PERGAMON

Corrosion Science 44 (2002) 2291–2302

CORROSION SCIENCE

www.elsevier.com/locate/corsci

Corrosion control using regenerative biofilms (CCURB) on brass in different media

D. Örnek [a], T.K. Wood [a], C.H. Hsu [b], F. Mansfeld [b,*]

[a] *Bioremediation and Applied Biotechnology Laboratory, Department of Chemical Engineering, University of Connecticut, Storrs, CT 06269-3222, USA*

[b] *Corrosion and Environmental Effects Laboratory (CEEL), Department of Materials Science and Engineering, University of Southern California, Los Angeles, CA 90089-0241, USA*

Received 11 July 2001; accepted 8 January 2002

Abstract

The corrosion behavior of cartridge brass (UNS C26000) exposed to artificial seawater (AS) and Luria Bertani (LB) medium has been studied using electrochemical impedance spectroscopy. Tests were performed in sterile media and in the presence of a *Bacillus subtilis* bacterial biofilm. Additional tests were performed in the presence of a *B. subtilis* bacterial biofilm that produced polyaspartate or a *B. licheniformis* biofilm that produced γ-polyglutamate. A significant reduction of corrosion rates and an ennoblement of the corrosion potential were observed 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.

1. Introduction

The great interest in microbiologically influenced corrosion (MIC) is documented in the proceedings of numerous international conferences [1,2] and symposia on this subject as well as the large number of publications dealing with MIC. Little et al. [3] have given a review of MIC of different materials. The general concept of MIC is based on the assumption that microorganisms accelerate the kinetics of electrochemical

* Corresponding author. Tel.: +1-213-740-3016; fax: +1-213-740-7797.
E-mail address: mansfeld@usc.edu (F. Mansfeld).

0010-938X/02/$ - see front matter
PII: S0010-938X(02)00038-0

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. [4] that the often-discussed ennoblement of stainless steels in seawater is due to the production of inhibitors by bacteria that are retained in the biofilm matrix. Jayaraman et al. [5–7] have demonstrated that protective biofilms decreased the corrosion rate of mild steel, apparently by reducing the oxygen concentration at the metal surface. Engineered protective biofilms secreting antimicrobial proteins active against corrosion-causing sulfate-reducing bacteria (SRB) were shown to reduce the corrosive attack of stainless steel by these deleterious bacteria [8]. These and other observations have led to a new approach of corrosion protection based on corrosion control using regenerative biofilms (CCURB) using both natural bacteria and engineered bacteria that secrete corrosion inhibitors. 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 large number of different bacterial communities. Results obtained for aluminum 2024-T3 exposed to artificial seawater (AS) in the absence and presence of a *Bacillus subtilis* bacterial biofilm have documented that pitting stopped when the biofilm was fully formed after an induction period of about two days during which active pit propagation was observed using electrochemical impedance spectroscopy [9,10]. Additional results for Al 2024 exposed to other media have been discussed elsewhere [11]. Preliminary results obtained for cartridge brass exposed to AS and Luria Bertani (LB) medium with or without a Bacillus biofilm have been presented [10,12]. A more detailed analysis and discussion of the experimental data will be given in the following.

2. Experimental approach

Cartridge brass (UNS C26000, 70Cu/30Zn) plates (10 cm × 10 cm squares, 2 mm thick) were cut from sheet stock and polished with 240 grit paper (Buehler, Lake Bluff, IL). One of the test environments was artificial seawater, also known as Väätänen nine salts solution (VNSS, [1] pH 7.5) [12]. The other test environment, Luria Bertani (LB, pH 6.5) medium, is a rich growth medium made from 10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter [13]. These test environments were used either in a sterile condition or after inoculation with one or more strains of

[1] VNSS: NaCl (17.6 g/l), $NaHCO_3$ (0.08 g/l), KBr (0.04 g/l), $CaCl_2 \cdot 2H_2O$ (0.41 g/l), $SrCl_2 \cdot 6H_2O$ (0.008 g/l), Na_2SO_4 (1.47 g/l), KCl (0.25 g/l), $MgCl_2 \cdot 6H_2O$ (1.87 g/l), H_3BO_3 (0.008 g/l), $FeSO_4 \cdot 7H_2O$ (0.01 g/l), Na_2HPO_4 (0.01 g/l), peptone (1.0 g/l), starch (0.5 g/l), glucose (0.5 g/l), yeast extract (0.5 g/l).

bacteria. *B. subtilis* WB600 [14], was obtained from Dr. Sui-Lam Wong of the University of Calgary and is a protease-deficient strain. It was transformed with pBE92 (a derivative of pBE60) [15], to become resistant to 50–100 μg/ml kanamycin and was used as a biofilm-forming negative control that does not secrete a corrosion inhibitor. Plasmid pBE92 was obtained from E.I. du Pont de Nemours (Wilmington, DE). The gene of 20 amino acid polyaspartate or polyglutamate and the *B. amyloliquefaciens apr* secretion signal was cloned downstream of the alkaline protease promoter of *B. amyloliquefaciens* in pBE92, and it was transformed to *B. subtilis* to create a strain that secretes constitutively either polyaspartate or polyglutamate *B. licheniformis* was obtained from the American type culture collection (Manassas, VA, strain 9945A). It produces and secretes 5–23 g/l γ-polyglutamate that is part of its capsule and is freely secreted into the growth medium [16]. γ-Polyglutamate is a homopolymer of glutamic acid that has amide linkages between the glutamate γ-carboxyl and α-amino group [16].

Biofilms on metal surfaces were developed in glass/Teflon cylindrical continuous reactors [5] (30 °C, liquid nutrient flow rate 0.2 ml/min, airflow 200 ml/min to headspace, working volume 100 ml, exposed surface area of test electrode 28.3 cm^2). A titanium counter electrode and autoclavable Ag/AgCl reference electrode (Ingold Silver Scavenger DPAS model 105053334, Metler-Toledo Process Analytical, Wilmington, MA) were also used. The test solutions passing through the continuous reactors were inoculated with 100 μg/ml kanamycin to ensure sterility or to ensure the presence of only the kanamycin-resistant *B. subtilis* WB600 strain. Kanamycin was not added for tests with *B. licheniformis*. The continuous reactors were inoculated with 1% by volume inoculum of an overnight culture of *Bacillus* strains grown from −80 °C glycerol stocks in 250 ml shake flasks with 25 ml LB medium at 37 °C and with shaking at 250 rpm (series 25 shaker, New Brunswick Scientific, Edison, NJ). Biofilms were allowed to develop for 15–18 h in batch mode. Sterile nutrients were then added continuously at a dilution rate of 0.12 h^{-1} to keep the biofilms on metal surface for 5–10 days.

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, West Lafayette, IN). Impedance spectra were collected once a day over a 10-day period for exposure to VNSS and over an 8-day period for exposure to LB medium.

3. Experimental results and discussion

We have shown previously using confocal scanning laser microscopy that bacteria cultivated under these conditions produce exopolysaccharide and form biofilms of about 15 μm attached to these metal surfaces [6]. We have also shown using these continuous fermentors that the corrosion inhibition of aluminum as well as that of mild steel is due to the biofilm on the metal surface and not due to the suspended cells [5,17].

Table 1
Experimental details for brass (UNS C26000) in VNSS and LB medium

Exp. #	Medium	pH	Strain	Secreted inhibitor
174	VNSS	7.5	Sterile	
239	VNSS	7.5	Sterile	
238	VNSS	7.5	*B. subtilis* WB600	
176	VNSS	7.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
175	VNSS	7.5	*B. licheniformis*	γ-Polyglutamate
133	LB	6.5	Sterile	
166	LB	6.5	Sterile	
130	LB	6.5	*B. subtilis* WB600	
131	LB	6.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
168	LB	6.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
132	LB	6.5	*B. licheniformis*	γ-Polyglutamate
167	LB	6.5	*B. licheniformis*	γ-Polyglutamate

The experiments carried out on cartridge brass in VNSS and LB medium are listed in Table 1 and were duplicated as indicated. The Bode plots obtained in sterile VNSS, pH = 7.5 after an exposure period of 1–5.5 days are shown in Fig. 1(a), while Fig. 1(b) shows the corresponding plots in the presence of *B. subtilis*. A comparison of the impedance spectra in Fig. 1 demonstrates qualitatively that the presence of the biofilm provided corrosion protection. Fig. 2(a) shows impedance spectra obtained for brass after 1, 3 and 10 days exposure in VNSS, while Fig. 2(b) and (c) show the spectra obtained in the presence of *B. subtilis* WB600/pBE92-polyasp, which produced polyaspartate (Fig. 2(b)), and in the presence of *B. licheniformis*, which produced γ-polyglutamate (Fig. 2(c)). In the very corrosive VNSS impedance data were low and several time constants were observed (Fig. 2(a)). However in the presence of biofilms, a large increase of the impedance was observed with mainly capacitive behavior (Fig. 2(b) and (c)). The time dependence of the normalized inverse polarization resistance $1/R_p$, which is proportional to the corrosion rate, and the capacitance C is shown in Figs. 3 and 4, respectively. Corrosion rates were lower and quite similar in the presence of the biofilms (Fig. 3). C was slightly lower for the sterile solution in the initial phase of the tests, however at the end of exposure very similar values of C were obtained for all three solutions.

The remarkable protective effect of the biofilms cannot be due solely to a reduction of the oxygen concentration at the brass surface in the presence of the biofilm since the corrosion potential E_{corr} was found to increase with time, i.e. ennoblement of brass was observed in AS in the presence of a biofilm (Fig. 5). After exposure for 10 days, E_{corr} was lower by about 100 mV in the sterile solution. Similar results have been observed for Al 2024-T3 in VNSS and LB medium [10,18]. 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 H_2SO_4/$Na_2Cr_2O_7$, 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.

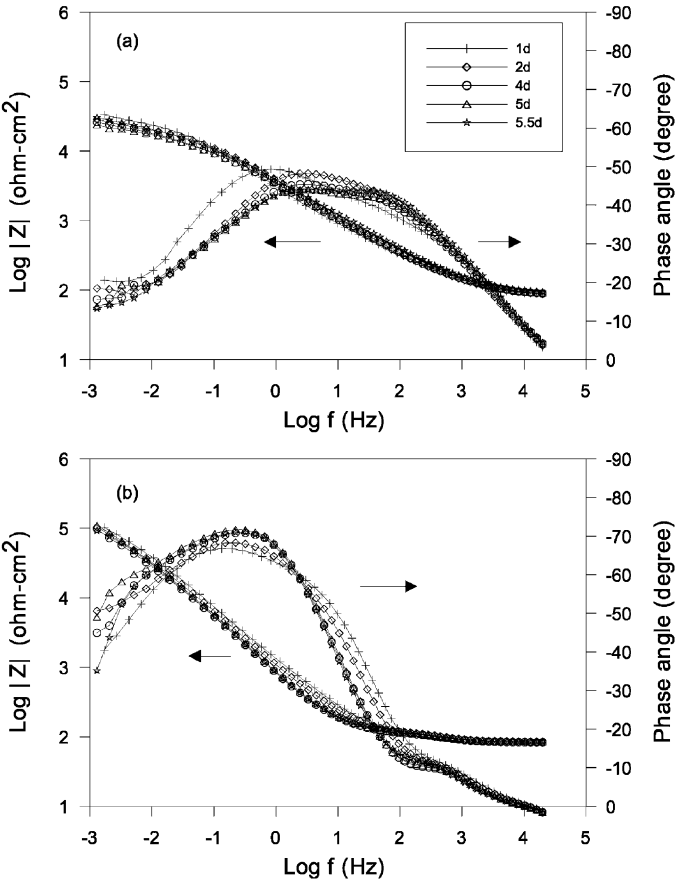

Fig. 1. Bode plots obtained for cartridge brass during exposure to sterile VNSS (a, test # 239) and VNSS containing *B. subtilis* (b, test # 238).

The experiments conducted in LB medium at pH = 6.5 (Table 1) produced similar results. The impedance spectra obtained in sterile LB medium (Fig. 6(a)) 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. 6(b)) or γ-polyglutamate (Fig. 6(c)), the impedance was much higher with essentially capacitive behavior similar to the results obtained in VNSS (Figs. 1 and 2). The time dependence of the relative corrosion rate expressed as $1/R_p$ and the capacitance C is shown in Figs. 7 and 8, respectively. Corrosion rates were more than an order of magnitude higher in the sterile LB medium than in the presence of the two different biofilms for which very similar corrosion rates were observed (Fig. 7). 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. 3). The average value of $R_p = 10^5$ ohm cm^2 corresponds to a uniform

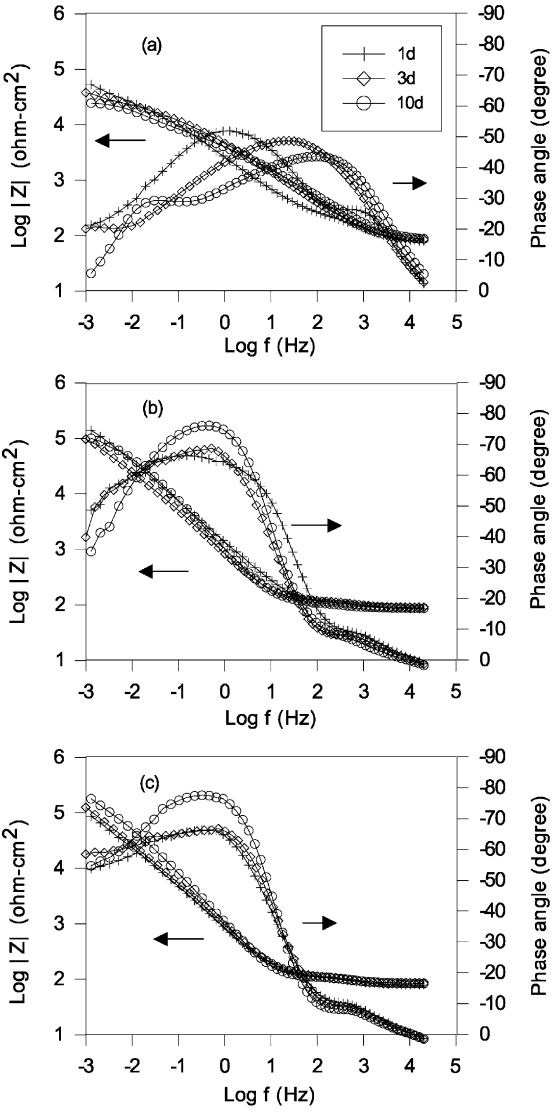

Fig. 2. Bode plots obtained for cartridge brass during exposure to VNSS; (a) sterile solution (test # 174), (b) *B. subtilis* WB600/pBE92-polyaspartate (test #176), (c) *B. licheniformis* (test # 175).

corrosion rate of about 2 μm/year, which is quite low. The capacitance values were similar for all exposure conditions of Table 1 in LB medium (Fig. 8). Duplicate tests (expts. #131 and #168; and expts. #132 and #167) resulted in comparable values of R_p and C, respectively (Figs. 7 and 8). The results of Fig. 7 seem to indicate that formation of a biofilm prevents corrosive attack by a yet unknown mechanism. The production of polyaspartate or γ-polyglutamate did not provide the additional

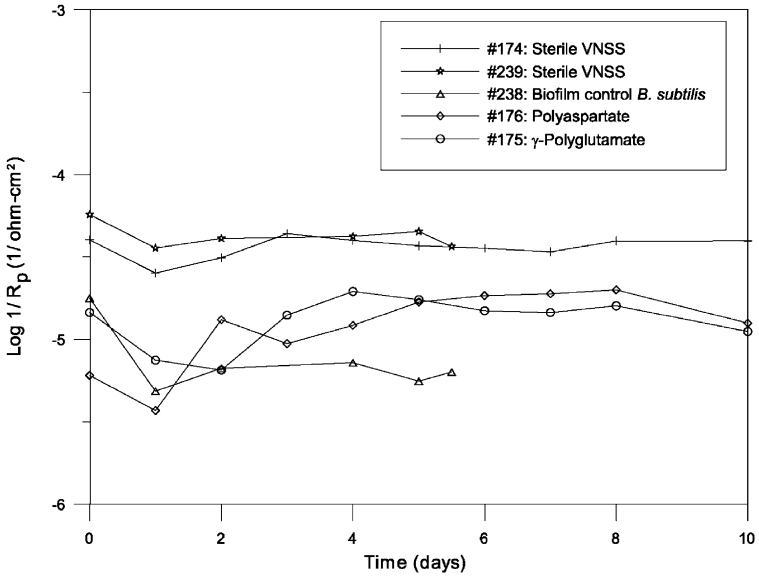

Fig. 3. The time dependence of the relative corrosion rate $1/R_p$ for cartridge brass exposed to VNSS under different conditions. Polyaspartate indicates the 20 amino acid polyaspartate secreted by a *B. subtilis* WB600/pBE92-polyaspartate biofilm, and γ-polyglutamate indicates the anionic polymer secreted by a *B. licheniformis* biofilm.

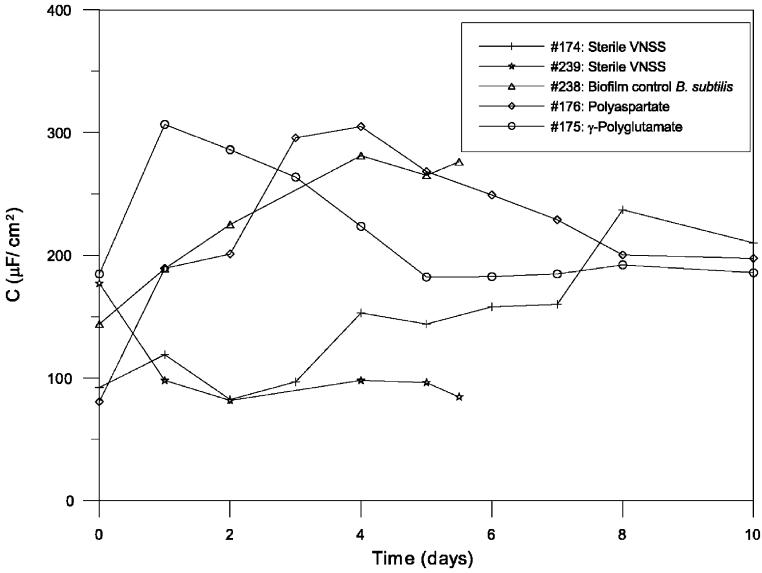

Fig. 4. The time dependence of the capacitance C for cartridge brass exposed to VNSS under different conditions. Symbols as defined in Fig. 3 caption.

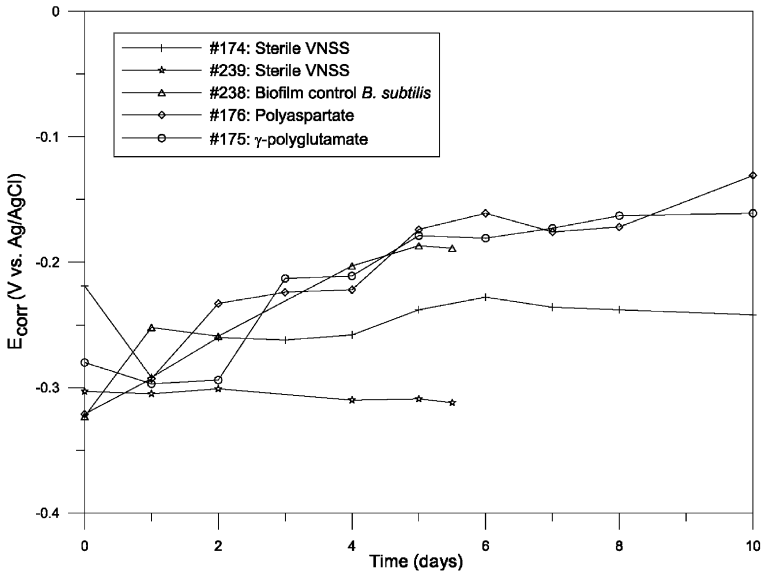

Fig. 5. The time dependence of E_{corr} for cartridge brass exposed to VNSS under different conditions. Symbols as defined in Fig. 3 caption.

corrosion protection for cartridge brass in VNSS that was observed for Al 2024-T3 in LB medium [10,11].

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 H_2SO_4/$Na_2Cr_2O_7$ no indication of localized attack was found. The samples used in the tests with bacteria remained untarnished and did not show any signs of corrosive attack. Ennoblement was also observed for these systems with a difference of E_{corr} of about 200 mV between the sterile solution (expts. # 133 and 166) and the solution containing *B. licheniformis* producing γ-polyglutamate (expts. # 132 and 167). The ennoblement in the latter solution was more pronounced than for *B. subtilis* WB600/pBE92-polyasp producing polyaspartate (expts. # 131 and 168) over most of the test period (Fig. 9).

4. Conclusions

The microorganisms used in this study of the corrosion behavior of cartridge brass in artificial seawater and LB medium were able to provide significant reduction of corrosion damage. 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 the production of so far unidentified inhibitive species by the bacteria contained in the biofilms that protected the brass surface from corrosion. The production of polyaspartate or γ-polyglutamate by the biofilms did not provide the additional

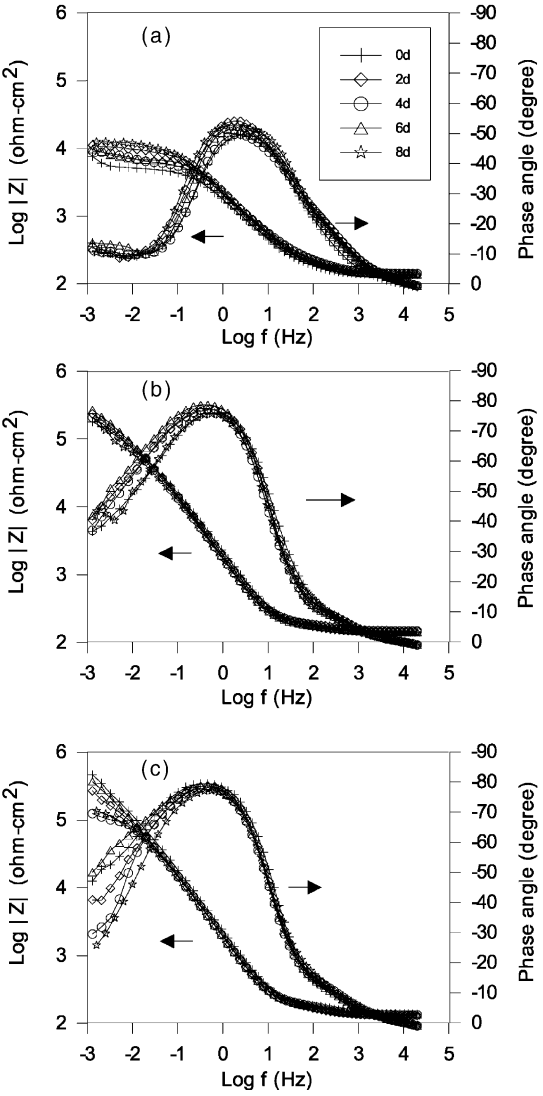

Fig. 6. Bode plots obtained for cartridge brass during exposure to LB medium; (a) sterile solution (test #166), (b) *B. subtilis* WB600/pBE92-polyaspartate (test #168), (c) *B. licheniformis* (test #167).

corrosion protection observed for Al 2024 in VNSS reported elsewhere [9,10]. The observed CCURB cannot be due solely to a significant reduction of the oxygen concentration at the brass surface which would have produced a shift of E_{corr} in the negative direction. Instead ennoblement of brass was observed in both media, similar to previous results for Al 2024 in artificial seawater [9,10,18] and in other media [9–11,18]. For a corrosion process under diffusion control, reduction of the corrosion

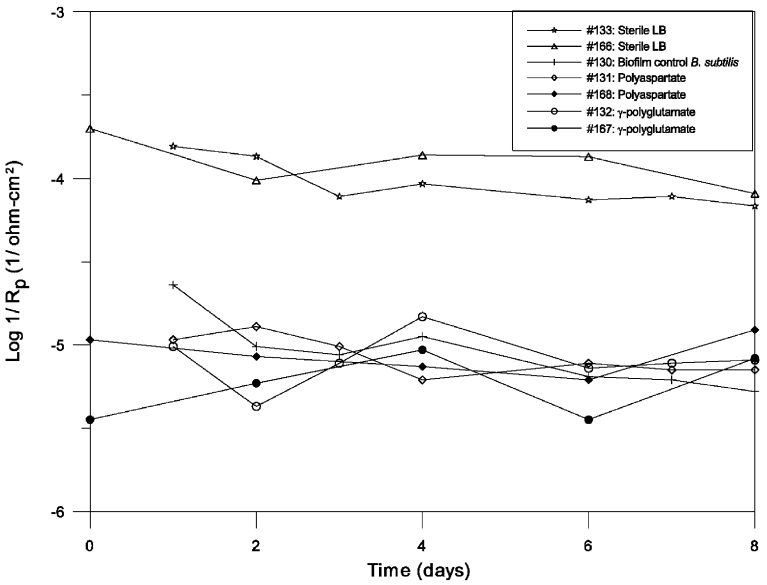

Fig. 7. The time dependence of the relative corrosion rate $1/R_p$ for cartridge brass exposed to LB medium under different conditions. Polyaspartate indicates the 20 amino acid polyaspartate secreted by a *B. subtilis* WB600/pBE92-polyaspartate biofilm, and γ-polyglutamate indicates the anionic polymer secreted by a *B. licheniformis* biofilm.

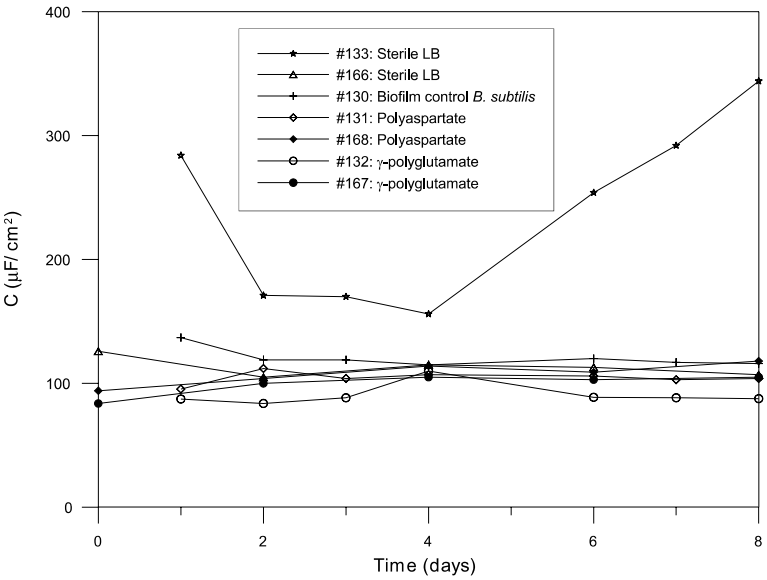

Fig. 8. The time dependence of the capacitance C for cartridge brass exposed to LB medium under different conditions. Symbols as defined in Fig. 7 caption.

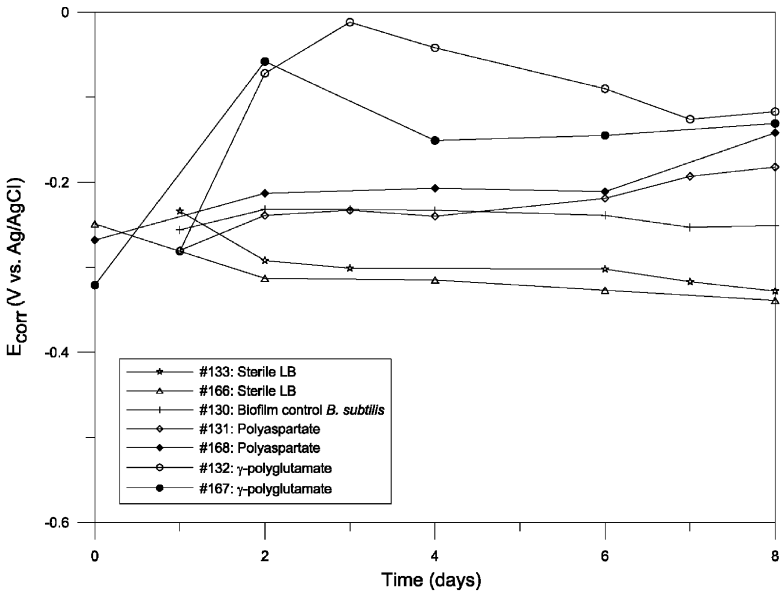

Fig. 9. The time dependence of E_{corr} for cartridge brass exposed to LB medium under different conditions. Symbols as defined in Fig. 7 caption.

rate accompanied by ennoblement can only be explained by simultaneous reduction of the rates of the cathodic reaction, i.e. oxygen reduction, and the anodic reaction, i.e. metal dissolution.

Acknowledgements

This project is being supported by the Electric Power Research Institute (Contract WO8044-05). The authors appreciate the critical review of this document by the project manager Dr. B.C. Syrett.

References

[1] Proceedings of International Congress on Microbiologically Influenced Corrosion and Biodeterioration, October 1990, MIC Consortium, Knoxville, TN, 1990.
[2] Proceedings of International Conference on Microbiologically Influenced Corrosion, May 1995, AWS and NACE, New Orleans, LA, 1995.
[3] B. Little, P. Wagner, F. Mansfeld, Int. Mater. Rev. 36 (6) (1991) 1.
[4] M. Eashwar, S. Maruthamutu, S. Sathyanarayanan, K. Balakrishnan, in: Proceedings of 12th International Corrosion Congress, September 1993, vol. 5b, NACE, Houston, TX, 1993, p. 3708.
[5] A. Jayaraman, E.T. Cheng, J.C. Earthman, T.K. Wood, Appl. Microbiol. Biotechnol. 48 (1997) 11.
[6] A. Jayaraman, E.T. Cheng, J.C. Earthman, T.K. Wood, J. Ind. Microbiol. Biotechnol. 18 (1997) 396.
[7] A. Jayaraman, J.C. Earthman, T.K. Wood, Appl. Microbiol. Biotechnol. 47 (1997) 62.

[8] A. Jayaraman, F. Mansfeld, T.K. Wood, J. Ind. Microbiol. Biotechnol. 22 (1999) 167.
[9] D. Örnek, A. Jayaraman, T.K. Wood, Z. Sun, C.H. Hsu, F. Mansfeld, Corros. Sci. 43 (2001) 21.
[10] F. Mansfeld, C.H. Hsu, D. Örnek,T.K. Wood, B.C. Syrett, in: The Electrochem. Soc., Proc. vol. 2000-24, 2001, p. 99; J. Electrochem. Soc. (in press).
[11] D. Örnek, T.K. Wood, C.H. Hsu, F. Mansfeld, Corrosion, submitted.
[12] G. Hernandez, V. Kucera, D. Thierry, A. Pedersen, M. Hermansson, Corros. Sci. 50 (1994) 603.
[13] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1982.
[14] X.-C. Wu, W. Lee, L. Tran, S.-L. Wong, J. Bacteriol. 173 (1991) 4952.
[15] N. Vasantha, A. Helene, C. Mario, R. Joachim, Gene 114 (1992) 121.
[16] Y.H. Ko, R.A. Gross, Biotechnol. Bioeng. 57 (1998) 430.
[17] A. Jayaraman, D. Örnek, D.A. Duarte, C.C. Lee, F.B. Mansfeld, T.K. Wood, Appl. Microbiol. Biotechnol. 52 (1999) 787.
[18] F. Mansfeld, Z. Sun, C.H. Hsu, D. Örnek, T.K. Wood, Corrosion (in press).