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Corrosion Science 47 (2005) 279–287
CORROSION SCIENCE
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Letter

The importance of live biofilms in corrosion protection

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Received 25 August 2004; accepted 27 September 2004

Abstract

As observed before, Al 2024 was passive in artificial seawater (AS) in the presence of a protective biofilm of *Bacillus subtilis* WB600. When antibiotics were added to the AS to kill the bacteria in the biofilm, pitting occurred within a few hours as indicated by characteristic changes in the impedance spectra. The corrosion potential E_{corr} decreased at the same time to values observed in sterile AS. Addition of the antibiotics to sterile AS had no effect on corrosion behavior.

Keywords: A. Aluminium; B. EIS; C. Microbiological corrosion; C. Pitting corrosion

1. Introduction

Jayaraman et al. [1,2] have shown previously that live biofilms are required for the corrosion protection of mild steel in a growth medium containing *P. fragi* or *E. coli*.

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doi:10.1016/j.corsci.2004.09.006

The biofilm bacteria were completely killed after addition of kanamycin and a significant increase of corrosion rates was observed within one week [1]. With mild steel in AS, (also known as Väätänen Nine Salts Solution) [2], the corrosion potential, E_{corr}, and the polarization resistance decreased over 24 h when the biofilm was killed [2]. The use of beneficial biofilms was then applied by Wood and coworkers to several new metals including brass [6] and copper and aluminum [3] where significant reductions in corrosion rates were found in growth medium and where the pitting of Al 2024 was discovered to be completely inhibited in AS [5]. More recently, Naguib and Mansfeld [4] studied the corrosion behavior of Al 2024, cartridge brass, and mild steel in AS containing *Shewanella ana*. When the biofilm was killed, E_{corr} increased towards values observed in sterile AS in a short time. However, corrosion rates as estimated from polarization resistance values obtained from impedance spectra only increased after several days. In order to determine in more detail the importance of live biofilms and the time frame during which the corrosion behavior changes after addition of an antibiotic, the corrosion behavior of Al 2024 exposed to AS in the presence of *Bacillus subtilis* WB600 was investigated. This system has been studied in detail previously [5,9,10] and it has been found that Al 2024 was passive in the presence of a live biofilm. Since E_{corr} had increased in the presence of the biofilm, it was concluded that inhibition of pitting was due to a reduction of the rate of the anodic reaction and an increase of the pitting potential E_{pit} [5,7,9,10]. The mechanism of corrosion inhibition of Al 2024 in AS is different in the presence of *S. ana* and *B. subtilis* since in the former case prevention of pitting is accompanied by a decrease of E_{corr} suggesting that corrosion inhibition is due mainly to a decrease of the rate of oxygen reduction at the metal surface [4].

2. Experimental approach

Aluminum 2024 coupons (UNS A92024, 10 × 10 cm, 1.2 mm thick) were cut from sheet stock (Yarde Metals, Bristol, CT), polished with 240 grit polishing paper (Buehler, Lake Bluff, IL), and rinsed with distilled water. As described previously [7], continuous, autoclavable reactors with the aluminum coupon as the test sample were used. The corrosion behavior of Al 2024 exposed to AS was followed by recording of impedance spectra at E_{corr} for about one week. Four different experiments were carried out in order to determine the importance of live biofilms in preventing pitting corrosion of Al 2024. In the first experiment, Al 2024 was exposed to sterile AS (test #1), while in the second experiment *B. subtilis* WB600 was added to the AS and allowed to develop biofilm in a batch mode for 1 day before continuous operation began (test #2). In the third experiment, the live biofilm was killed by adding penicillin G (2.5 mg/ml) and neomycin (1 mg/ml) simultaneously after exposure to AS for 90.5 h (test #3). In the fourth test antibiotics were added to the sterile AS in order to determine whether the antibiotics had an effect on corrosion behavior other than killing bacteria (test #4). After the experiments were finished, the aluminum coupons were washed under tap water and scrubbed with rubber stopper to remove any cell debris and corrosion products before the pitted area fraction was determined.

Another set of experiments was conducted independently but under the same conditions as above, to show that the biofilm cells were effectively killed by the antibiotics. The aluminum coupons were rinsed with sterile dH_2O to remove the suspension cells and were sonicated (FS3 sonicator, Fisher, Hanover Park, IL) to detach the biofilm cells from the metal surface which were then spread on solid LB plates to determine the cell number. The percentage of the biofilm cells killed by the antibiotics was obtained by comparing the cell numbers on the plates for the biofilm exposed to the antibiotics and the biofilm without addition of the antibiotics.

3. Results and discussion

Figs. 1 and 2 give a comparison of impedance spectra observed in sterile AS (Fig. 1) and AS containing *B. subtilis* (Fig. 2). The spectra obtained in the sterile solution have the typical features described by the pitting model proposed by Mansfeld et al. [11,12], i.e., a second time constant at the lowest frequencies which is due to the presence of active pits (Fig. 1). In the presence of the live biofilm, passive behavior was found, i.e., the spectra contained only one time constant (Fig. 2). In Figs. 1 and 2 only the spectra recorded after 91 h are shown. Spectra recorded before this time were very similar.

The addition of antibiotics killed about 99.2% of the biofilm cells as measured on solid plates. The effect of adding antibiotics to AS containing bacteria is shown in Fig. 3, where the addition of neomycin and penicillin G was made after 90.5 h. The spectrum recorded about 0.5 h after addition of the antibiotics indicated that Al 2024 was still passive, however the spectrum recorded 7 h later showed indications of pitting as seen by the minimum of the phase angle at about 0.1 Hz (Fig. 3). The next two spectra recorded at 113.5 h and 124 h after start of the test were unstable at the lowest frequencies, however the following five tests resulted in stable spectra similar to the ones recorded in sterile AS (Fig. 1). The spectra obtained in sterile media did not change when the antibiotics were added after 90.5 h (Fig. 4).

The impedance spectra recorded for the four tests described above were analyzed using the ANALEIS software developed by Mansfeld et al. [11,12]. Figs. 5 and 6 show the time dependence of E_{corr} and the polarization resistance R_p for passive surfaces or the pit polarization resistance R_{pit} for Al 2024 undergoing pitting corrosion [11,12], respectively. As observed previously [5,7,9,10], E_{corr} was more positive in the presence of the live biofilm (Fig. 5). After addition of the antibiotics, E_{corr} decreased in a very short time to the values observed in the sterile solution. As shown in Fig. 3, pitting initiated in a very short time after addition of the antibiotics with R_{pit} reaching values which were close to those observed in the sterile solution (Fig. 6). No changes of R_{pit} occurred when the antibiotics were added to the sterile AS proving that the antibiotics do not affect the corrosion behavior of Al 2024 in the sterile solution (Fig. 6).

It should be noted that the R_p and R_{pit} data shown in Fig. 6 have not been normalized by the areas of the exposed passive surface A_t and the total pitted area A_{pit}, respectively. In principle A_{pit} can be determined by analysis of the impedance spectra using the pitting model [12]. As demonstrated previously for the same system as studied here [5,7,9], the normalized parameters R_p^0 and R_{pit}^0 had typical values of about

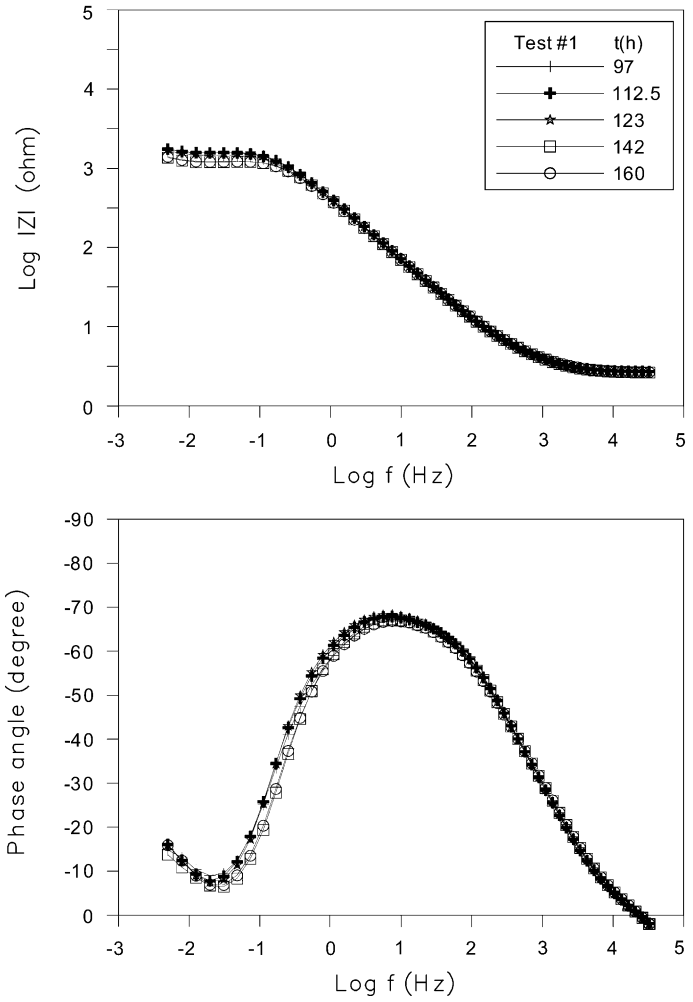

Fig. 1. Bode plots for Al 2024 exposed to sterile AS.

$10^6\,\Omega\mathrm{cm}^2$ and less than $1000\,\Omega\mathrm{cm}^2$, respectively. In the present tests, this procedure was not applied and R_{pit} is used as a qualitative measure of pit growth rates. At the end of the four tests the samples were examined under a microscope to observe the extent of pitting attack. A_{pit} was determined using a digital camera and appropriate software for image analysis based on the pitted area fraction F as $A_{pit} = 2FA_t$ assuming hemispherical pits [5,9,12]. The F-values were 0.3% for test #1 (sterile control), 0% for test #2 (*B. subtilis* biofilm), 2.2% for test #3 (*B. subtilis* biofilm killed at 90.5 h), and 0.4% for test #4 (sterile control with antibiotics added). R_p^0 was $2.05 \times 10^5\,\Omega\mathrm{cm}^2$ for test #2, while R_{pit}^0 was $416\,\Omega\mathrm{cm}^2$ for test #1, $955\,\Omega\mathrm{cm}^2$ for test #3, and $201\,\Omega\mathrm{cm}^2$ for test #4 based on the experimental R_p and R_{pit} values determined at the end of each test.

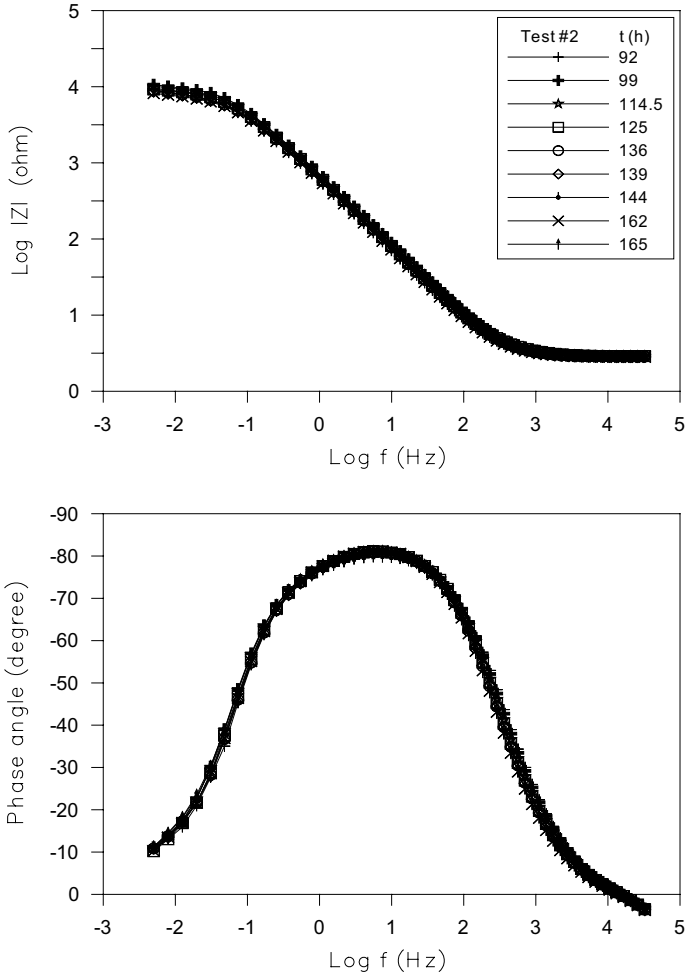

Fig. 2. Bode plots for Al 2024 exposed to AS containing *B. subtilis*.

It will be noted that F and therefore A_{pit} were the largest for test #3 in which the bacteria were killed after 90.5 h. However, R^0_{pit} had the largest value for this test which suggests that although the total number of pits was larger than in the two tests without bacteria (tests #1 and #4), the pits grew at a slower rate.

4. Conclusions

The results discussed above have shown that the protective action of the live biofilm disappears in a very short time (0.5–7 h) after the bacteria are killed. This result raises questions concerning the mechanism(s) by which the bacteria prevent pitting

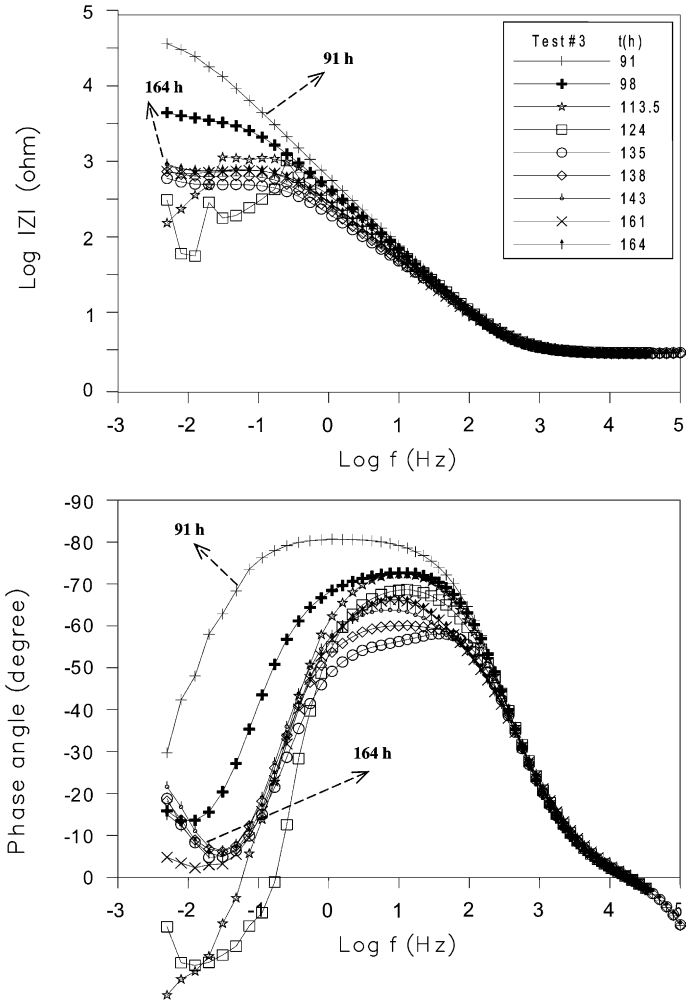

Fig. 3. Bode plots for Al 2024 exposed to AS containing *B. subtilis*, addition of antibiotics after 90.5 h.

attack of Al 2024 in AS. The observation that corrosion protection results in an ennoblement of E_{corr} suggests that (1) the biofilm produces an inhibitor or (2) that inhibitors increase E_{pit} to higher values than E_{corr}. In the sterile solution, E_{pit} and E_{corr} have similar values. Although the diffusion barrier for the penetration of some antibiotics in biofilms has been shown to probably not be important [13], a simple Fick's Second Law ($\frac{\partial C_A}{\partial t} = D_{AB}\frac{\partial^2 C_A}{\partial y^2}$) analysis shows that the antibiotics (penicillin G and neomycin) would diffuse to the bottom of the biofilm in less than 2 min. This is based on the solution $\frac{C_A}{C_{A0}} = 1 - \text{erf}\frac{y}{\sqrt{4D_{AB}t}}$, where C_A is the antibiotic concentration at the bottom of the biofilm, and C_{A0} is the concentration of antibiotics in the bulk medium. Using a relatively slow diffusion constant (D_{AB}) of 10^{-6} cm^2/s [13] and a

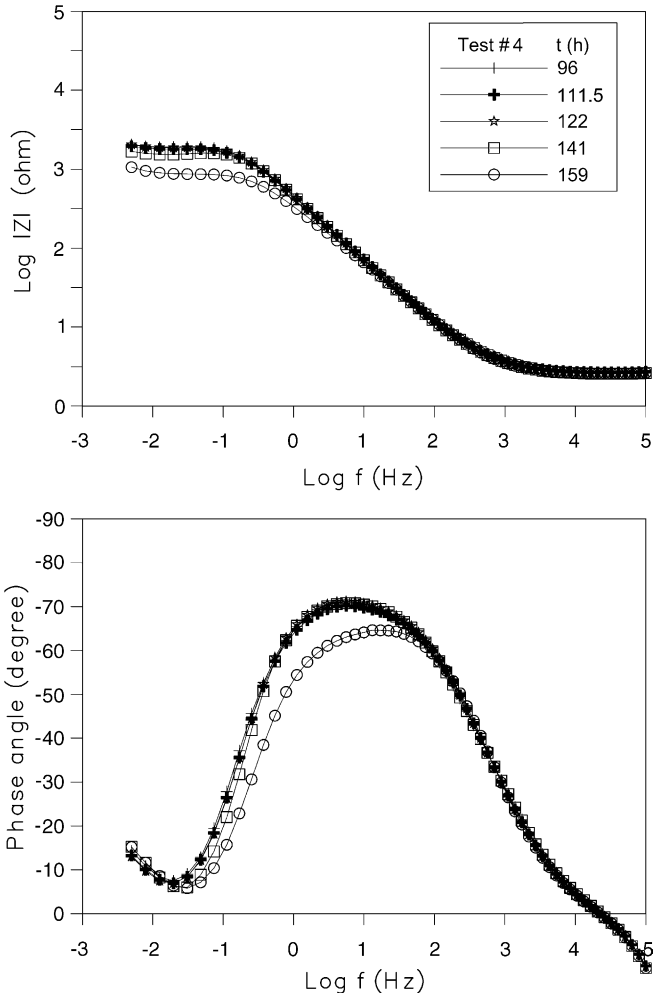

Fig. 4. Bode plots for Al 2024 exposed to sterile AS, addition of antibiotics after 90.5 h.

B. subtilis biofilm thickness (y) of about 20 μm as was seen by us in similar systems [8], only 1.6 min is needed for the concentration of antibiotics at the bottom of the biofilm to reach 90% of the bulk fluid concentration. Since the corrosion protection is lost in a very short time (0.5–7 h), and since any inhibitor that might be made by the live biofilm would have time to diffuse away (just like the antibiotic), it is difficult to speculate on the role of any possible inhibitor, if one is made at all. However, the corrosion protection may be due to loss of the negative charge of the bacteria: it is possible that the negatively charged bacteria [14] make the biofilm repel chloride ions and thereby increase E_{pit} [15]. Once the bacteria are killed (within minutes due to rapid diffusion of the antibiotic), this negative charge could disappear. It is also possible that corrosion protection is due to the extracellular polymeric substance (EPS)

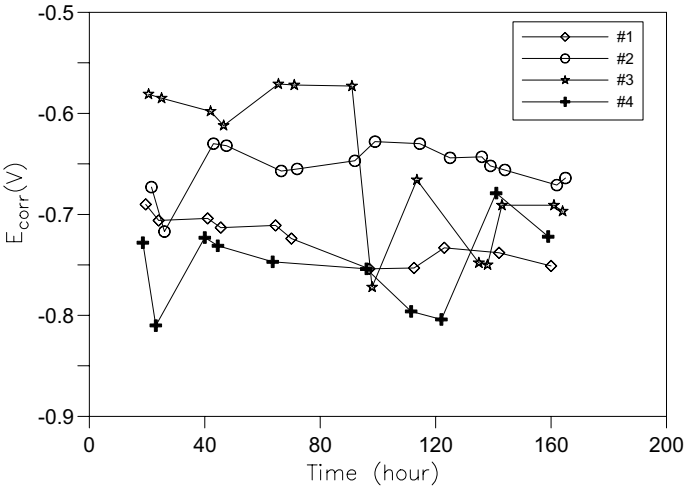

Fig. 5. Time dependence of E_{corr} for four different tests.

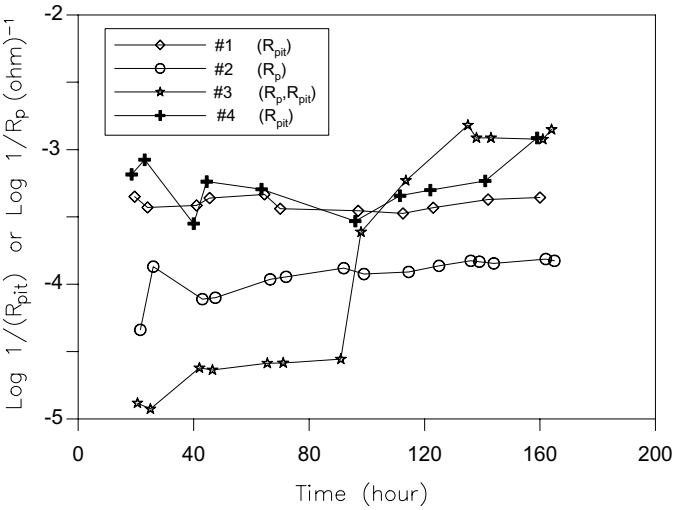

Fig. 6. Time dependence of R_p or R_{pit} for four different tests.

of the biofilm which is negatively charged for most bacteria species [16]. However, while this charge might disappear once the bacteria are killed, it is unlikely that the EPS layer would lose its protective properties which are considered to be similar to those of a paint layer immediately after the bacteria have been killed.

The present results have shown clearly that corrosion protection of Al 2024 in AS occurs only in the presence of a live biofilm. The mechanism(s) by which *B. subtilis* and other bacteria [4,5,7,9,10] protect metallic surfaces from corrosion remain(s) unclear.

Acknowledgment

This project is supported by the Electric Power Research Institute (Contract EP-P5388-C2665). We thank Dacheng Ren for the determination of the biofilm cell numbers.

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