



## Corrosion Control Using Regenerative Biofilms on Aluminum 2024 and Brass in Different Media

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The corrosion behavior of Al 2024-T3 and C26000 brass exposed to artificial seawater and Luria Bertani medium has been studied using electrochemical impedance spectroscopy. Tests were performed in sterile media and in the presence of three strains of bacteria. A *Bacillus subtilis* biofilm was genetically engineered to produce polyaspartate or polyglutamate, a *B. licheniformis* biofilm naturally produced the anionic polymer  $\gamma$ -polyglutamate, and *E. coli* was genetically engineered to produce polyphosphate. A significant reduction of active pit growth rates and an ennoblement of the corrosion potential  $E_{\text{corr}}$  were observed for Al 2024 in both media in the presence of the biofilms. The lowest corrosion rates of Al 2024 exposed to LB medium were observed in the presence of the *B. subtilis* bacterial biofilms producing polyaspartate and the *E. coli* bacterial biofilm producing polyphosphate in which  $E_{\text{corr}}$  was more positive by about 400 mV than in the sterile solution. A significant reduction of corrosion rates and an ennoblement of  $E_{\text{corr}}$  were also observed for brass in both media in the presence of the biofilms. Samples exposed in the presence of biofilms remained untarnished and unattacked for time periods exceeding one week, while samples exposed in the sterile solutions were covered with a dark film of corrosion products.

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The great interest in microbiologically influenced corrosion (MIC) is documented in the proceedings of numerous international conferences<sup>1,2</sup> and symposia on this subject as well as the large number of publications dealing with MIC. Little *et al.* have given a review of MIC of different materials.<sup>3</sup> The general concept of 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.

It is surprising that only very few publications have dealt with the possibility that bacteria can influence corrosion reactions in a beneficial way, *i.e.*, cause microbiologically influenced corrosion inhibition. One of the few exceptions is the suggestion by Eashwar *et al.*<sup>4</sup> that the often-discussed ennoblement of stainless steels in seawater is due to the production of inhibitors by bacteria retained in the biofilm matrix. Jayaraman *et al.* have suggested that protective biofilms decreased the corrosion rate of mild steel by reducing the oxygen concentration at the metal surface.<sup>5-7</sup> Protective biofilms secreting antimicrobial proteins active against sulfate-reducing bacteria (SRB) were shown to reduce the corrosive attack of stainless steel by these deleterious bacteria.<sup>8</sup> These 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.

The CCURB concept is being evaluated at present in detail for a number of materials such as mild steel, stainless steel, brass, and aluminum alloys in the presence of a number of different bacterial communities. Results obtained for UNS C26000 brass exposed to artificial seawater (AS) and Luria Bertani (LB) medium in the absence and presence of a *Bacillus subtilis* bacterial biofilm, which was genetically engineered to produce the polypeptides polyaspartate or polyglutamate, have documented that corrosion and tarnishing of brass was much reduced in the presence of a biofilm.<sup>9,10</sup> Analyses of electrochemical impedance spectroscopy (EIS) data obtained for Al 2024 exposed to AS and LB medium have shown that in the presence of biofilms active pit growth stopped after about two

days.<sup>11,12</sup> A more detailed analysis of the data obtained for Al 2024 and brass exposed to both media is given in the following.

### Experimental

Aluminum alloy 2024-T3 plates (10 × 10 cm squares, 2 mm thick) were cut from sheet stock and polished with 240 grit paper. Cartridge brass (UNS C26000, 70Cu/30Zn) plates (10 × 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 Vätäänen nine salts solution (VNSS, pH 7.0).<sup>13</sup> The other test solution, LB medium, is a rich growth medium. Table I gives the chemical composition of these two solutions. These test environments were used either in a sterile condition or after inoculation with bacteria such as *B. subtilis* WB600<sup>14</sup> obtained from Dr. Sui-Lam Wong of the University of Calgary which is a protease-deficient strain. Another strain, *Escherichia coli* XLI (Blue), was purchased from Stratagene (La Jolla, CA). Plasmid pBE92 containing the alkaline

Table I. Composition of VNSS and LB medium.

Compound	Concentration (g/L)	
	VNSS	LB medium
NaCl	17.6	10
NaHCO <sub>3</sub>	0.08	
KBr	0.04	
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.41	
SrCl <sub>2</sub> ·6H <sub>2</sub> O	0.008	
Na <sub>2</sub> SO <sub>4</sub>	1.47	
KCl	0.25	
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.87	
H <sub>3</sub> BO <sub>3</sub>	0.008	
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01	
Na <sub>2</sub> HPO <sub>4</sub>	0.01	
Peptone	1.0	
Starch	0.5	
Glucose	0.5	
Yeast extract	0.5	5
Tryptone		10

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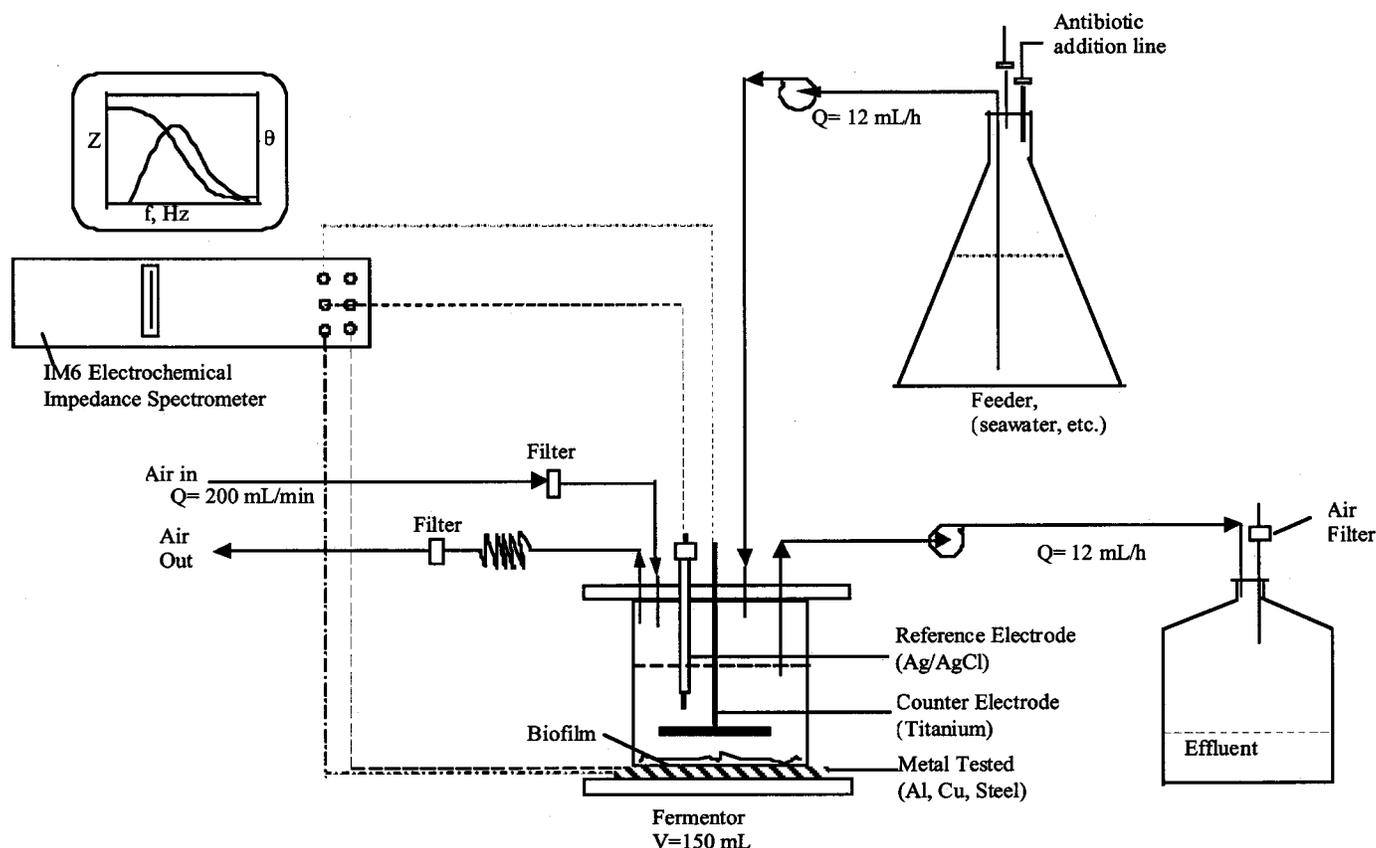


Figure 1. Schematic of biofilm apparatus.

protease (apr) promoter, constitutive apr signal sequence, and the alkaline phosphatase reporter gene were obtained from E. I. du Pont de Nemours Inc. (Wilmington, DE).

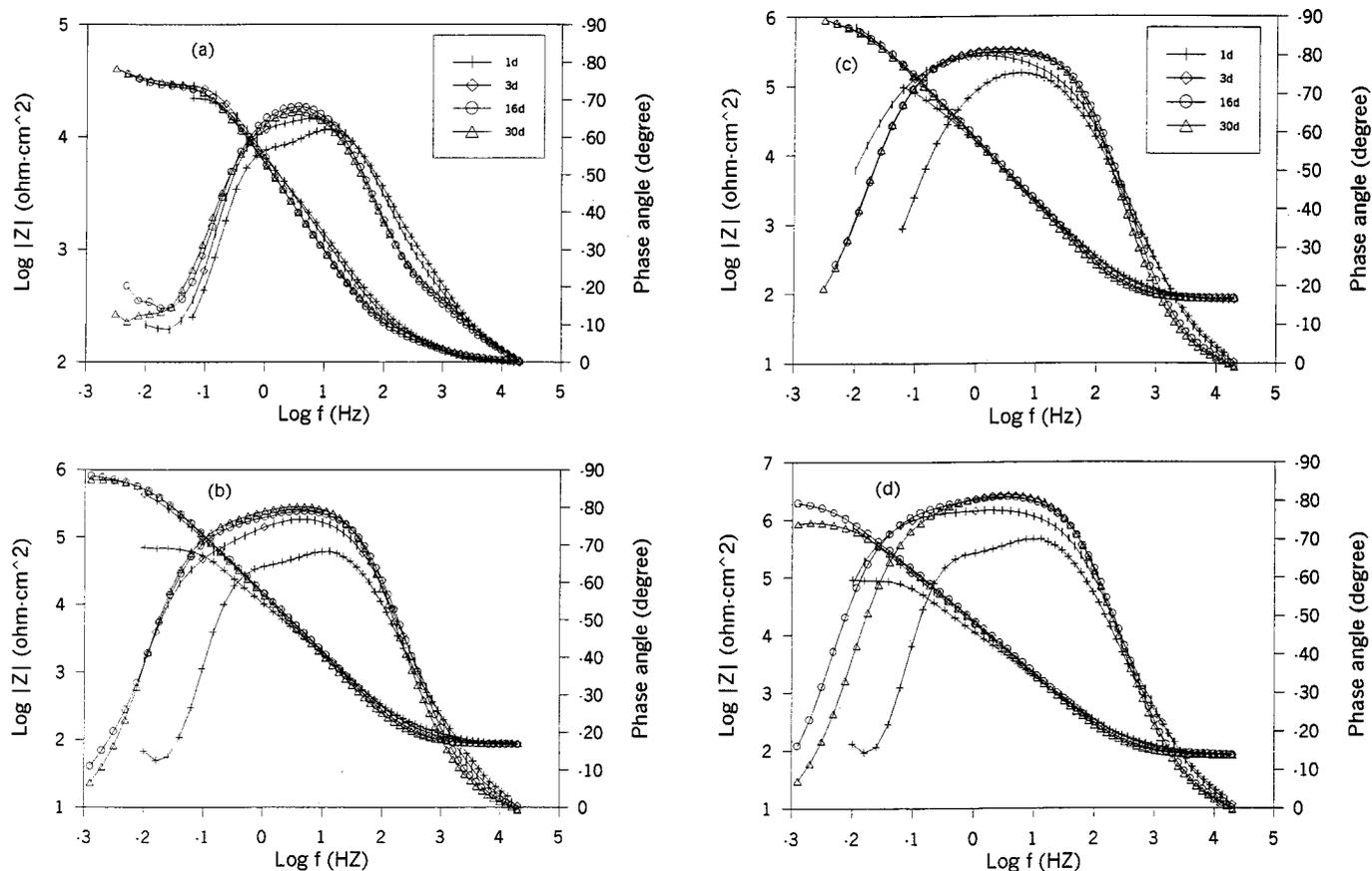
*B. subtilis* WB600 was engineered to secrete either polyaspartate or polyglutamate as 20 amino acid polymers by using recombinant DNA methods as described previously.<sup>11,12</sup> *E. coli* XLI (Blue) transformants containing the correct insert (pBE92-Asp or pBE92-Glu) were screened as white colonies on LB agar plates containing 100  $\mu\text{g/mL}$  of ampicillin and 40  $\mu\text{g/mL}$  of 5-bromo-4-chloro-3-indolyl phosphate (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, *Hind* III, and *Eco* RI. *E. coli* XLI (Blue) cells were made electrocompetent according to the method of Smith and Iglewski<sup>15</sup> and electroporated using a gene pulser/pulse controller (Bio-Rad Laboratories, Hercules, CA). *B. subtilis* WB600 strains

were transformed with the two plasmids according to the two-step method of Cutting and Vander Horn<sup>16</sup> and plated on LB agar plates containing 50  $\mu\text{g/mL}$  kanamycin.

Biofilms on metal surfaces were developed in glass/Teflon 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 27.3 or 44.2 cm<sup>2</sup>) as shown in Fig. 1. The metal sample formed the bottom of the reactor, the four corners of the metal sample, were not part of the reactor. A glass cylinder (5.5 cm diam, 0.6 cm thick) containing an O-ring at its bottom formed the walls of the system, and a 1 cm thick Teflon plate (12.6  $\times$  12.6 cm) formed the roof of the reactor. The growth temperature was maintained at 30°C by heating tape wrapped around the reactor. Nutrient flow rates were maintained using a Masterflex precision standard drive with a 10-turn potentiometer (Cole-Parmer, Niles, IL). Biofilms were allowed to develop for 12 h in batch mode,

Table II. Experiments for Al 2024 in LB medium.

Test no.	Medium	pH	Strain	Secreted inhibitor
111	LB	6.5	Sterile	
158	LB	6.5	Sterile	
102	LB	6.5	<i>B. subtilis</i> WB600	
109	LB	6.5	<i>B. subtilis</i> WB600	
101	LB	6.5	<i>B. subtilis</i> WB600/pBE92-polyaspartate	Polyaspartate
110	LB	6.5	<i>B. subtilis</i> WB600/pBE92-polyaspartate	Polyaspartate
157	LB	6.5	<i>B. subtilis</i> WB600/pBE92-polyglutamate	Polyglutamate
86	LB	6.5	<i>B. licheniformis</i>	$\gamma$ -Polyglutamate
107	LB	6.5	<i>B. licheniformis</i>	$\gamma$ -Polyglutamate
170	LB	7.0	<i>E. coli</i>	
171	LB	7.0	<i>E. coli</i>	Polyphosphate

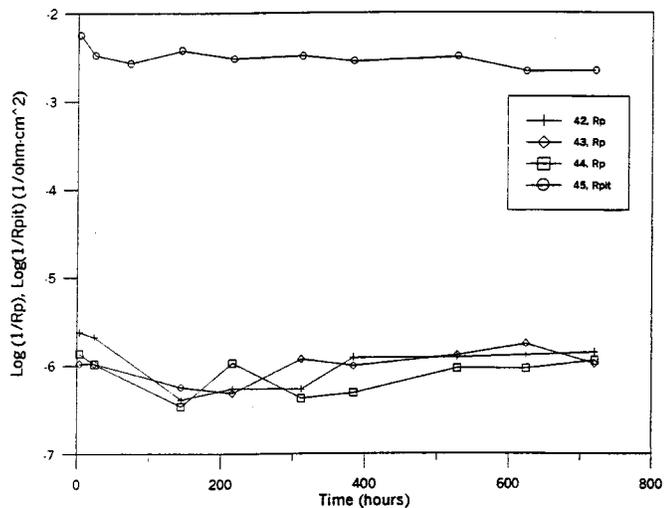


**Figure 2.** Bode plots for Al 2024 exposed to VNSS for different time periods; (a) sterile solution (test 45), (b) VNSS containing a *B. subtilis* WB600 biofilm (test no. 42), (c) VNSS containing a biofilm of *B. subtilis* WB600/pBE92 producing polyglutamate (test no. 43), (d) VNSS containing a biofilm of *B. subtilis* WB600/pBE92 producing polyaspartate (test no. 44).

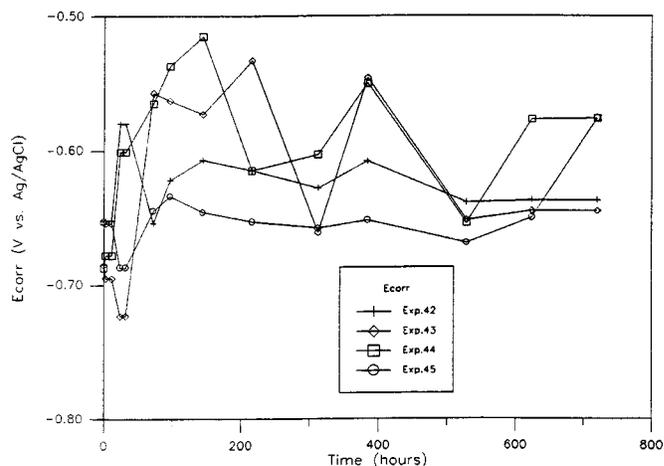
then nutrients were added continuously. A titanium counter electrode and autoclavable Ag/AgCl reference electrode ( $E^{\circ} = 0.208 \text{ V vs. standard hydrogen electrode}$ ) were used for the electrochemical measurements. The continuous reactors (sterile and inoculated) were conducted with  $50 \mu\text{g/mL}$  kanamycin to ensure sterility or the pres-

ence of the engineered kanamycin-resistant strain. A 1 vol % bacterial inoculum from a turbid, 16 h culture was used for all continuous experiments.

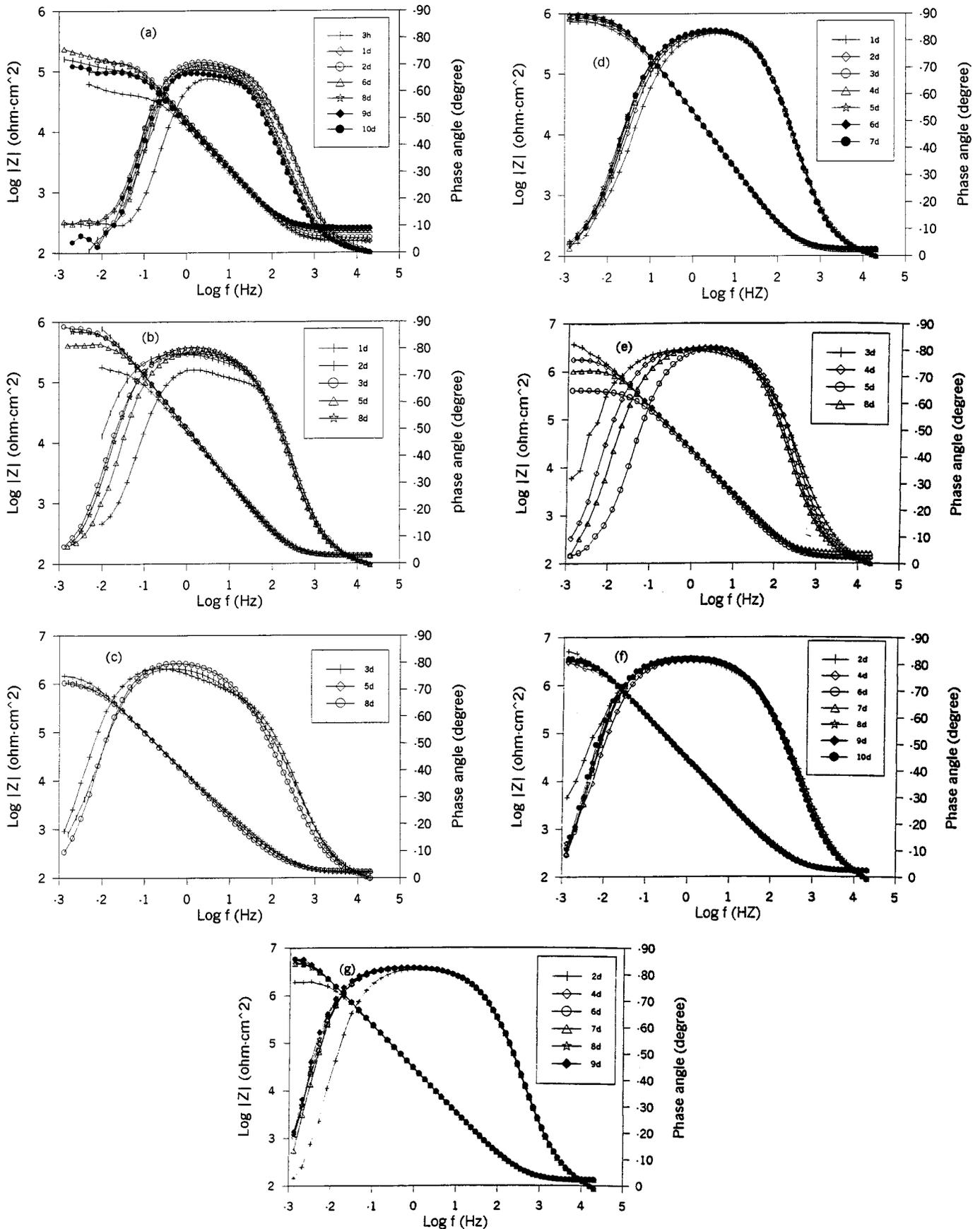
Electrochemical impedance data were obtained at the open-circuit potential,  $E_{\text{corr}}$ , in the frequency range of 20 kHz to 0.5 mHz using a Zahner IM6 electrochemical impedance analyzer with a 16 channel cell multiplexer. Impedance spectra were collected once a



**Figure 3.** Time dependence of  $R_{\text{pit}}^{\circ}$  and  $R_{\text{p}}^{\circ}$  in sterile VNSS (test no. 45) and in VNSS containing a biofilm of *B. subtilis* WB600/pBE92 (tests no. 42-44).



**Figure 4.** Time dependence of  $E_{\text{corr}}$  in sterile VNSS (test no. 45) and in the presence of a biofilm of *B. subtilis* WB600/pBE92 (test no. 42, 43, and 44).



**Figure 5.** Bode plots for Al 2024 exposed to LB medium for different time periods: (a) sterile solution, (b) *B. subtilis* WB600 biofilm, (c) *B. subtilis* WB600/pBE92-polyaspartate biofilm, (d) *B. subtilis* WB600/pBE92-polyglutamate biofilm, (e) *B. licheniformis*  $\gamma$ -polyglutamate biofilm, (f) *E. coli* biofilm, (g) *E. coli* polyphosphate biofilm.

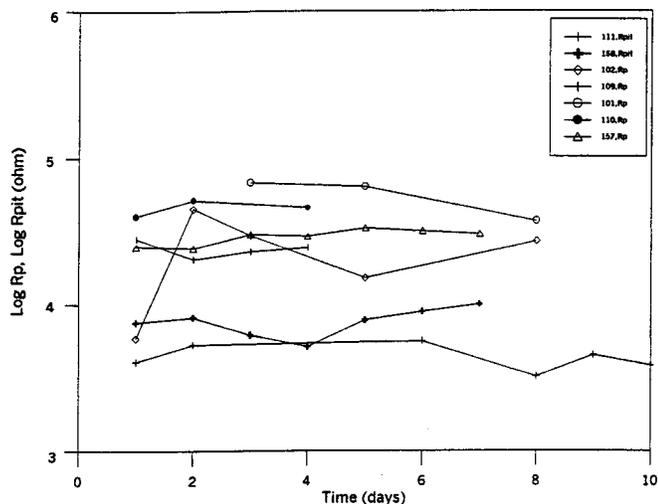


Figure 6. Time dependence of  $R_p$  or  $R_{pit}$  for Al 2024 exposed to LB medium with and without *B. subtilis* WB600.

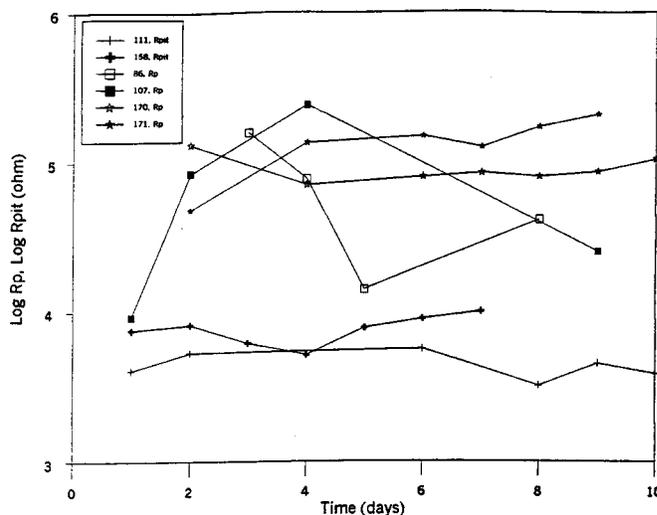


Figure 8. Time dependence of  $R_p$  or  $R_{pit}$  for Al 2024 exposed to LB medium with and without *Bacillus* or *E. coli*.

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.*<sup>17,18</sup>

Results and Discussion

In the apparatus shown in Fig. 1, the bacteria developed biofilms with a thickness of approximately 15  $\mu\text{m}$ .<sup>6</sup> The oxygen concentration was reduced considerably by the aerobically respiring biofilm, but was not zero since previous experiments have shown that the corrosion rates with the aerobic biofilms approach those of anaerobic systems.<sup>5</sup>

Figure 2a 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 Fig. 2a. The spectra suggest that pitting occurred during the entire test period as evidenced by the typical low frequency minimum of the phase angle  $\Phi$  which is partially masked by the scatter of the data points below 0.01 Hz. Nevertheless, the spectra in Fig. 2a are in agreement with the pitting model proposed by Mansfeld *et al.*<sup>17,18</sup> Qualitatively it can be observed that the polarization resistance of active pits,  $R_{pit}$ ,

which is close to the impedance value  $|Z|$  at the phase angle minimum at low frequencies, increased with increasing exposure time as the pit growth rate decreased.<sup>17,18</sup>

In the presence of a *B. subtilis*, biofilm pitting also occurred in the first two 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  $C$ .<sup>17,18</sup> The fairly high values of  $R_p$ , which approach the  $\text{M}\Omega \text{ cm}^2$  range, suggest that pits formed in the initial stages of exposure have become passivated (Fig. 2b). Very similar results were obtained in the presence of *B. subtilis* producing polyglutamate (Fig. 2c) or polyaspartate (Fig. 2d). The slightly increased  $R_p$  values suggest that the inhibitors produced by the bacteria provided a small degree of additional corrosion protection.

Figure 3 illustrates the time dependence of the relative corrosion rates expressed as  $1/R_{pit}^o$  for the tests in the absence of bacteria and  $1/R_p^o$  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 total pitted area,  $A_{pit}$ , determined by analysis of the impedance spectra as explained elsewhere.<sup>11</sup> For the tests in the absence of bacteria  $R_p$  could not be determined due to the lack of sufficient low frequency data (Fig. 2a). Figure 3 clearly demonstrates the inhibition of pitting corrosion in the presence of *B. subtilis* and the increased corrosion resistance in the early stages of exposure due to the inhibitors produced by the bacteria.

For the tests in the presence of *B. subtilis* (tests no. 42-44) the values of  $A_{pit}$  determined at the end of the exposure period by analysis of photographs of the exposed surface taken with a digital camera and using image analysis software 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 no. 42, 43, and 44 were 0.07, 0.16, and 0.06%, respectively, which is close to the detection limit of the electrical impedance spectroscopy technique.<sup>18</sup>

The inhibition of pitting in the presence of bacteria could be due to exclusion of oxygen from the metal surface which would reduce the rate of the cathodic reduction resulting in a significant decrease of the corrosion potential,  $E_{corr}$ , below the pitting potential  $E_{pit}$ , while in the sterile solution both values are very similar. 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 (Fig. 4). 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

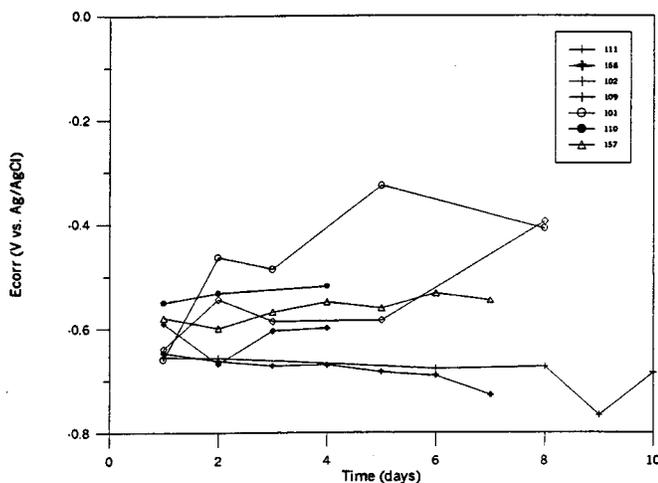
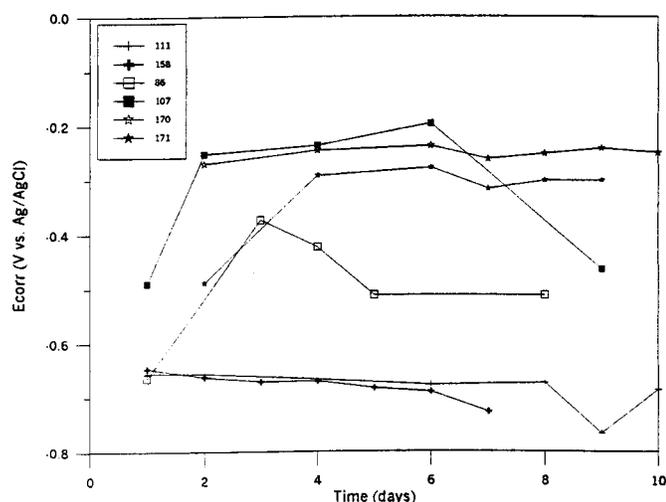


Figure 7. Time dependence of  $E_{corr}$  for Al 2024 exposed to LB medium with and without *B. subtilis* WB600.



**Figure 9.** Time dependence of  $E_{\text{corr}}$  for Al 2024 exposed to LB medium with and without *B. licheniformis* or *E. coli*.

presence of a biofilm leading to a significant increase of  $E_{\text{pit}}$ . The beneficial effect was apparent even when the biofilm contained bacteria that were not engineered to produce inhibitors. Indeed, the observation that pitting occurred in all cases in the first two days of exposure clearly suggests that formation of a stable biofilm is needed to stop growth of active pits.

The impedance spectra obtained in LB medium are shown in Fig. 4 as a function of exposure time for the tests listed in Table II. Most tests in Table II were conducted in duplicate. Only one of these sets is shown in Fig. 5. Pitting was indicated by the frequency dependence of the impedance spectra at low frequencies in the sterile LB medium (Fig. 4a) similar to the results obtained in sterile VNSS (Fig. 2a).

The time dependence of  $R_{\text{pit}}$  for the test in sterile AS and  $R_p$  for the tests in the presence of a *B. subtilis* biofilm is shown in Fig. 6. Similar low values of  $R_{\text{pit}}$  were observed in the sterile solution (tests no. 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 no. 101 and 110 in Fig. 6. Similar to the results obtained in AS,  $E_{\text{corr}}$  was more positive in presence of *B. subtilis* than in the sterile solution (Fig. 7). The most pronounced ennoblement was observed in the presence of polyaspartate produced by the biofilm.

The time dependence of  $R_{\text{pit}}$  for the test in sterile LB medium and  $R_p$  for all tests in the presence of the biofilm that produces the natural polyanionic polymer  $\gamma$ -polyglutamate of *B. licheniformis*, or for the biofilm of *E. coli* producing polyphosphate is shown in Fig. 8. While there is some scatter in the  $R_p$  values obtained for tests in

the presence of *B. licheniformis* producing  $\gamma$ -polyglutamate (tests no. 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 no. 171) were higher than those in test no. 170 suggesting that the polyphosphate produced an additional increase in the corrosion resistance of Al 2024 exposed to LB medium (Fig. 8).  $R_p$  values determined in the presence of the *E. coli* biofilm producing polyphosphate were about a factor of ten higher than those in the sterile solution. The average values of  $R_p^0 = 2 \times 10^6 \Omega \text{ cm}^2$  correspond to a very low corrosion rate of about  $0.1 \mu\text{m}/\text{year}$ . Ennoblement was also observed in these tests (Fig. 9). In the presence of *E. coli*,  $E_{\text{corr}}$  was about 400 mV more positive than in the sterile solution.

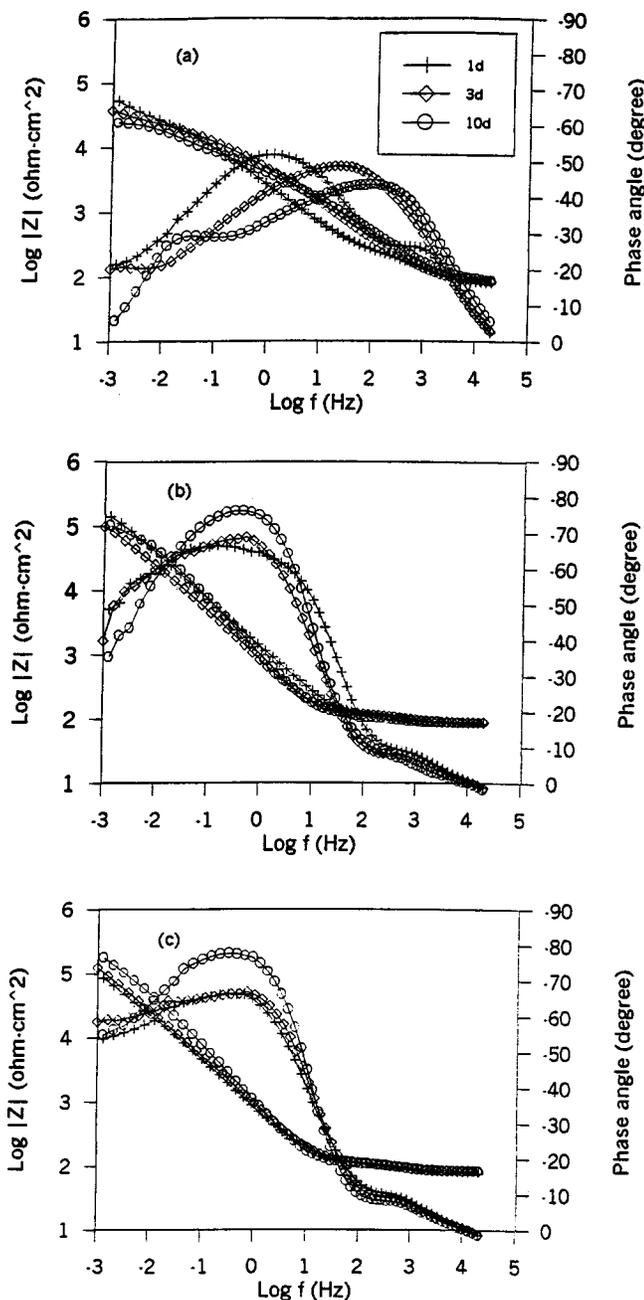
The experiments carried out for C26000 brass in VNSS and LB medium are listed in Table III. Some tests have been performed in duplicate. Figure 10a shows impedance spectra obtained for brass after 1, 3, and 10 days exposure in VNSS, while Fig. 10b and c shows the spectra obtained in the presence of a biofilm of *B. subtilis* WB600/pBE92-polyaspartate, which produced polyaspartate (Fig. 10b), and in the presence of a *B. licheniformis* biofilm producing  $\gamma$ -polyglutamate (Fig. 10c). In the very corrosive sterile VNSS, impedance data were low and several time constants were observed (Fig. 10a). However in the presence of biofilms, a large increase of the impedance was observed with mainly capacitive behavior (Fig. 10b and c). The time dependence of the normalized inverse polarization resistance,  $I/R_p$ , which is proportional to the corrosion rate, is shown in Fig. 11. Corrosion rates were lower and quite similar in the presence of the biofilms.

The remarkable protective effect of the biofilms cannot be due to a reduction of the oxygen concentration at the brass surface in the presence of the biofilm, because  $E_{\text{corr}}$  was found to increase with time, i.e., ennoblement of brass was observed in artificial seawater in the presence of a biofilm (Fig. 12). After exposure for 10 days,  $E_{\text{corr}}$  was lower by about 100 mV in the sterile solution. The sample exposed to VNSS was covered by a dark film, while the samples exposed to the same solution containing bacteria remained untarnished and did not show signs of corrosive attack. After removal of the corrosion products in a solution of  $\text{H}_2\text{SO}_4/\text{Na}_2\text{Cr}_2\text{O}_7$ , no indication of localized attack was found for the sample exposed to sterile AS. The corrosion process is assumed to have progressed by the commonly accepted mechanism of dezincification of brass.

The experiments conducted in LB medium at pH 6.5 (Table III) produced similar results. The impedance spectra obtained in sterile LB medium (Fig. 13a) were similar to those observed for diffusion controlled processes which are described by the Warburg impedance in series with  $R_p$  (Randles circuit). In the presence of biofilms producing polyaspartate (Fig. 13b) or  $\gamma$ -polyglutamate (Fig. 13c), the impedance was much higher with essentially capacitive behavior similar to the results obtained in VNSS (Fig. 10). The time dependence of the relative corrosion rate expressed as  $I/R_p$  is shown in Fig. 14. Corrosion rates were more than an order of magnitude higher in the sterile LB medium than in the presence of the two

**Table III.** Experiments for brass in VNSS and LB media.

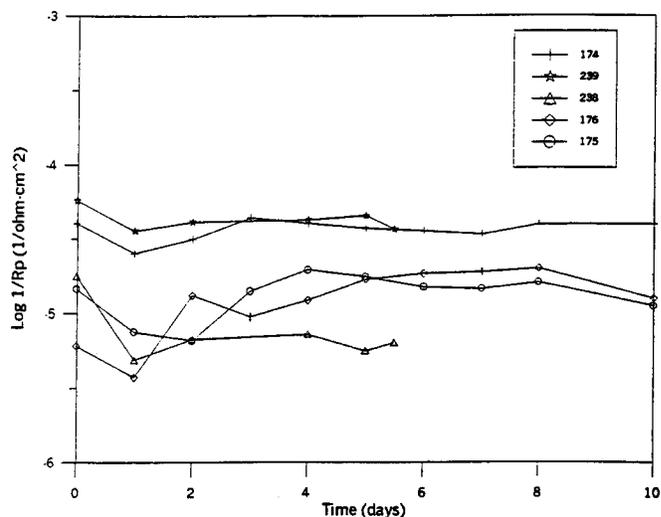
Test no.	Medium	pH	Strain	Secreted inhibitor
174	VNSS	7.5	Sterile	
239	VNSS	7.5	Sterile	
238	VNSS	7.5	<i>B. subtilis</i> WB600	
176	VNSS	7.5	<i>B. subtilis</i> WB600/pBE92-polyaspartate	Polyaspartate
175	VNSS	7.5	<i>B. licheniformis</i>	$\gamma$ -Polyglutamate
166	LB	6.5	Sterile	
130	LB	6.5	<i>B. subtilis</i> WB600	
131	LB	6.5	<i>B. subtilis</i> WB600/pBE92-polyaspartate	Polyaspartate
168	LB	6.5	<i>B. subtilis</i> WB600/pBE92-polyaspartate	Polyaspartate
132	LB	6.5	<i>B. licheniformis</i>	$\gamma$ -Polyglutamate
167	LB	6.5	<i>B. licheniformis</i>	$\gamma$ -Polyglutamate



**Figure 10.** Bode plots obtained for brass during exposure to VNSS: (a) sterile solution (test no. 174), (b) *B. subtilis* WB600/pBE92-polyaspartate (test no. 176), (c) *Bacillus licheniformis* producing  $\gamma$ -polyglutamate (test no. 175).

different biofilms for which very similar corrosion rates were observed (Fig. 14). The  $R_p$  values determined in LB medium in the presence of the biofilms were similar to those observed for the same conditions in VNSS (Fig. 10). The average value of  $R_p = 10^5 \Omega \text{ cm}^2$  corresponds to a very low corrosion rate of about 2  $\mu\text{m}/\text{years}$ . Duplicate tests resulted in comparable values of  $R_p$  (Fig. 14). The results of Fig. 14 seem to indicate that formation of a biofilm prevents corrosive attack by a yet unknown mechanism. The production of polyaspartate or  $\gamma$ -polyglutamate did not provide the additional corrosion protection for cartridge brass in VNSS that was observed for Al 2024-T3 in LB medium.<sup>9,10</sup>

Ennoblement was also observed for these systems with a difference in  $E_{\text{corr}}$  of about 200 mV between the sterile solution (test



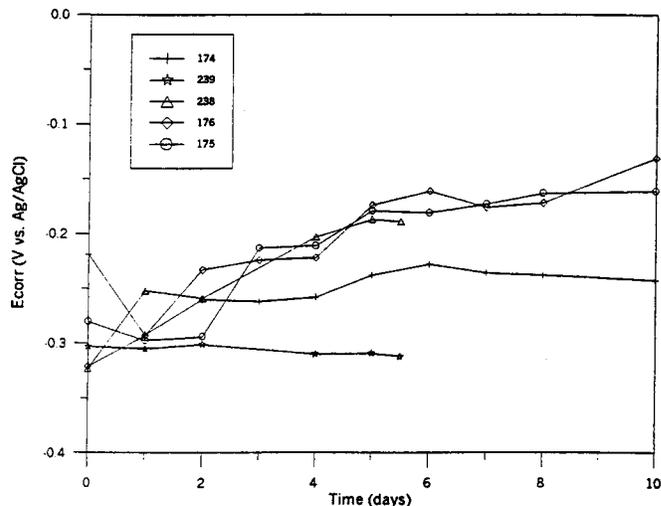
**Figure 11.** The time dependence of the relative corrosion rate  $1/R_p$  for brass exposed to VNSS under different conditions.

no. 166) and the solution containing bacillus producing  $\gamma$ -polyglutamate (tests no. 132 and 167) for which ennoblement seemed to be more pronounced than for *B. subtilis* WB600/pBE92-polyaspartate producing polyaspartate (tests no. 131 and 168) (Fig. 15).

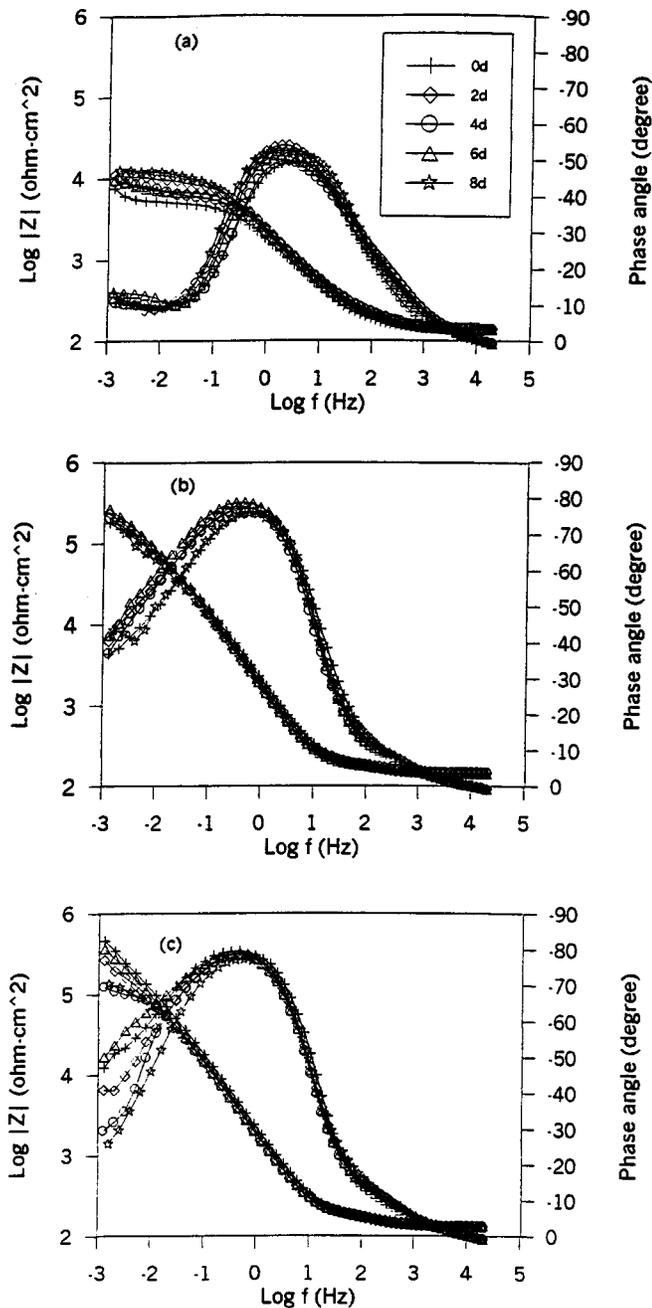
At the end of exposure to sterile LB medium, the sample was covered by a dark film of corrosion products and after removal of this film in a solution of  $\text{H}_2\text{SO}_4/\text{Na}_2\text{Cr}_2\text{O}_7$  no indication of localized attack was found. The samples used in the tests with bacteria remained untarnished and showed no signs of corrosive attack.

**Conclusions**

The corrosion behavior of Al 2024 and brass was monitored by recording of impedance spectra in AS and LB medium. In the sterile solutions, pitting occurred for Al 2024 during the entire exposure period. In the presence of *B. subtilis*, WB600 pitting was also observed initially in AS; however, after about 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



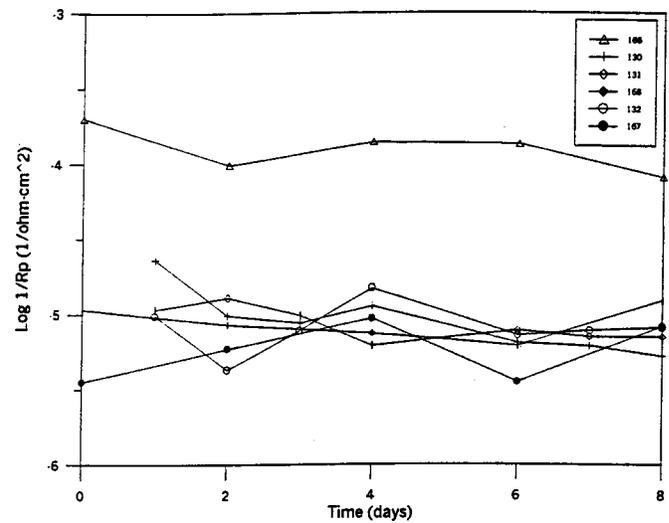
**Figure 12.** The time dependence of  $E_{\text{corr}}$  for brass exposed to VNSS under different conditions.



**Figure 13.** Bode plots obtained for brass during exposure to LB medium: (a) sterile solution (test no. 166), (b) *B. subtilis* WB600 pBE92-polyaspartate (test no. 168), (c) *B. licheniformis* producing  $\gamma$ -polyglutamate (test no. 167).

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 micrometer per year range.

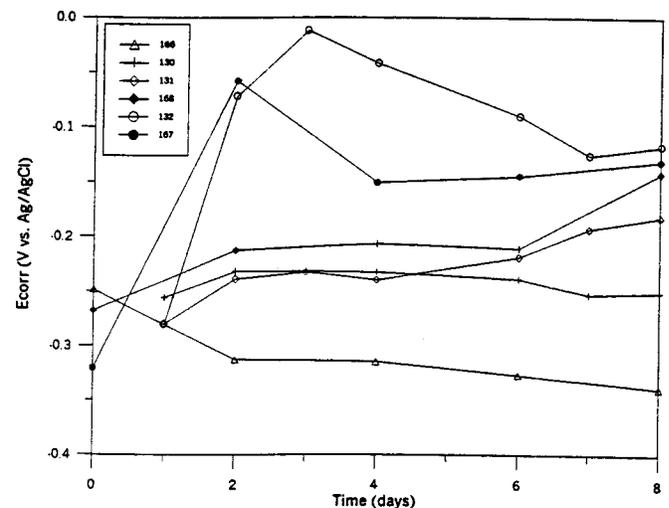
$E_{\text{corr}}$  reached its most noble values after about one week of exposure to AS containing bacterial biofilms producing inhibitors. In LB medium ennoblement of about 400 mV compared to the sterile solution was observed for *E. coli* producing polyphosphate. These results are similar to the ennoblement of stainless steels in seawater and natural waters although the increase of  $E_{\text{corr}}$  was less dramatic in most cases discussed here.



**Figure 14.** The time dependence of the relative corrosion rate  $1/R_p$  for brass exposed to LB medium under different conditions.

Since, even in the presence of the bacteria that did not produce inhibitors,  $E_{\text{corr}}$  increased beyond  $E_{\text{pit}}$  which for Al 2024 in sterile AS equals  $E_{\text{corr}}$ , it is likely that *B. subtilis* WB600 produced a chemical species that was able to stop pitting after a certain incubating period. The observed ennoblement of  $E_{\text{corr}}$  rules out the possibility that CCURB in AS was only due to anaerobic conditions at the Al alloy surface.

The microorganisms used in this study in AS and LB medium were also able to provide significant reduction of corrosion damage for brass. The black film of corrosion products formed in sterile media was not observed in the presence of the bacteria. This result is considered to be due to so far unidentified inhibitive species produced by the bacteria contained in the biofilms that protected the brass surface from corrosion. The production of polyaspartate or the anionic polymer  $\gamma$ -polyglutamate by the biofilms did not provide the additional corrosion protection observed for Al 2024 in VNSS. The observed CCURB cannot be solely due to a significant reduction of the oxygen concentration at the brass surface which would have produced a shift of  $E_{\text{corr}}$  in the negative direction. Instead ennoblement of brass was observed in both media similar to the results for



**Figure 15.** The time dependence of  $E_{\text{corr}}$  for brass exposed to LB medium under different conditions.

Al 2024 in AS and in LB medium. The fact the ennoblement was observed for Al 2024 and brass in AS and LB medium suggests that this phenomenon might be more common than previously realized.<sup>19</sup>

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