Technical Note: Ennoblement—A Common Phenomenon?

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

ABSTRACT

A shift of the corrosion potential in the positive direction ("ennoblement") has been observed for cartridge brass and Al 2024 (UNS A92024) exposed to artificial seawater or Luria Bertani growth medium in the presence of bacteria such as B. subtilis, B. licheniformis, *or* E. coli. *Since ennoblement was always accompanied by a reduction of corrosion rates, it can be assumed that natural biofilms produce inhibitive species. The production of polypeptides and polyphosphates by biogenetically engineered bacteria provided additional corrosion protection.*

KEY WORDS: Al 2024, biofilms, cartridge brass, ennoblement, potential, seawater

INTRODUCTION

Probably no other phenomenon has fascinated those studying microbiologically influenced corrosion (MIC) more than ennoblement (i.e., an increase of the corrosion potential [E_{corr}] caused by the formation of a biofilm). Ennoblement has been observed mainly for stainless steels (SS) exposed to natural seawater (NS). Mansfeld and Little have reviewed experimental results and various attempts to explain the observed ennoblement phenomena.[1] Early explanations used thermodynamic arguments, which suggested that the reversible potential (E°) of the oxygen electrode increased in the presence of biofilms because of either an increase in the partial pressure of oxygen or a decrease in the surface pH. Since E° increases only very slightly with an increase of oxygen pressure and since it is unlikely that acidification would increase passivity, these explanations need to be rejected. Formation of hydrogen peroxide (H_2O_2) has also been suggested as causing an increase of E°.[2] The possibility that the biofilm causes an increase of the exchange current density for oxygen reduction has apparently not been considered. Johnsen and Bardal suggested that ennoblement was caused by a change in the cathodic properties of the SS as a result of microbiological activity on the surface.[3] The very interesting and elegant explanation by Lewandowski and coworkers concerning ennoblement observed for SS in river water involving formation of manganese dioxide (MnO_2) (which has an E° close to the observed ennobled E_{corr} of SS) deals with a special case of ennoblement.[4-5]

The possibility that formation of a biofilm can decrease the passive current density, which would also lead to ennoblement, has not been considered thus far.[6] One of the few exceptions is the suggestion by Eashwar, et al.,[7] that ennoblement of SS in seawater is the result of the production of inhibitors by bacteria that are retained in the biofilm matrix. An important observation, which has not been explained, is the fact that E_{corr} for SS exposed to NS can exceed the pitting potential (E_{pit}) measured in sterile seawater yet suffer no pitting. This result could be attributed to the formation of inhibiting species by the biofilm (as suggested by Eashwar, et al.)[7] or to the reduction of the chloride concentration at the SS

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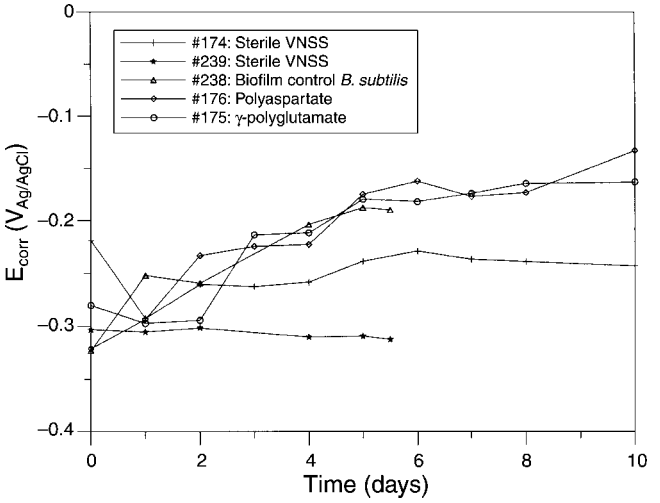

FIGURE 1. *Time dependence of E_{corr} for UNS 26000 brass exposed to VNSS under different conditions. Polyaspartate indicates the 20-amino-acid polyaspartate secreted by a* B. subtilis *WB600/pBE92-polyaspartate biofilm, and γ-polyglutamate indicates the anionic polymer secreted by a* B. licheniformis *biofilm.*

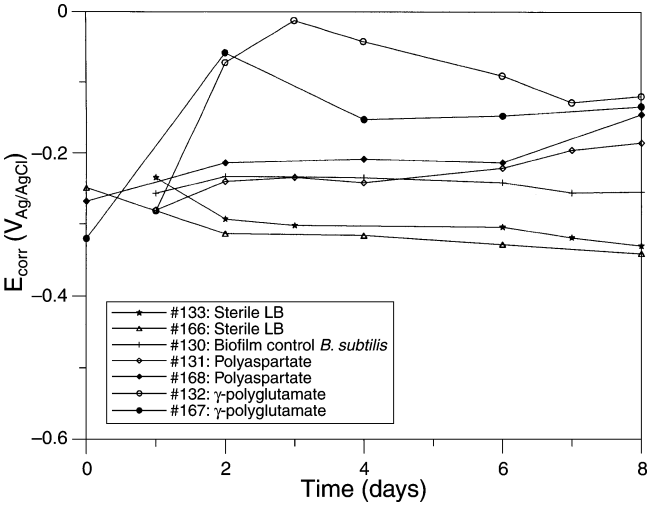

FIGURE 2. *Time dependence of E_{corr} for UNS 26000 brass exposed to LB medium under different conditions. Polyaspartate indicates the 20-amino-acid polyaspartate secreted by a* B. subtilis *WB600/pBE92-polyaspartate biofilm, and γ-polyglutamate indicates the anionic polymer secreted by a* B. licheniformis *biofilm.*

surface covered by the biofilm. These and other observations have led to the evaluation of a new approach to corrosion protection based on Corrosion Control Using Regenerative Biofilms (CCURB), using both natural bacteria and engineered bacteria that secrete corrosion inhibitors.[8]

The CCURB concept is being evaluated at present in detail for a number of materials such as mild steel, SS, brass, and aluminum alloys in the presence of many different bacterial communities. Results obtained for cartridge brass exposed to artificial seawater (AS) and nutrient-rich Luria Bertani (LB) medium in the absence and presence of a *Bacillus subtilis* bacterial biofilm have shown that corrosion and tarnishing of the brass were reduced in the presence of a biofilm.[9] Analyses of electrochemical impedance spectroscopy (EIS) data obtained for Al 2024 (UNS A92024)[(1)] exposed to AS and LB medium have shown that, in the presence of biofilms, active pit growth stopped after ~2 days.[10-11] For the remainder of the exposure time, the impedance spectra showed that the Al alloy was passive with very low corrosion rates, which is quite unusual considering the great susceptibility of Al 2024-T3 to pitting in chloride media. Obviously, successful implementation of CCURB could reduce expenditures for biocides and corrosion inhibitors in many practical applications.

RESULTS AND DISCUSSION

Results obtained for UNS C26000 cartridge brass (70Cu/30Zn) and Al 2024-T3 revealed that ennoblement occurred in all cases. Figures 1 through 4 show the time dependence of E_{corr} for brass exposed to AS (Figure 1) and LB medium (Figure 2) as well as for Al 2024 exposed to AS (Figure 3) and LB medium (Figure 4). AS was prepared from Väätänen nine salts solution (VNSS) (17.6 g/L sodium chloride [NaCl], 0.08 g/L sodium bicarbonate [$NaHCO_3$], 0.04 g/L potassium bromide [KBr], 0.41 g/L calcium chloride [$CaCl_2 \cdot 2H_2O$], 0.008 g/L strontium chloride [$SrCl_2 \cdot 6H_2O$], 1.47 g/L sodium sulfate [Na_2SO_4], 0.25 g/L potassium chloride [KCl], 1.87 g/L magnesium chloride [$MgCl_2 \cdot 6H_2O$], 0.008 g/L boric acid [H_3BO_3], 0.01 g/L ferrous sulfate [$FeSO_4 \cdot 7H_2O$], 0.01 g/L disodium phosphate [Na_2HPO_4], 1.0 g/L peptone, 0.5 g/L starch, 0.5 g/L glucose [$C_6H_{12}O_6$], and 0.5 g/L yeast extract) at pH = 7.5[12] and LB medium (10 g/L NaCl, 5 g/L yeast extract, and 10 g/L tryptone).[13] Biofilms on metal surfaces were developed in autoclavable continuous reactors with AS or LB medium, as described elsewhere.[14] The working volume of the reactor was 100 mL or 150 mL with an airflow rate of 200 mL/min (monitored with a FM1050[†] series flowmeter). The exposed surface area of the test electrode was 28.3 cm^2 or 45.4 cm^2. A titanium counter electrode and an autoclavable silver/silver chloride (Ag/AgCl) reference electrode were also used for measurement of impedance spectra at E_{corr}. The growth temperature was maintained at 30°C for *Bacillus* and 37°C for *Escherichia coli* by heating tape wrapped around the reactor. Sterile medium used in each experiment was fed at a nutrient flow rate of 12 mL/h using a Masterflex[†] precision standard drive with a 10-turn potentiometer (Cole-Parmer[†]). Sterile

(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.

† Trade name.

control experiments were conducted with 100 µg/mL kanamycin ($C_{18}H_{36}O_{11}N_4$) to ensure sterility. *B. licheniformis* was grown without antibiotics. *B. subtilis* cultures were grown in 100 µg/mL kanamycin, recombinant *E. coli* was grown in 25 µg/mL chloramphenicol ($C_{11}H_{12}O_5N_2Cl_2$) and 50 µg/mL ampicillin ($C_{16}H_{19}N_3O_4S$), and natural *E. coli* was grown in 10 µg/mL tetracycline ($C_{22}H_{24}O_8N_2$) to retain the plasmids. The reactors were inoculated with overnight cultures of *Bacillus* or *E. coli* strains grown from –80°C glycerol stocks in 250-mL shake flasks with 25 mL LB medium at 37°C and were shaken at 250 rpm with a New Brunswick Scientific Series 25† shaker. Biofilms were allowed to develop for 15 h to 18 h in batch mode. Then, sterile nutrients were added continuously. Additional experimental details are given elsewhere.[9-11] The tests performed for brass are listed in Table 1, while the tests for Al 2024 are summarized in Table 2 (duplicate experiments are indicated).

B. licheniformis was obtained from the American Type Culture Collection (Strain 9945A). It produces 5 g/L to 23 g/L γ-polyglutamate that is part of its capsule and is freely secreted into the growth medium.[15] γ-Polyglutamate is a homopolymer of glutamic acid ($C_5H_9O_4N$) that has amide linkages between the glutamate γ-carboxyl and α-amino groups.[15]

B. subtilis WB600[16] was obtained from S.-L. Wong of the University of Calgary and is a protease-deficient strain. It was transformed with pBE92, a derivative of pBE60,[17] to become resistant to 50 µg/mL to 100 µg/mL kanamycin and was used as a biofilm-forming negative control that does not secrete a corrosion inhibitor. pBE92 was obtained from E.I. du Pont de Nemours, Inc.

The genes encoding the 20-amino-acid polyaspartate or polyglutamate peptide and the *B. amyloliquefaciens apr* secretion signal were cloned downstream of the alkaline protease promoter of *B. amyloliquefaciens* in pBE92 plasmid using published techniques.[13,18] pBE92-polyaspartate and pBE92-polyglutamate were transformed into *B. subtilis* so that these strains secreted constitutively either polyaspartate or polyglutamate.

E. coli MV1184, with plasmids pBC29 and pEP02.2, were obtained from Ohtake of Hiroshima University.[19] The recombinant *E. coli* (pBC29, pEP02) produces and releases polyphosphate. Polyphosphate is a linear polymer of orthophosphate residues (P_i) linked by high-energy, phosphoanhydride bonds with a chain length of up to 1,000 or more.[20] To produce and secrete polyphosphate,[19,21] 0.1 g/L to 5 g/L phosphate (K_2HPO_4) and 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) (Fisher Scientific Co.) were added to the LB medium.[13]

The remarkable protective effect of the biofilms observed for brass in AS[9] could not have been caused solely by a reduction of the oxygen concentration at

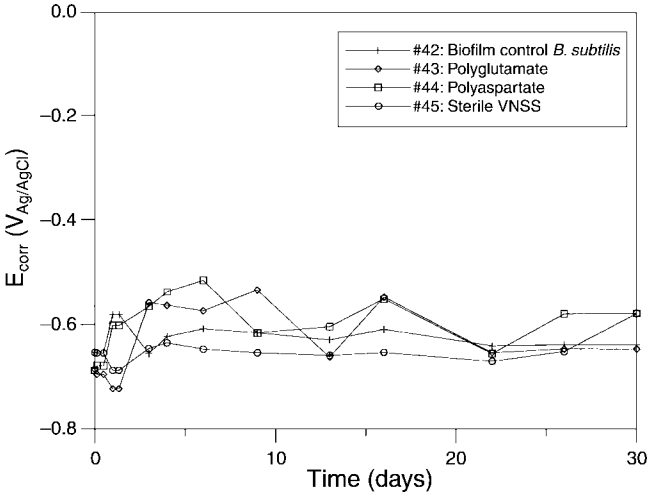

FIGURE 3. *Time dependence of E_{corr} for Al 2024 in sterile VNSS medium and in the presence of* B. subtilis *WB600. Polyaspartate indicates the 20-amino-acid polyaspartate secreted by a* B. subtilis *WB600/pBE92-polyaspartate biofilm, and polyglutamate indicates the 20-amino-acid polyglutamate secreted by* B. subtilis *WB600/pBE92-polyglutamate biofilm.*

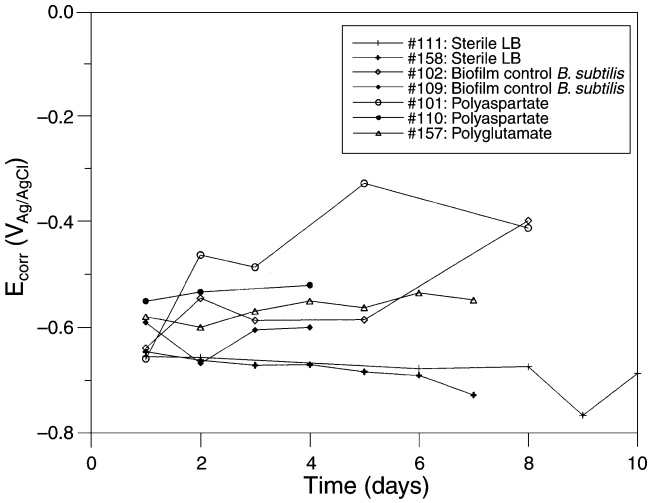

FIGURE 4. *Time dependence of E_{corr} for Al 2024 exposed to LB medium with and without* B. subtilis *WB600. Polyaspartate indicates the 20-amino-acid polyaspartate secreted by a* B. subtilis *WB600/pBE92-polyaspartate biofilm, and polyglutamate indicates the 20-amino-acid polyglutamate secreted by* B. subtilis *WB600/pBE92-polyglutamate biofilm.*

the brass surface in the presence of the biofilm since E_{corr} was found to increase with time (i.e., ennoblement of brass was observed in VNSS in the presence of a biofilm [Figure 1]). E_{corr} was almost constant and lower in sterile VNSS (Tests 174 and 239, Table 1). However, in the presence of *B. subtilis* (Tests 238 and 176) or *B. licheniformis* (Test 175), ennoblement was observed (Figure 1). After exposure for 10 days, E_{corr} was more noble by ~100 mV in the solutions containing bacteria than in the sterile solutions. The sample

TABLE 1
Experiments for Brass UNS C26000 in VNSS and LB Media

Test	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

TABLE 2
Experiments for Al 2024 in VNSS and LB Media

Test	Medium	pH	Strain	Secreted Inhibitor
45	VNSS	7.5	Sterile	—
42	VNSS	7.5	*B. subtilis* WB600	—
44	VNSS	7.5	*B. subtilis* WB600/pBE92-polyaspartate	Polyaspartate
43	VNSS	7.5	*B. subtilis* WB600/pBE92-polyglutamate	Polyglutamate
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

that had been exposed to sterile VNSS was covered by a dark film while the samples that had been exposed to the same solution containing bacteria remained untarnished and did not show any signs of corrosive attack.

Ennoblement of brass was also observed in LB medium with a difference in E_{corr} of ~200 mV between the sterile solution (Test 166, Table 1) and the solution containing *B. licheniformis*-producing γ-polyglutamate (Tests 132 and 167), for which ennoblement seemed to be more pronounced than for *B. subtilis* WB600/pBE92-producing polyaspartate (Tests 131 and 168) (Figure 2). At the end of the exposure of brass to sterile LB medium, the sample was covered by a dark film of corrosion products. The samples used in the tests with bacteria remained untarnished and did not show any signs of corrosive attack.

The observed inhibition of pitting of Al 2024 in VNSS in the presence of bacteria could have been caused by the exclusion of oxygen from the metal surface,[10-11] which would reduce the rate of the cathodic reduction, resulting in a decrease of E_{corr} to below 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 inhibition of pit growth and ennoblement were observed in the presence of all biofilms, it has to be concluded that the observed CCURB is a result of a passivation effect, which occurs even in the presence of bacteria that are not engineered to produce inhibitors from a biofilm. 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.[10-11]

E_{corr} was more positive in LB medium in the presence of *B. subtilis* than in the sterile solution (Figure 4). The most pronounced ennoblement was observed in the presence of polyaspartate produced by the biofilm (Tests 101 and 110). Ennoblement was also observed in the tests with *B. licheniformis* or *E. coli* (Table 2, Figure 5). In the presence of *E. coli*, E_{corr} was ~400 mV more positive than in the sterile solution (Test 170). Corrosion rates of Al 2024 in LB medium in the presence of *E. coli*-producing polyphosphates

(Test 171) were ~0.1 μm/y, as estimated from the analysis of the impedance spectra.[10-11]

CONCLUSIONS

❖ Since ennoblement was always accompanied by a reduction of corrosion rates and degree of tarnishing, it can be concluded that natural biofilms produce inhibitive species, as suggested by Eashwar.[7] The degree of corrosion protection and ennoblement of Al 2024 were different in the presence of different microorganisms (Figures 4 and 5). Therefore, it can be assumed that the natures of the inhibitive species produced naturally by the different biofilms were different. The production of polypeptides and polyphosphate by biofilms provided additional corrosion protection, as evaluated in the present study under the CCURB project.

❖ Results discussed here clearly demonstrate that ennoblement is a phenomenon that seems to be more common than previously assumed. It occurs not only for SS in seawater and natural waters, but, as shown here, also for Al 2024 and brass exposed to AS or LB medium in the presence of certain bacteria.

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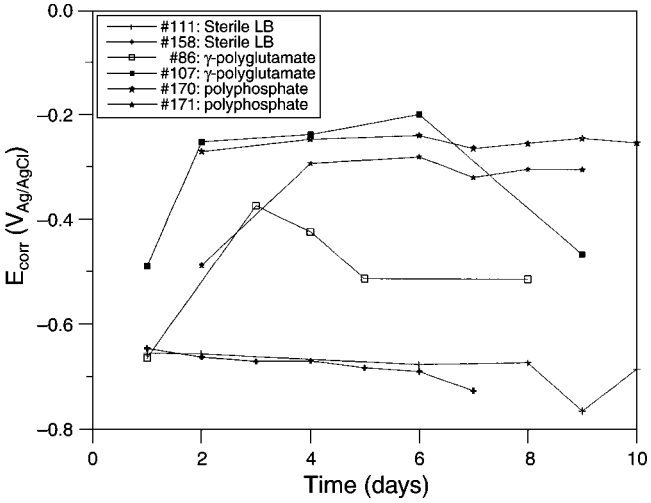

FIGURE 5. *Time dependence of E_{corr} for Al 2024 exposed to LB medium with and without* B. licheniformis *or* E. coli. *γ-polyglutamate indicates the anionic polymer secreted by a* B. licheniformis *biofilm, and polyphosphate indicates the anionic polymer secreted by an* E. coli *MV1184 (pBC29, pEP02.2) biofilm.*

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