Corrosion control using regenerative biofilms (CCURB): an update

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Corrosion Control Using Regenerative Biofilms (Corrosion CURB or, simply, CCURB), a phenomenon that has only recently been studied to any significant degree, involves the formation of protective surface films by bacteria. One advantage of these biofilms is that they repair themselves when damaged. Aerobic bacteria have been studied that can form protective biofilms on the iron, copper, and aluminum alloys used in power plants, giving rise to quite significant decreases in corrosion rate. One reason for the reduced corrosion rates is that the aerobic bacteria in the surface of the biofilm consume oxygen in the water before it can reach the metal surface and participate in the corrosion reaction. In addition, the beneficial aerobic bacteria can be genetically engineered to produce and release corrosion-inhibiting compounds or antimicrobial compounds. The latter kills potentially deleterious anaerobic bacteria, such as sulfate reducing bacteria (SRB). Concurrent field testing is underway in several power plant service water systems. Biofilm samples from these plants are being examined to determine the nature and abundance of naturally occurring bacteria. One or more of these bacteria will be chosen as "hosts" that will be engineered to provide corrosion protection to metals and alloys, first in an adjacent instrumented closed-loop sidestream, but eventually in the service water system itself.

Parole chiave: corrosione, trattamenti superficiali

INTRODUCTION

When metal surfaces are exposed to natural aqueous environments, they are rapidly colonized by aerobic (oxygen loving) microorganisms present in the bulk water, such as bacteria, fungi, and microalgae. These microbes form biofilms composed of same-species and mixed-species microcolonies that have a distinct architecture and even a mechanism of communication (chemical signals). The upper layers of this biofilm are typically aerobic -- because the water contains dissolved oxygen -- while the regions near the metal surface are typically anaerobic due to the consumption of oxygen by the aerobic microorganisms in the outer layers of the biofilm. Sulfate-reducing bacteria (SRB) can colonize these anaerobic niches and can play an important role in microbially influenced corrosion (MIC) of the nearby metal. SRB have been implicated in the deterioration of a wide range of materials, like carbon-steels, stainless steels, and copper alloys, and in a wide range of environments. Affected systems include pipelines and off-shore oil rigs in the oil and shipping industries; cooling water recirculation systems in industrial systems; sewage treatment facilities and pipelines; jet fuel tanks in the aviation industry; and the power generation industry. Because of this and other forms of MIC, bacteria in biofilms are believed to be responsible for $4 - 6 billion of corrosion damage in the U.S every year.

Corrosion of a metal (M) involves at least one anodic and one cathodic reaction at the metal/environment interface. In the anodic reaction, the metal is dissolved or oxidized with the resulting release of electrons (e^-) into the metal and, for example, metal ions (M^{n+}) into the environment:

$$M \rightarrow M^{n+} + ne^- \qquad (1)$$

where n is the valence of the metal ion produced. The supporting cathodic reaction consumes the electrons produced by the anodic reaction by reducing another chemical species. In many neutral waters, the cathodic reaction is reduction of dissolved oxygen:

$$O_2 + 2\,H_2O + 4e^- \rightarrow 4\,OH^- \qquad (2)$$

The destruction of the metal at anodic sites, Equation (1), cannot occur without the supporting reduction reaction occurring at cathodic sites, Equation (2), so, in the case shown, corrosion can be stifled by deaerating the water thereby removing the cathodic reactant, oxygen.

Conventional corrosion prevention strategies aim to prevent or reduce the rate of one or both of these half-reactions. For instance, corrosion may be reduced or halted by control of water chemistry (pH, redox potential, purity), application of organic or inorganic coatings, cathodic protection, or addition of biocides or corrosion inhibitors. Each approach has its shortcomings. For instance, protective coatings have been used extensively but they are not permanent: the cost of maintaining and replacing them is quite high. Biocides and corrosion inhibitors have also been widely used but, in such systems as open cooling towers, the environmental impact and cost of adding large quantities of inorganic compounds is huge.

CORROSION CONTROL USING REGENERATIVE BIOFILMS (CCURB)

In an attempt to counter the limitations and costs of current corrosion prevention technology, a new approach is being

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developed by the Electric Power Research Institute (EPRI). This paper reports the concept of Corrosion Control Using Regenerative Biofilms (Corrosion-CURB, or, simply, CCURB) being developed by a team of scientists from EPRI and four U.S. universities. In 1994, Wood and Earthman, had the intriguing idea that biofilms need not be deleterious and that they should be capable of being engineered to reduce corrosion in industrial settings. As detailed below, genetically engineered aerobic bacteria have now been developed that are capable of forming a biofilm and limiting corrosion by one or more mechanisms. For instance, aerobic bacteria consume oxygen in the outer layers of the film, thereby preventing this cathodic reactant from reaching the metal surface and participating in the corrosion reaction, Equation (2). In addition, some aerobic bacteria can be modified to over produce antimicrobial compounds that are capable of inhibiting the growth of otherwise damaging SRB. In more recent experiments, genetically engineered bacteria have been developed that secrete corrosion inhibiting compounds within the biofilm.

MILD STEEL AND STAINLESS STEEL CORROSION CURB

Many reports have indicated that bacteria can stimulate corrosion (MIC) and, until recently, beneficial effects of bacteria were unknown. However, in the early phases of the current work, tests on mild steel in corrosive waters showed that the presence of some types of aerobic bacteria reduced corrosion rates considerably compared to the rates measured in sterile controls (1-9). For instance, Fig. 1 illustrates that, in simple immersion tests in rich nutrient broth (10 g/L NaCl, 10 g/L peptone, 5 g/L yeast extract) at pH 6.5, a biofilm containing *P. fragi* reduces weight loss of mild steel by a factor of about 10 after 4-weeks compared with weight loss in a similar, but sterile, solution or in a solution containing non-film-forming bacteria (*S. lividans*).

Electrochemical impedance spectroscopy (EIS) measurements in a continuous bioreactor demonstrated that live cells are required to reduce corrosion rates and that bacterial fermentation products alone do not decrease corrosion damage (1,3). Fig. 2 shows that *P. fragi* can considerably reduce corrosion rates of mild steel in Väätänen nine-salt solution (simulating seawater) so long as the bacteria are alive. In one test, the *P. fragi* were killed with a biocide after about 190 hours of exposure resulting in an immediate increase in the corrosion rate to values more typical of the sterile solution. The dead biofilm was not able to provide corrosion protection.

Mass loss measurements and surface examinations using scanning electron microscopy and confocal laser microscopy confirmed these EIS results and demonstrated that the CCURB phenomenon was not restricted to just a few bacteria (2,3). Tests on 15 different bacteria demonstrated that the extent of corrosion inhibition depends on the nature of the biofilm: the greater the proportion of live cells, the lower was the corrosion rate. Corrosion inhibition is greatest in the presence of bacteria that create continuous biofilms and only a small layer of active, respiring cells is required to inhibit corrosion. The beneficial biofilms reduce corrosion rates of mild steel by reducing the oxygen concentration at the metal surface and, possibly, by naturally expressing a corrosion inhibiting chemical compound.

After these early encouraging results, work was directed towards studying how beneficial bacteria might be engineered to secrete antimicrobial compounds capable of inhibiting growth of deleterious bacteria, such as SRB, that could be co-present in the biofilm. To this end, Jayaraman et al. (5) developed gram-positive *Bacillus* strains that formed continuous biofilms and secreted one of two peptide compounds (indolicidin or bactenecin) which preliminary tests demonstrated had antimicrobial properties. These genetically engineered *Bacillus* strains were tested in suspension in a concentrated culture broth in the co-presence of one of two representative strains of SRB known to stimulate corrosion, *Desulfovibrio vulgaris* and *Desulfovibrio gigas*. The *Bacillus* strains killed 83 percent of the SRB whereas, when the *Bacillus* strain was not engineered to produce the antimicrobial, there was no reduction in the viability of these bacteria.

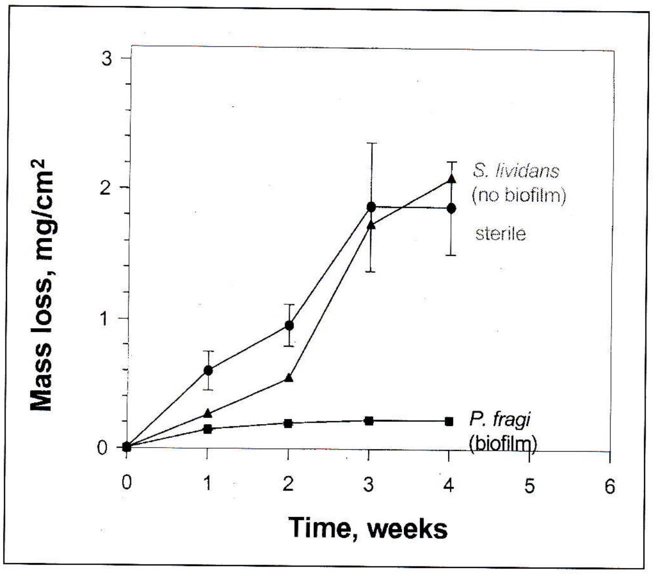

Fig. 1. Carbon-steel mass loss as a function of time in sterile rich nutrient broth (Luria-Bertani medium) exposed to air and in the same solution after batch inoculations of P. fragi or S. lividans (3).

Fig. 1. Perdita in massa dell'acciaio al carbonio in funzione del tempo in bagno nutriente sterile (soluzione Luria-Bertani) esposto all'aria e nella stessa soluzione dopo inoculazione di P. fragi o S. lividans (3).

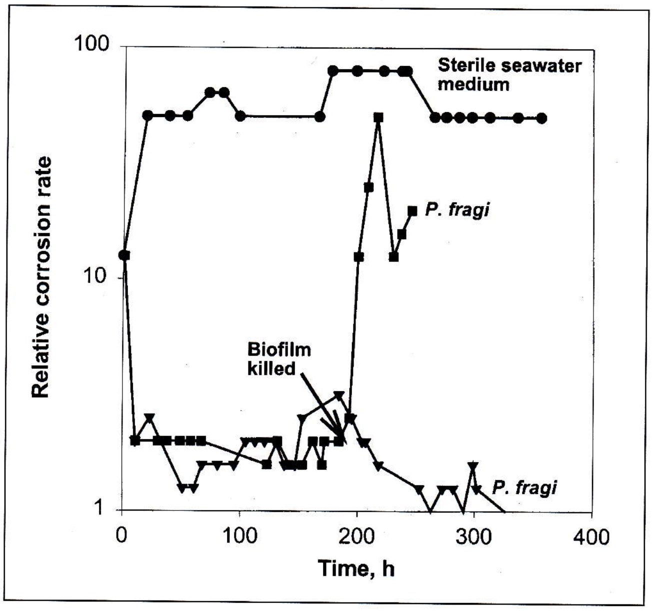

Fig. 2. Corrosion rate of mild steel in sterile Väätänen nine-salt solution (simulating seawater) and in the same solution after continuous inoculation of P. fragi. In one test, the P. fragi were killed by addition of a biocide after ~190 hours (1).

Fig. 2. Tasso di corrosione di acciaio dolce in soluzione sterile Väätänen (che simula l'acqua marina) e nella stessa soluzione dopo inoculazione continua di P. fragi. In un test, i batteri di P. fragi sono stati uccisi mediante aggiunta di biocidi dopo ~190 ore (1).

To test the ability of the *Bacillus* constructs to kill SRB within a biofilm, the number of viable SRB was measured after five days in a biofilm formed on 304 stainless steel. Compared with biofilms containing unaltered bacteria (without a cloned antimicrobial gene), the biofilm formed by the bactenecin-producing *Bacillus* strain resulted in an almost 60-fold decrease in SRB. The ability to kill SRB was not quite as good when this *Bacillus* strain produced indolicidin instead of bactenecin but, even here, SRB were reduced by 37%. Hence, these results clearly indicate that the growth of SRB can be inhibited in the biofilm by the expression of the antimicrobials.

Further corroboration of the ability of the engineered, protective, *Bacillus* biofilms to inhibit growth of SRB was obtained by measuring the corrosion rate of mild steel in shake flasks. Upon addition of SRB to a non-antimicrobial-producing *Bacillus* culture, a strong odor of hydrogen sulfide was detected in less than 60 hours. This was accompanied by the formation of a black iron sulfide precipitate which indicated growth and colonization of SRB in the aerobic biofilm growing on the metal surface (3). In contrast, both antimicrobial-producing bacteria were able to delay the onset of SRB-induced corrosion by an additional 96 hours.

The ability of the *Bacillus* biofilms, engineered to secrete antimicrobials, to inhibit growth of SRB was also verified using EIS. EIS measures polarization resistance (R_p) which is inversely proportional to corrosion rate. Since corrosion was induced by the SRB in the biofilm, any measured reduction in corrosion rate was related to the decrease in the population of SRB in the biofilm. Addition of SRB to the non-engineered *Bacillus* biofilms on type 304 stainless steel increased the corrosion rate (as indicated by a decrease in the polarization resistance (R_p) to 1/7 of the value obtained in an SRB-free solution). However, no such increase in the corrosion rate was observed upon addition of SRB to the biofilm when the antimicrobial, bactenecin, was produced in the biofilms. Therefore, these results also indicate that the expression and secretion of bactenecin significantly inhibited the growth of SRB in the biofilm.

BRASS AND ALUMINUM CORROSION CURB

Recent laboratory tests have shown for the first time that the presence of a biofilm can significantly decrease corrosion in brass (10). The corrosion rate of brass (UNS C26000) in the presence of one of two *Bacillus* strains in rich nutrient broth at pH 6.5 was compared with the rate in the equivalent sterile medium in continuous reactors during an 8-day test period. Corrosion rates, determined by EIS in terms of reciprocal polarization resistance ($1/R_p$), are shown in Fig. 3 as a function of time. A comparison of the average $1/R_p$ values during the last 4 days of the test suggests that the corrosion rate of brass in the presence of *Bacillus* strains #1 and #2 was, respectively, about 1/40 and 1/50 of the rate in the sterile solution. In the sterile solution (no biofilm), the surface of the brass was completely black whereas, in the presence of the *Bacillus* cultures, it was totally free of tarnish. Similar results have been obtained in an artificial seawater environment.

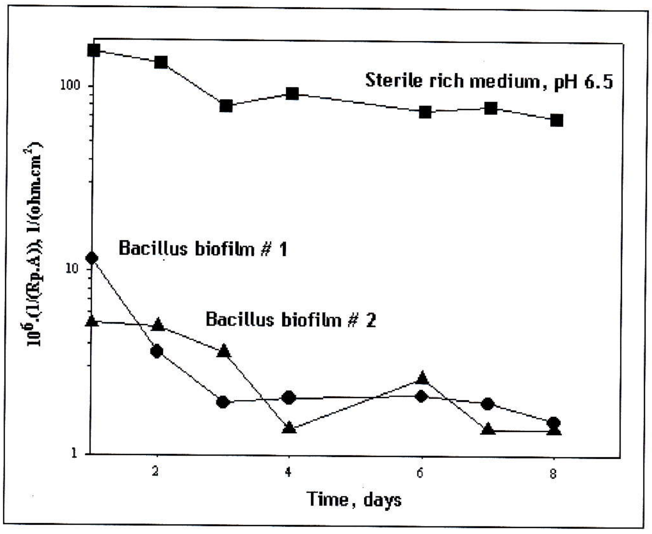

Fig. 3. Comparison of corrosion rates ($1/R_p$) for brass in a continuous aerobic reactor containing sterile rich medium at pH 6.5 with rates in a similar medium containing either Bacillus strain #1 or #2.

Fig. 3. Confronto dei tassi di corrosione ($1/R_p$) per l'ottone in un reattore aerobico continuo contenente soluzione sterile con valore di pH 6,5 e in una soluzione simile contenente ceppo di Bacillo #1 o #2.

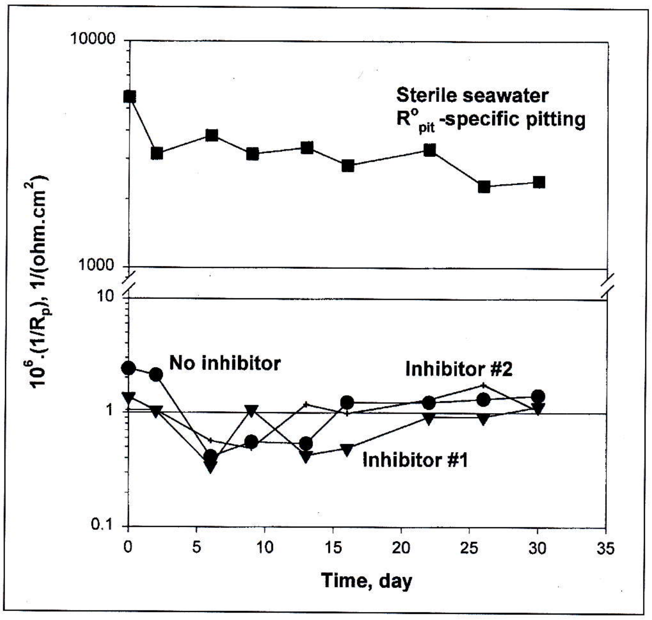

Fig. 4. Comparison of relative corrosion rates ($1/R_p$) for aluminum alloy 2024 in simulated seawater (pH 7.5) containing one of three Bacillus strains with the rate of pit growth ($1/R_{pit}$) in a similar sterile medium. Two of the Bacillus strains were engineered to secrete a corrosion inhibiting compound ("inhibitor #1" or "inhibitor #2") while the third ("no inhibitor") was not engineered.

Fig. 4. Confronto dei relativi tassi di corrosione ($1/R_p$) per una lega di alluminio 2024 in una acqua marina artificiale (pH 7,5) contenente uno dei tre ceppi di Bacilli con tasso di sviluppo di vaiolatura ($1/R_{pit}$) in una soluzione sterile similare. Due dei ceppi di Bacillo sono stati studiati per secernere un complesso inibitore della corrosione ("inibitore #1" o "inibitore # 2") mentre il terzo ("senza inibitore") no.

Other recent tests have demonstrated for the first time that pitting of aluminum can be reduced substantially by the presence of a protective biofilm (10,11). EIS was used to monitor corrosion rates of aluminum alloy 2024 (UNS A92024) during 30-day tests in simulated seawater -- either sterile or seawater containing one of three *Bacillus* strains. Two of the strains were genetically engineered to secrete corrosion inhibiting compounds and the third formed a protective biofilm but was not engineered. Pitting was observed throughout the test in sterile seawater but occurred for only about two days in the presence of the *Bacillus* strains. After the first two days of exposure, the biofilm on the metal surface became protective, the aluminum alloy stopped pitting and remained passive thereafter. Visual inspection at the end of the

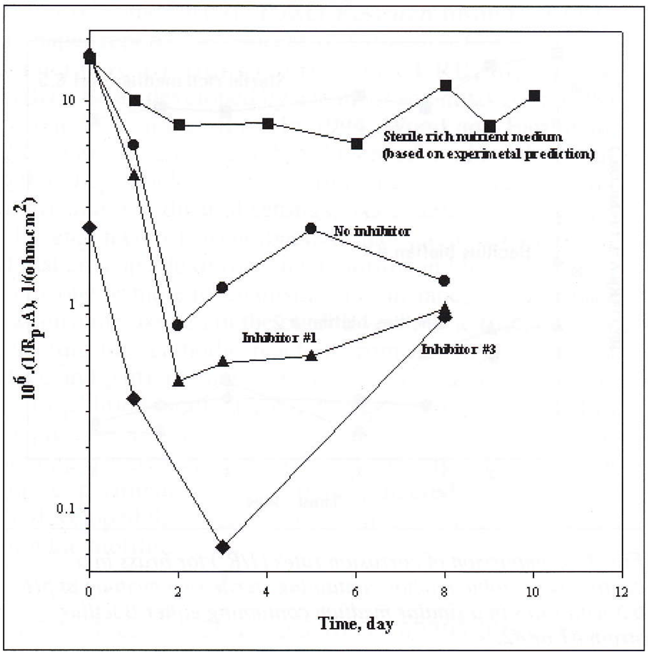

Fig. 5. Comparison of corrosion rates ($1/R_p$) for aluminum 2024 in sterile rich nutrient (Luria-Bertani) medium at pH 6.5 with rates in a similar medium containing one of three Bacillus strains; two of these strains secreted a corrosion inhibiting compound ("inhibitor #1" and "inhibitor 3"). Tests were performed in a continuous aerobic reactor for 8 days.

Fig. 5. Confronto fra i tassi di corrosione ($1/R_p$) dell'alluminio 2024 in soluzione nutriente sterile (Luria-Bertani) con pH 6,5 e in una soluzione similare contenente ono dei tre ceppi di Bacillo; due di questi ceppi hanno generato un complesso inibitore della corrosione ("inibitore #1" e "inibitore # 3). I test sono stati condotti in un reattore aerobico continuo per 8 giorni.

tests showed that the percentage (P) of the exposed surface that was pitted in the presence of the biofilm (P = 0.06-0.17%) was about one-tenth the percentage (P = 1.04%) found in the absence of a biofilm.

Corrosion rates in the presence of one of the three *Bacillus* strains, as expressed by the normalized reciprocal polarization resistance [$1/(R_p.A)$ $ohm^{-1}.cm^{-2}$, where A is the exposed area], are shown in Fig. 4 as a function of time. Also shown are the normalized corrosion rates of the active pits [$1/(R_{pit}.A_{pit})$ $ohm^{-1}.cm^{-2}$, where Apit is the pitted area] formed in sterile simulated seawater (11). The results in Fig. 4 demonstrate that, when aluminum alloy 2024 is covered by any of the *Bacillus* biofilms, it is less susceptible to damage by pitting corrosion than when exposed to sterile seawater.

Fig. 4 shows that the *Bacillus* strains that secreted corrosion inhibitors did not provide aluminum alloy 2024 with any more resistance to *pitting* corrosion than the parent (no inhibitor) strain.

However, recent tests in non-pitting environments have shown for the first time that genetically-engineered *Bacillus* strains secreting corrosion inhibitors within a biofilm can significantly decrease the *general* corrosion rate of aluminum (10). Corrosion rates of aluminum 2024 are shown in Fig. 5 in terms of reciprocal polarization resistance ($1/R_p$) versus time in sterile rich nutrient broth at pH 6.5 and in the same broth containing one of three *Bacillus* strains. The data clearly demonstrate that the *Bacillus* biofilms reduce general corrosion rates, especially when inhibitor #1 or #3 is secreted by the bacteria. In the latter case, the corrosion rate ($1/R_p$) in the presence of the biofilm was as little as 1/100 of the rate in the sterile solution.

FLOW LOOP TESTS

Initial field-testing is underway in a sidestream flow loop connected to the 6-million-gallon circulating chilled water system running through the University of California, Irvine (UCI) campus (Fig. 6).

Construction materials in this system are known to suffer from MIC despite the addition of biocides, such as bromine and gluteraldehyde. The chilled water system at UCI is quite similar to service water systems found in power generation facilities, but additional testing will also be performed in utility power plants. To this end, a transportable sidestream test loop was designed,

built, and tested in the UCI laboratory and this has served as a blueprint for two similar sidestream test loops, nearing completion at GPU Nuclear Inc.'s Three Mile Island plant. All of these loops have parallel sections that allow simultaneous testing at different flow rates.

Fig. 7 shows that each section houses small, flush-mounted, disc electrodes (the exposed flat ends of cylindrical rods) and 2-inch-diameter 5-inch-long (50.8 mm x 127 mm) pipe specimens.

The disc electrodes are used for corrosion rate monitoring and other electrochemical measurements; and both disc and pipe specimens are used for studying biofilm characteristics. An example of electrochemical measurements made at different locations ("clock positions") in the pipe in the UCI chilled water test loop is shown in Fig. 8.

Here, the reciprocal of the pore resistance ($1/R_{po}$ ohm^{-1}) is a

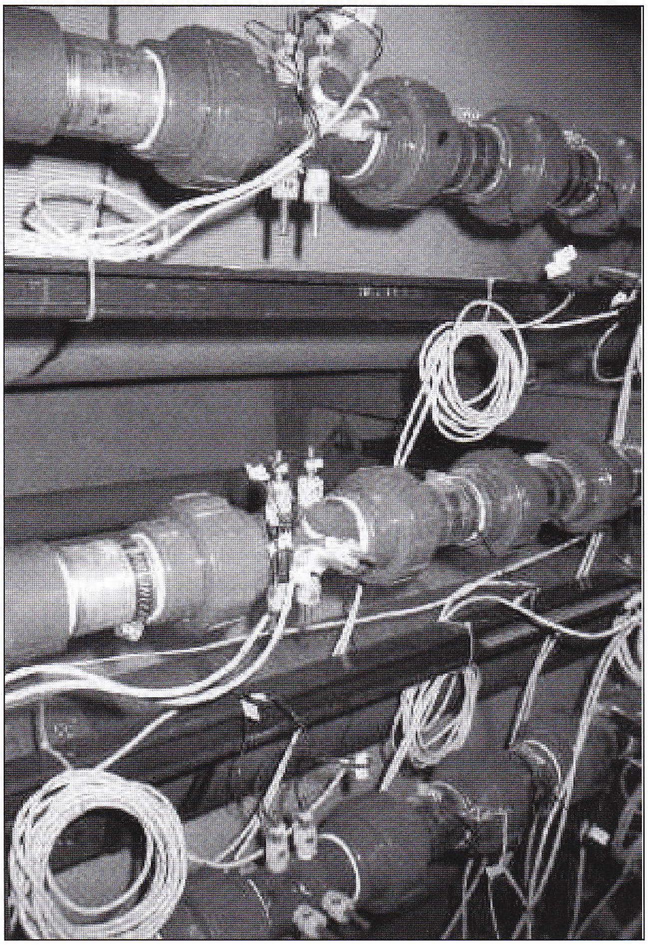

Fig. 6: Pipe specimens and cylindrical electrodes in the sidestream test loop in the UCI chilled water system. The three parallel branches of the loop allow simultaneous testing at three water flow rates.

Fig. 6: Campioni di tubi e di elettrodi cilindrici in un sistema di flussi paralleli ad acqua raffreddata UCI. Le tre linee parallele del circuito permettono l'analisi simultanea con tre diversi flussi.

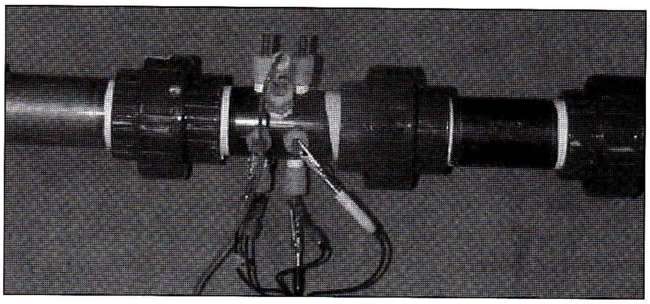

Fig. 7. Close-up of pipe specimens and small cylindrical electrodes in the flow loops at UCI and at Three Mile Island nuclear plant. Materials being tested include mild steel, type 316 stainless steel and brass. [Source of photo: Russ Green, GPU Nuclear Inc.]

Fig. 7. Immagine ravvicinata dei campioni tubi ed elettrodi cilindrici nel sistema a circuito UCI e nell'impianto nucleare di Three Mile island. Il materiale analizzato include acciaio dolce, acciaio inossidabile 316 e ottone. [Fonte dell'immagine: Russ Green, GPU Nuclear Inc.]

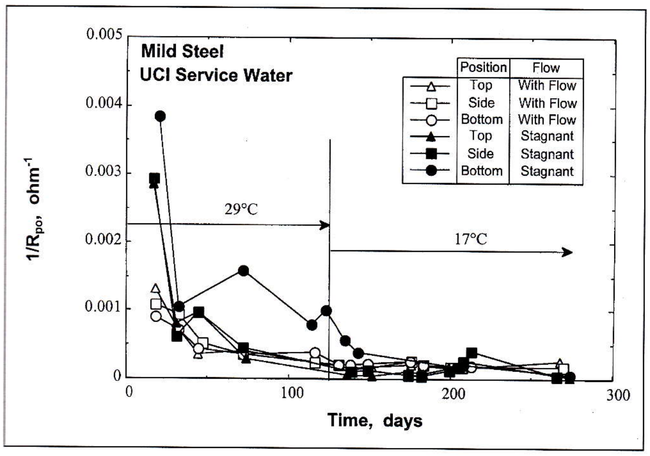

Fig. 8. Comparison of reciprocal pore resistance ($1/R_{po}$ values) for mild steel specimens in three orientations and at two flow rates (stagnant and 0.9 m/s) over a period of 250 days in the transportable sidestream system. The temperature was lowered from 29°C to 17°C after 125 days.

Fig. 8. Confronto fra le relative resistenze dei pori (valori 1/Rpo) per campioni di acciaio dolce con tre orientamenti e due tassi di flusso (stagnante e 0.9 m/s) durante un periodo di 250 giorni nel sistema a circuito parallelo trasportabile. La temperatura è stata abbassata da 29°C a 17°C dopo 125 giorni.

measure of the area of the "pores" or less resistive areas in the surface film that formed during exposure to the inhibitor-containing water.

Concurrent with the development of the field test flow loops, work is being conducted at five power plants to characterize the bacteria and biofilms that occur naturally in service waters and fire protection systems. After these systems are characterized, naturally occurring bacteria will be identified that are predisposed to providing corrosion protection to the materials exposed to those waters. These bacteria will then be genetically engineered to improve their natural corrosion protection tendencies or to create anti-corrosion properties not possessed by unaltered versions of those strains. The modified bacteria will be introduced into the sidestream test loops, and their impact on corrosion rate and type will be monitored and compared with baseline data that were generated before the introduction of the engineered bacteria. The eventual aim is to apply the successful CCURB technology to the actual (full-scale) service water or fire protection system.

CONCLUSIONS

This work has shown for the first time that control of SRB is possible by adding strains of bacteria that secrete peptides, a new class of environmentally benign antimicrobials for this application. Several classes of alloys, such as mild steel, stainless steel, brass and aluminum, have been shown to be protected by biofilms. Of particular note is the discovery that pitting corrosion (of aluminum) can be inhibited by a biofilm. Work is continuing in the laboratory to investigate other bacteria-based approaches to corrosion control.

Field studies are underway to determine whether this technology can be transferred from the very controlled conditions of a laboratory to real-world conditions. If this study demonstrates that bacteria can indeed control corrosion under field conditions, the future of this technology is potentially enormous. For instance, the electric power utility industry alone could realize huge cost savings if it could eliminate the need for chemicals added to service water and fire protection systems for corrosion control. Furthermore, industries outside power generation could use a similar bacteria-based approach to corrosion control and also realize huge cost savings.

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ABSTRACT

CONTROLLO DELLA CORROSIONE MEDIANTE BIOFILM RIGENERATIVI (CCURB). UN AGGIORNAMENTO

Il controllo della corrosione mediante biofilm rigenerativi (Corrosion Control Using Regenerative Biofilm o CCURB), che prevede la formazione di film superficiali protettivi per mezzo di batteri, costituisce un argomento che soltanto recentemente è stato studiato in modo significativo. Un vantaggio di questi biofilm consiste nel fatto che essi si auto-riparano quando danneggiati. In test di laboratorio condotti dall'EPRI, sono stati sviluppati batteri aerobici, prodotti geneticamente, in grado di formare biofilm protettivi sulle leghe impiegate nelle centrali elettriche; essi portano a una diminuzione del tasso di corrosione di 30 volte. I dati indicano che il motivo principale della riduzione del tasso di corrosione è dovuta al fatto che i batteri aerobici presenti alla superficie del biofilm consumano l'ossigeno contenuto nell' acqua prima che questo possa raggiungere la superficie del metallo e partecipare alla reazione di corrosione.

Lo svantaggio di tale sistema consiste nel potenziale sviluppo di uno strato anaerobico all' interfaccia di metallo/biofilm in cui i batteri anaerobici deleteri, quali i batteri che riducono il solfato (SRB) tendono a prosperare. Tuttavia, ulteriori dati hanno indicato che i batteri aerobici benefici possono essere alterati geneticamente al fine di produrre e rilasciare complessi antimicrobici che eliminano gli SRB. Il CCURB è possibile, quindi, anche in presenza di tali batteri anaerobici deleteri.

Numerosi test in campo sono attualmente in corso contemporaneamente presso diversi sistemi di centrali elettriche. I campioni di Biofilm provenienti da questi impianti sono stati esaminati per determinare la natura e la quantità di batteri generati naturalmente. Uno o più di questi batteri vengono scelti come "ospite" e verranno applicati per fornire protezione contro la corrosione a metalli e leghe nei sistemi di approvvigionamento d' acqua o linee antincendio. Nel futuro immediato, gli effetti dei batteri prodotti geneticamente saranno studiati in ambienti più controllati come possono essere ad esempio tubi a circuito chiuso e strumentati, paralleli alla rete idrica. I dati preliminari dei test in loco saranno presto disponibili.

La protezione offerta da questi batteri aerobici ha permesso di aumentare la vita dei componenti nelle centrali elettriche, nelle raffinerie ed in altri impianti industriali. I microbi protettivi hanno consentito di ridurre notevolmente l'impiego di inibitori di corrosione e biocidi, con un risparmio potenziale su base annua di miliardi di dollari oltretutto eliminando l' effetto di questi prodotti chimici sull' ambiente.