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Axenic aerobic biofilms inhibit corrosion of copper and aluminum

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Abstract The corrosion behavior of unalloyed copper and aluminum alloy 2024 in modified Baar's medium has been studied with continuous reactors using electrochemical impedance spectroscopy. An axenic aerobic biofilm of either *Pseudomonas fragi* K or *Bacillus brevis* 18 was able to lessen corrosion as evidenced by a consistent 20-fold increase in the low-frequency impedance value of copper as well as by a consistent four- to seven-fold increase in the polarization resistance of aluminum 2024 after six days exposure compared to sterile controls. This is the first report of axenic aerobic biofilms inhibiting generalized corrosion of copper and aluminum. Addition of the representative sulfate-reducing bacterium (SRB) *Desulfovibrio vulgaris* (to simulate consortia corrosion behavior) to either the *P. fragi* K or *B. brevis* 18 protective biofilm on copper increased the corrosion to that of the sterile control unless antibiotic (ampicillin) was added to inhibit the growth of SRB in the biofilm.

Introduction

Aerobic bacteria have been shown to decrease the rate of mild steel corrosion due to biofilm formation (Jack et al. 1992; Jayaraman et al. 1997a–c; Obuekwe et al. 1987; Pedersen and Hermansson 1989, 1991; Potekhina 1984). Jayaraman et al. have reported that axenic *Pseudomonas fragi* biofilms on SAE 1018 steel decreased the corrosion rate compared to sterile controls by two- to ten-fold over a period of four weeks in batch reactors (Jayaraman et al. 1997a) and by 40-fold in continuous reactors (Jayaraman et al. 1997c). The mechanism for this general reduction in corrosion was found to be due to oxygen depletion at the metal surface by respiring cells (Jayaraman et al. 1997c). This is a general phenomenon in that 15 other aerobic bacteria decreased corrosion of mild steel if they formed a biofilm (Jayaraman et al. 1997b). Pedersen and Hermansson (1989, 1991) have also observed an eight-fold corrosion inhibition of mild steel by aerobic bacteria under favorable conditions.

The toxicity of copper to microorganisms has led to the belief that microbially-induced corrosion of copper is insignificant (Iverson 1987). However, the ammonia generated by microorganisms, sulfuric acid generated by *Thiobacillus*, and hydrogen sulfide generated by sulfate-reducing bacteria (SRB) can cause corrosion of copper alloys (Iverson 1987; Wagner and Little 1993). Wagner and Little (1993) observe that the presence of a biofilm on copper creates differential aeration cells and chloride gradients, which can cause pitting. Corrosion of copper alloys is a problem in heat exchanger tubing, ship seawater piping, and aircraft fuel tanks (Iverson 1987; Miller 1981). Iverson (1987) mentions that the corrosion of copper in fresh water and seawater was inhibited by the addition of bacteria but corrosion increased after the bacteria died.

Formation of a passive oxide film on aluminum enhances its corrosion resistance (Iverson 1987; Wagner and Little 1993). *Pseudomonas* and *Cladosporium* species have been commonly associated with the microbiologically influenced corrosion of aluminum and its alloys (Iverson 1987). The production of corrosive organic compounds by *P. aeruginosa* can remove zinc and magnesium from aluminum alloys, and cause corrosion. The pitting of aluminum by three strains of SRB has been reported and a 100-fold increase in weight loss

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compared to sterile controls was observed (Iverson 1987).

This study reports corrosion inhibition of both unalloyed copper and aluminum alloy 2024 by axenic, aerobic biofilms of either *P. fragi* or *Bacillus brevis*. Corrosion rates for unalloyed copper are found to increase to those of a sterile control when the protective biofilm is challenged by SRB.

Materials and methods

Bacterial strains and growth conditions

P. fragi K is a kanamycin-resistant derivative of *P. fragi* (Jayaraman et al. 1997a), and *B. brevis* 18 is a gramicidin S-overproducing strain (Azuma et al. 1992), resistant to kanamycin. *Desulfovibrio vulgaris* (ATCC 29579) was obtained from the American Type Culture Collection and cultivated in 15-ml screwcap tubes with 10 ml modified Baars' medium (ATCC medium 1249) supplemented with 100 µl each of the oxygen-scavengers 4% sodium sulfide and Oxyrase (Oxyrase, Mansfield, Ohio). Initial cultures were grown from −85 °C glycerol stocks; all subsequent cultures were grown with a 3% inoculum from the initial culture maintained at 30 °C without shaking. *D. vulgaris* was periodically checked with 0.1% ferrous ammonium sulfate (black iron sulfide formed) and by the desulfoviridin assay (red color under UV light) as described previously (Jayaraman et al. 1999b).

Biofilms on metal surfaces were developed in continuous reactors (flowrate 12 ml/h except for washout experiments, working volume 100 ml) with modified Baar's medium since this medium supports the growth of aerobes and SRB. Sterile continuous reactor controls were conducted with 50 µg/ml kanamycin to ensure sterility, while 10–50 µg/ml was used for continuous cultures with *P. fragi* K and 10 µg/ml for *B. brevis* 18. A 1% (v/v) bacterial inoculum was used for all continuous experiments, the aerobic bacteria being inoculated from a turbid, 16-h culture, and SRB (culture age 24–48 h) added after 3–5 days of aerobic biofilm development. Where indicated, ampicillin (100 µg/ml) was added simultaneously to the feed bottle and the reactor (before SRB had colonized the metal) to inhibit SRB in the biofilm while not inhibiting *P. fragi* K (which is naturally resistant to ampicillin).

Sample preparation

Unalloyed copper and aluminum alloy 2024 plates ($7.5 \times 7.5 \times 0.12$ cm) were cut from sheet stock, polished with 240 grit paper (Buehler, Lake Bluff, Ill.), and stored as described previously (Jayaraman et al. 1997c).

Continuous corrosion rates using EIS

Twenty-three continuous reactors (in at least duplicate experiments) were used to develop biofilms on metal surfaces (27.3 cm^2 of exposed surface area); electrochemical impedance spectroscopy (EIS) was used to obtain impedance data as described earlier (Jayaraman et al. 1997c). Impedance data (at 1.4 mHz–20 kHz) were obtained using a Solarton-Schlumberger electrochemical measurement unit (SI 1280, Schlumberger Technical Instruments Division, San Jose, Calif.) interfaced to a Macintosh computer (PowerMac 7100/80, Apple Computers, Cupertino, Calif.) running EISIS electrochemical experimentation software (University of California, Irvine). The open-circuit potential (OCP) was measured as the potential between the metal specimen and the reference electrode (Ag/AgCl), and the polarization resistance (R_p) was determined as the direct-current (low-frequency) limit of the impedance using the ANALEIS software developed by Mansfeld et al. (1992); equivalent electrical circuits were used to determine the capacitance (C) and corrosion potential (E_{corr}).

Results

EIS is a non-invasive, in situ method that allows accurate and sensitive quantification of the corrosion rate of metal (Mansfeld 1995; Mansfeld et al. 1992); hence, it enables one to study the impact of a biofilm on the metal surface without disturbing it (Jayaraman et al. 1997c). R_p is obtained from the low-frequency value of the impedance (or more rigorously from equivalent circuit models as used in this report). This parameter is inversely proportional to the continuous culture corrosion rates based on the Stern-Geary equation, $R_p = B/i_{corr}$, where B is a parameter depending on the Tafel slopes and i_{corr} is the corrosion current density which can be converted into a corrosion rate using Faraday's law (Mansfeld 1976).

EIS was combined with continuous cultures in modified Baar's medium at 30 °C to measure the corrosion data (impedance, polarization resistance, capacitance, and corrosion potential) for copper and aluminum (Table 1). The 23 continuous reactor experiments were performed for a combined total of over 5400 h (average 236 h each) with the impedance spectrum measured on average every 11.6 h. Hence, 467 impedance spectra were generated and only representative spectra are shown in Figs. 1 and 2.

Table 1 Polarization resistance (R_p), capacitance (C), and corrosion potential (E_{corr}) of aluminum 2024 alloy and unalloyed copper in modified Baar's medium at 30 °C with *Pseudomonas* or *Bacillus* biofilms, determined by equivalent electric circuits. Data are from a representative experiment (minimum of two independent experiments)

Biofilm	R_p (Ohm · cm^2)	C (F/cm^2)	E_{corr} (mV vs. Ag/AgCl)	Metal sample
Sterile	3.0×10^4	1.78×10^{-5}	−670	Aluminum 2024
P. fragi K	13.2×10^4	4.05×10^{-5}	−520	Aluminum 2024
B. brevis 18	21.3×10^4	1.69×10^{-5}	−512	Aluminum 2024
Sterile	[a]	[a]	−171	Copper
P. fragi K	[b]	[b]	−118	Copper
B. brevis 18	9.66×10^5	3.60×10^{-4}	−177	Copper
B. brevis 18 + SRB	1.43×10^5	1.65×10^{-3}	−385	Copper

[a] Not possible to estimate parameters based on available equivalent circuit models
[b] Impedance suggests pitting ($R_p = 2.97 \times 10^5$ Ohm · cm^2, $C = 8.1 \times 10^{-5}$ F/cm^2, $R_{pit}/F = 3.52 \times 10^3$ Ohm)

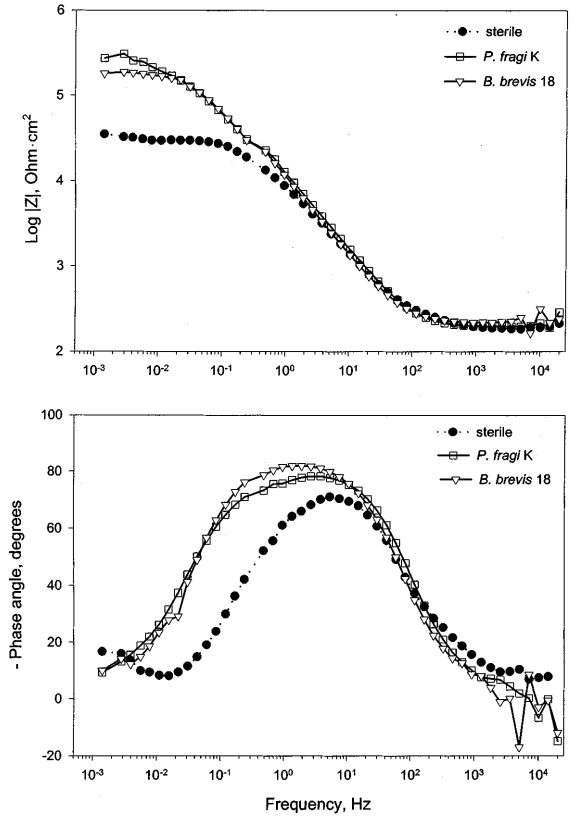

Fig. 1 Representative impedance spectra for aluminum alloy 2024 in modified Baar's medium at 30 °C with either a *Pseudomonas fragi* K or a *Bacillus brevis* 18 protective biofilm (minimum of two independent experiments for each condition)

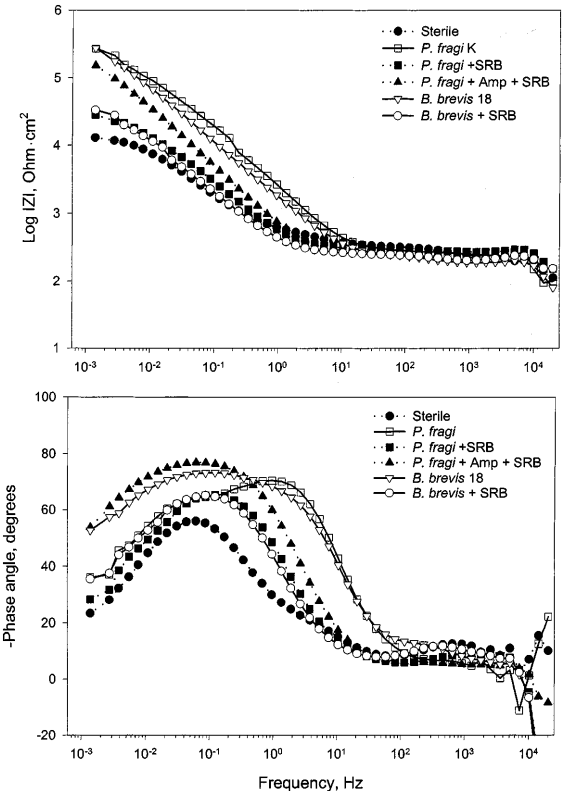

Fig. 2 Representative impedance spectra for unalloyed copper in modified Baar's medium at 30 °C with a *P. fragi* K or *B. brevis* 18 protective biofilm (minimum of two independent experiments for each condition). Where indicated, *D. vulgaris* was added (as a representative sulfate-reducing bacterium SRB) after the protective biofilm had become established, and ampicillin was added prior to SRB inoculation

For aluminum alloy 2024, the impedance spectra obtained with sterile modified Baar's medium in continuous reactors (two independent experiments) consistently showed a maximum phase angle of 72 ± 2° at the low frequencies during ten days exposure and a low-frequency value of the impedance of 1314 ± 500 Ohm (Fig. 1). When a *P. fragi* K biofilm was developed on the aluminum alloy for 6 days (five independent experiments), the maximum phase angle shifted to 81 ± 3°, and a seven-fold increase in the low-frequency value of impedance was seen in the spectra (Fig. 1, R_p = 9400 ± 2400 Ohm). Also, a 4.3-fold increase in R_p was calculated (Table 1). This increase in R_p corresponds to a 4.3-fold decrease in the corrosion rate based on the Stern-Geary equation. This increase in R_p and decrease in corrosion with the *P. fragi* K biofilm was corroborated by the aerobic *B. brevis* 18 biofilm (three independent experiments) which also increased the R_p of aluminum 2024 by seven-fold (Table 1) and the phase angle by 10° (Fig. 1) under similar conditions (R_p = 9400 ± 2200 Ohm, 82 ± 2°).

For the corrosion of unalloyed copper, sterile reactors (five independent experiments) had a maximum phase angle of 46 ± 11° and R_p of 470 ± 220 Ohm during 10 days of exposure (Fig. 2). A *P. fragi* K biofilm grown on copper (five independent experiments) increased the impedance by 20-fold at the lowest frequency measured (1.4×10^{-3} Hz, Fig. 2) in the same time period (R_p = 9160 ± 2200 Ohm). This decrease in corrosion was also corroborated by an increase in the phase angle (72 ± 4° vs 56°). Similar changes in the impedance spectra (two independent experiments) relative to the sterile control were also observed when a *B. brevis* 18 biofilm was developed on copper (Fig. 2) since the phase angle increased to 73 ± 2° and the low-frequency impedance increased 19-fold (R_p = 9000 ± 3700 Ohm).

It is important to ensure that the observed corrosion inhibition for copper and aluminum are due to the attached biofilm and not the planktonic (suspended) cells. To verify this, the nutrient flowrates in two continuous reactors with *Bacillus* strains and aluminum alloy were increased after 6 days from 12 ml/h to 125 ml/h for 24 h. The flowrate was then increased further to 400 ml/h for 5 h in one of the reactors but kept at 125 ml/h for another 24 h in the other. This increased the dilution rate from 0.12/h to 1.25/h and 4.0/h (1.4 and 4.4 times the maximum specific growth rate). Under these conditions, washout of unattached cells occurred (the supernatant became clear of bacteria) and only the biofilm affected EIS. During washout, no significant difference in the EIS

spectra was seen in either reactor; therefore, the impedance effects observed were due to the biofilm. Note also that previous work using scanning laser confocal microscopy to study biofilms formed on metal coupons under similar quiescent conditions indicated that robust, uniform biofilms of about 10–20 μm were formed (Jayaraman et al. 1997b, 1998).

Since natural biofilms usually contain many kinds of bacteria including SRB, the ability of the biofilm to protect the copper metal during SRB attack was evaluated. As shown in Fig. 2, addition of SRB to the continuous reactors containing either a *B. brevis* 18 or *P. fragi* biofilm resulted in a decrease in the impedance (relative to experiments with a non-SRB, protective biofilm) to nearly that of the sterile control; hence, corrosion was increased by a factor of ten (factor of seven based on impedance model results shown in Table 1). This quantitative indicator of enhanced corrosion due to SRB growth was corroborated by a strong smell of hydrogen sulfide. However, if ampicillin was added prior to SRB addition, the impedance was not changed significantly (Fig. 2) since *D. vulgaris* is inhibited by ampicillin (Saleh et al. 1964).

Discussion

The similar reduction in corrosion of aluminum and copper obtained by the two different biofilms (*B. brevis* 18 and *P. fragi*) suggests the protection of these metal surfaces is a general phenomenon which occurs due to oxygen removal. This hypothesis is based on previous results with mild steel which showed that corrosion inhibition with protective biofilms was comparable to the inhibition seen with sterile medium and anaerobic conditions, that live (respiring) cells were necessary for the corrosion inhibition, that fermentation products alone do not decrease corrosion, and that good biofilm-forming bacteria of various genera were capable of corrosion reduction (Jayaraman et al. 1997b, c). In addition, the observed increases in R_p and the changes in the impedance spectra for copper and aluminum (relative to sterile controls) are similar to the 35° increase in the phase angle (unpublished) and 40-fold increase in R_p reported by Jayaraman et al. (1997c) for SAE 1018 mild steel with an axenic *P. fragi* K biofilm (these mild steel results were verified by weight loss determinations of metal coupons).

Based on the results presented here, it may be possible to decrease corrosion of aluminum and copper through oxygen reduction using a protective biofilm. However, this film must also be able to inhibit the growth of deleterious bacteria such as SRB (perhaps by producing anti-SRB antimicrobials in situ in the biofilm). This engineered-biofilm approach is feasible given that Jayaraman et al. (1999a, b) have shown that the generation of antimicrobials within engineered biofilms can be used to inhibit the colonization of SRB and decrease the corrosion of 304 stainless steel and SAE 1018 mild steel.

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