pBE92 (Tests 42 through 44).*

explained elsewhere.[18] For the tests in the absence of bacteria, R_p could not be determined because of the lack of sufficient low-frequency data (Figure 1[a]). Figure 2 clearly demonstrates the inhibition of pitting corrosion in the presence of *B. subtilis* and the increased corrosion resistance in the early stages of exposure caused by the inhibitors produced by the bacteria. The lowest corrosion rates were observed for the biofilm producing polyaspartate.

For the experiments in the presence of *B. subtilis* (Tests 42 through 44), the values of A_{pit}, determined by visual observation at the end of the exposure period, were much less than those determined in the absence of bacteria for which the pitted area fraction, F, was 1.04%. The final F values for Tests 42, 43, and 44 were 0.07%, 0.16%, and 0.06%, respectively.

The inhibition of pitting in the presence of bacteria could be a result of the exclusion of oxygen from the metal surface, which would reduce the rate of the cathodic reduction resulting in a decrease of the corrosion potential, E_{corr}, below the pitting potential, E_{pit}. However, the experimental values of E_{corr} had the lowest values in the absence of bacteria, while a certain degree of ennoblement was observed in the presence of bacteria (Figure 3). Since the highest values of E_{corr} were observed in the presence of inhibitors, it can be concluded that the observed CCURB is due to a passivation effect that occurs in the presence of a biofilm. This beneficial effect is apparent even when the biofilm contains bacteria that were not engineered to produce inhibitors. Indeed, the observation that pitting occurred in all cases in the first 2 days of exposure clearly suggests that formation of a stable biofilm is needed to stop the growth of active pits.

The impedance spectra obtained in LB medium are shown in Figure 4 as a function of exposure time for the tests listed in Table 2. Most tests in Table 2 were conducted in duplicate. Only one of these sets is shown in Figure 4. Pitting is indicated by the frequency dependence of the impedance spectra at low frequencies in the sterile LB medium (Figure 4[a]), similar to the results obtained in sterile VNSS (Figure 1[a]).

The time dependence of R_{pit} for the test in sterile LB medium and R_p for the tests in the presence of *B. subtilis* is shown in Figure 5. Similar low values of R_{pit} were observed in the sterile solution (Tests 111 and 158), while the R_p values in solutions containing *B. subtilis* were all higher. Polyaspartate produced by the biofilm seemed to provide some additional corrosion protection, as suggested by the high R_p values for Tests 101 and 110 in Figure 5. Similar to the results obtained in AS, E_{corr} was more positive in the presence of *B. subtilis* than in the sterile solution (Figure 6). The most pronounced ennoblement was observed in the presence of polyaspartate produced by the biofilm.

The time dependence of R_{pit} for the test in sterile LB medium and R_p for all tests in the presence of *B. licheniformis* or *E. coli* is shown in Figure 7. While there is some scatter in the R_p values obtained for tests in the presence of *B. licheniformis* producing γ-polyglutamate (Tests 86 and 107), the average values were significantly higher than those determined in the sterile solution. The R_p values obtained in the presence of *E. coli* producing polyphosphate (Test 171) were higher than those in Test 170, suggesting that polyphosphate produced an additional increase in the corrosion resistance of Al 2024 exposed to LB medium (Figure 7). R_p values determined in the presence of *E. coli* were about a factor of 10 higher than those in the sterile solution. The average values of $R_p^o = 2 \times 10^6$ Ω-cm^2 correspond to a very low corrosion rate of ~0.1 μm/y, similar to the corrosion rates de-

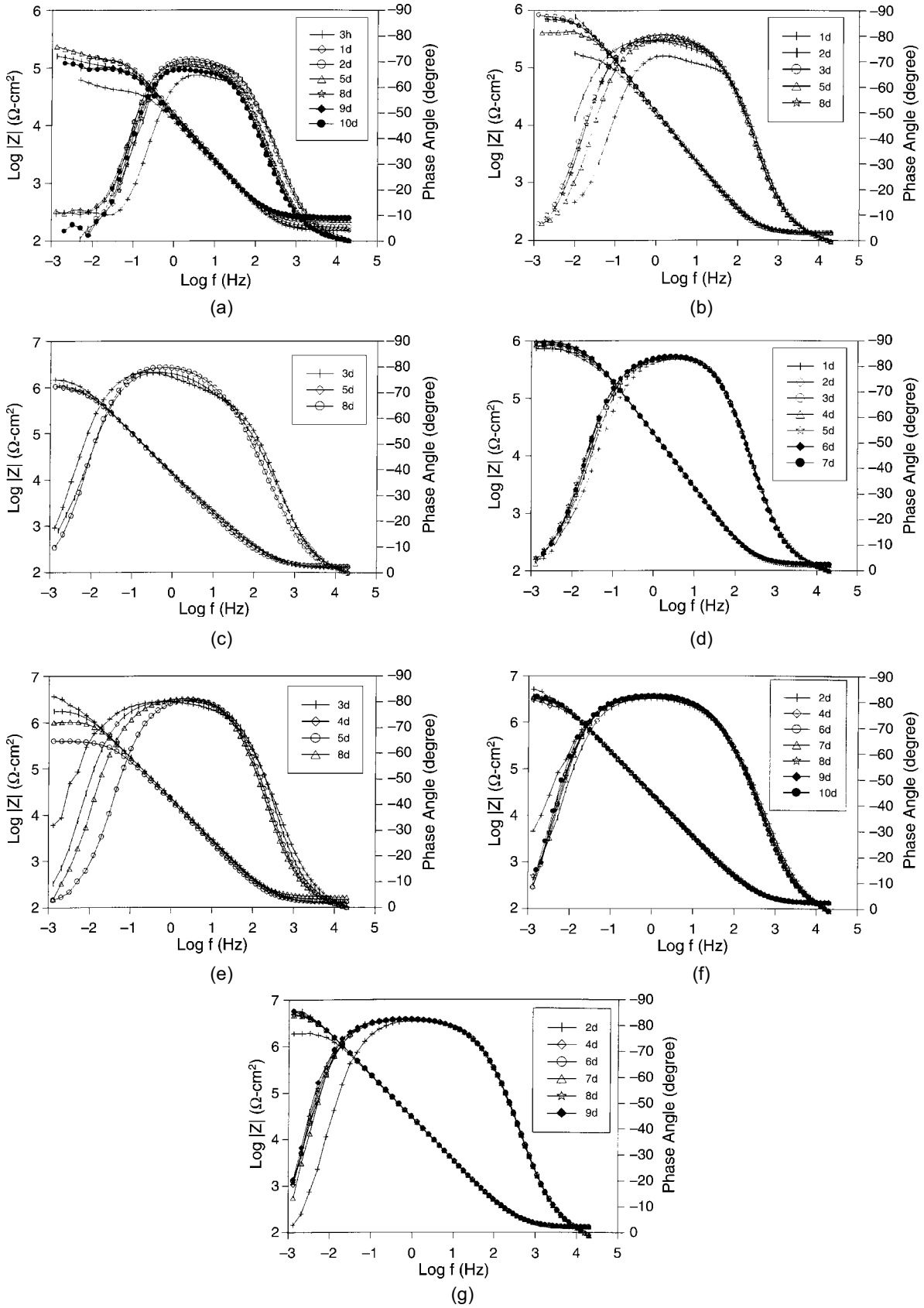

FIGURE 4. *Bode plots for Al 2024 exposed to LB medium for different time periods: (a) sterile solution, (b)* B. subtilis *WB600, (c)* B. subtilis *WB600/pBE92-polyaspartate, (d)* B. subtilis *polyglutamate, (e)* B. licheniformis-γ*-polyglutamate, (f)* E. coli, *and (g)* E. coli-*polyphosphate.*

TABLE 2
Experiments for Al 2024 in LB Medium

Test	Medium	pH	Strain	Secreted Inhibitor
111	LB	6.5	Sterile	–
158	LB	6.5	Sterile	–
102	LB	6.5	*B. subtilis* WB600	–
109	LB	6.5	*B. subtilis* WB600	–
101	LB	6.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
110	LB	6.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
157	LB	6.5	*B. subtilis* WB600/pBE92-polyglutamate	Polyglutamate
86	LB	6.5	*B. licheniformis*	γ-polyglutamate
107	LB	6.5	*B. licheniformis*	γ-polyglutamate
170	LB	7.0	*E. coli*	–
171	LB	7.0	*E. coli*-polyphosphate	Polyphosphate

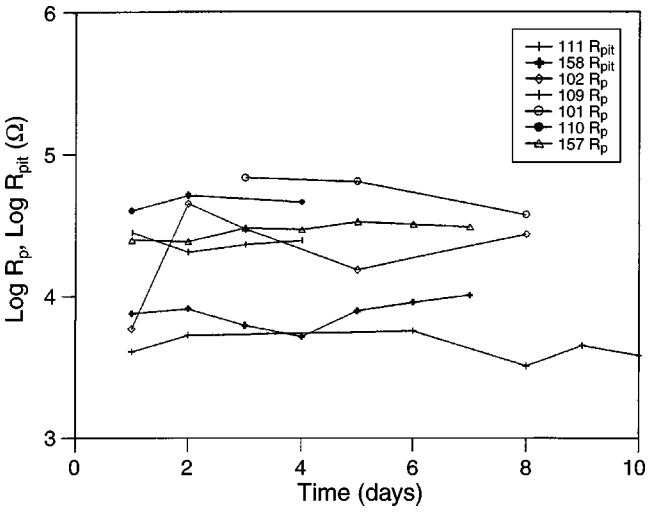

FIGURE 5. *Time dependence of R_p or R_{pit} for Al 2024 exposed to LB medium with and without* B. subtilis *WB600.*

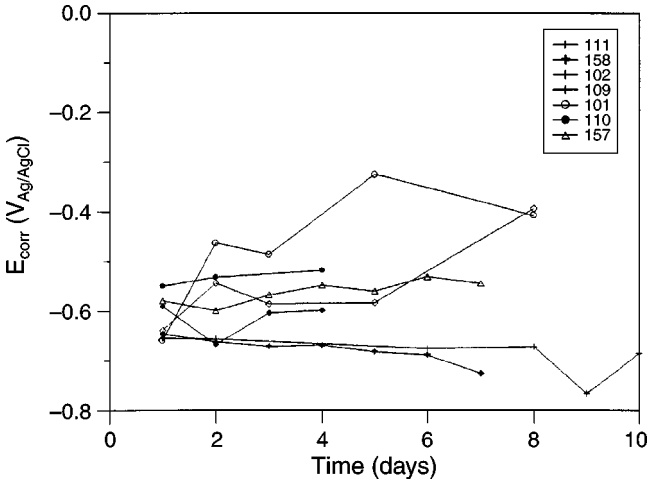

FIGURE 6. *Time dependence of E_{corr} for Al 2024 exposed to LB medium with and without* B. subtilis *WB600.*

termined for UNS C26000 brass exposed under the same conditions.[11] Ennoblement was also observed in these tests (Figure 8). In the presence of *E. coli*, E_{corr} was ~400 mV more positive than in the sterile solution.

CONCLUSIONS

❖ The corrosion behavior of Al 2024 was monitored by recording impedance spectra in AS and LB medium for 30 days. In the sterile solutions, pitting occurred during the entire exposure period. In the presence of *B. subtilis* WB600, pitting was also observed initially in AS; however, after ~3 days the impedance spectra demonstrated that pitting had stopped. CCURB was also achieved in the presence of the same type of bacteria that had been genetically altered to produce polyglutamate or polyaspartate as inhibitors. A small additional passivation effect was observed in these cases. Considering the susceptibility of Al 2024-T3 to pitting in seawater, the observed success of CCURB is quite remarkable. Similar results were observed during exposure to LB medium, where pitting occurred in sterile solutions, but inhibition was observed in the presence of bacterial biofilms producing inhibitors. Corrosion rates were quite low in the μm/y range.

❖ E_{corr} reached its most noble values after ~1 week of exposure to AS containing bacterial biofilm producing inhibitors. In LB medium, ennoblement of ~400 mV compared to the sterile solution was observed for *E. coli* producing polyphosphate. These results are similar to the ennoblement of stainless steels in seawater and natural waters, although the increase of E_{corr} was less dramatic in most cases discussed here. The fact that ennoblement was also observed for UNS C26000 brass in AS and LB medium[11,26] suggests that this phenomenon is more common than previously realized.

❖ Since even in the presence of the bacteria that did not produce polyglutamate or polyaspartate, E_{corr} increased beyond E_{pit} determined in the sterile solution, for Al 2024 in sterile AS equals E_{corr}; it is likely

that *B. subtilis* WB600 produced a chemical species that was able to stop pitting after a certain incubation period. The observed ennoblement of E_{corr} rules out the possibility that CCURB in AS was only caused by anaerobic conditions at the Al alloy surface. It is interesting to note that Nagiub and Mansfeld[27] reported that in the presence of *Shewanella algae* or *an*a inhibition of pitting of Al 2024 exposed to AS containing a growth medium was accompanied by a shift of E_{corr} in the negative direction. In this case, the bacteria created anaerobic conditions leading to a significant decrease of E_{corr} below E_{pit}, resulting in passive behavior instead of pitting. For brass in the presence of *Shewanella,* significantly reduced corrosion rates were accompanied by more negative values of E_{corr},[27] indicating that the mechanism of corrosion protection is different for *B. subtilis* and *Shewanella.*

ACKNOWLEDGMENTS

This project is being supported by the Electric Power Research Institute (Contract WO8044-05). The authors acknowledge many helpful discussions with the project monitor, B.C. Syrett. The generous gifts of *Bacillus* cloning vectors and advice by M.W. Chen and V. Nagarajan of E.I. du Pont de Nemours, Inc. are gratefully acknowledged.

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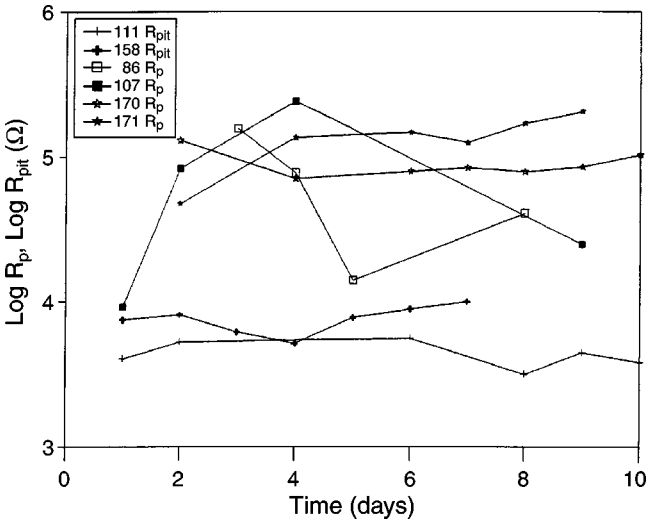

FIGURE 7. *Time dependence of R_p or R_{pit} for Al 2024 exposed to LB medium with and without* B. licheniformis *or* E. coli.

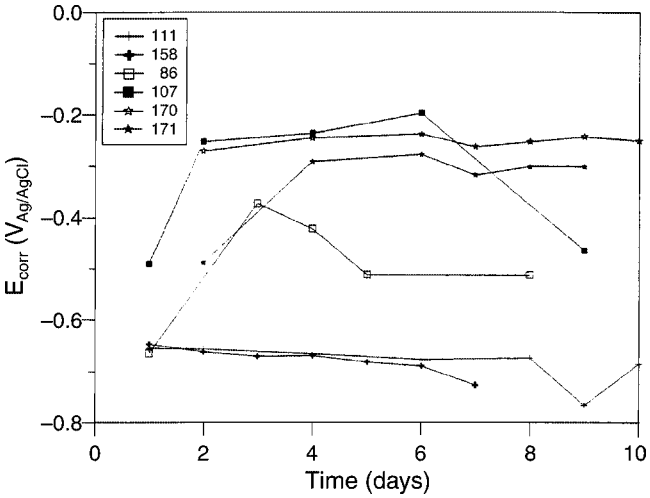

FIGURE 8. *Time dependence of E_{corr} for Al 2024 exposed to LB medium with and without* B. licheniformis *or* E. coli.

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