Corrosion inhibition in copper by isolated bacteria

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Abstract

Purpose – The purpose of this paper is to report a study of microbiological influenced corrosion (MIC) of copper due to bacteria strains isolated from potable water pipes and oxidation lagoons using electrochemical noise (EN) analysis and scanning electron microscopy (SEM).

Design/methodology/approach – Bacteria strains isolated from copper surfaces of potable water pipes and from oxidation lagoons were identified, based on the 16S rDNA gene sequence analysis. Corrosion studies were undertaken over a period of six weeks, placing copper electrodes inside an LB culture media with and without bacteria. The corrosion resistance was obtained using EN analysis. In all the cases, the corrosion type was identified. SEM images of the copper electrodes were taken to evaluate the surface condition.

Findings – The bacteria strains identified were: *Pantoea agglomerans*, *Alcaligenes faecalis*, *Bacillus cereus*, *Brucellaceae bacterium*, *Enterobacter cloacae*, *Delftia tsuruhatensis*, and *Pseudochrobactrum asaccharolyticum*. EN analysis gave noise resistance values in the range 1,036-5,040 Ωcm^2 for the control samples and in the range of 2,336-22,573 Ωcm^2 for samples that had been inoculated with bacteria. It was found that a decrease in the rate of corrosion took place due to the development of a biofilm by the microorganisms on the copper surface. SEM images corroborated the presence of the biofilm on the copper electrodes.

Practical implications – The isolated bacteria strain reduced the rate of corrosion on the copper electrodes, as shown by the SEM images and EN analysis results, due to the formation of a biofilm that can act as an anticorrosive coating.

Originality/value – Even though MIC is a known phenomenon, it has not been reported that isolated bacteria strains can reduce corrosion on the surface of copper potable water pipes and in oxidation lagoons.

Keywords Corrosion inhibitors, Electrodes, Copper, Pipes, Bacteria, Microbiology, MIC, Copper corrosion, Electrochemical noise, SEM, Biofilms

Paper type Research paper

1. Introduction

Corrosion can be defined as the chemical (or electrochemical) reaction between a material, usually a metal, and its environment, which leads to a change of the characteristics of the metal, that produces substantial impairment of the function of the metal, in accordance with the ISO standard 8044 (ISO 8044, 1999). Corrosion is a spontaneous phenomenon that takes place on the majority of metallic materials. It constitutes an ancient and difficult industrial problem, being the main cause of failure of metallic structures and objects (Askeland, 2004). The amount of money spent to prevent and repair structures attacked by corrosion is substantial, and in industrialized countries normally amounts to between 3 and 5 percent of the PIB (Uhlig, 2000). In particular, in the USA the direct cost of corrosion damage was almost $276 billion (Report FHWA-RD-01-156, 2001).

Thus, corrosion is a fundamental problem in material science and its characterization (e.g. uniform, pitting, crevice, etc.) is a transcendental problem (Kelly *et al.*, 2007; Winston and Uhlig, 2008). Corrosion type can be identified by visual inspection or microscopic techniques (scanning electron microscopy (SEM), TEM, etc.) but this gives only a qualitative approach (Winston and Uhlig, 2008). To get a quantitative analysis, the rate of corrosion is calculated, which is reported in terms of the average depth of penetration of corrosion per unit time and is often quoted in millimeters per year, mm/year (Uhlig, 2000). By measuring the corrosion current and voltage, it is possible to evaluate in real time the type and the initiation point of the corrosion attack, the corrosion rate and any transition from a localized to a generalized corrosion process (Kelly *et al.*, 2007). To measure it, the spontaneous fluctuations in potential

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(electrochemical potential noise) and current (electrochemical current noise) that can be observed on a pair of electrically coupled corroding specimens are monitored, and it is these fluctuations that are known collectively as electrochemical noise (EN) (Brusamarello *et al.*, 2000; García-Hernández *et al.*, 2008, 2009; Iverson, 1968; Mansfeld and Xiao, 1993; Stern and Geary, 1957; Tan, 2005; Tyagai, 1971). EN data can be analyzed through statistical and Fast Fourier Transform algorithms that estimate its power spectral density (PSD) in the frequency domain (Cottis, 2001; Eden and Rothwell, 1992). EN also can be used to help recognize the different types of corrosion (Legat and Dolecek, 1995).

Biocorrosion, also called microbially influenced corrosion (MIC), is an electrochemical process due to microorganisms that initiate, promote, enhance and/or accelerate the corrosion reaction (Videla, 1986; Javaherdashti, 1999; Parande *et al.*, 2005). When considering biocorrosion it is important to consider environmental conditions (abiotic factors) like temperature, pH, and chemical composition. Temperature changes the growth and health of the microorganisms; in particular, higher temperatures modify the enzymatic reactions of cellular processes such as the rate and speed of propagation. Moreover, proteins, nucleic acids, and other cellular components are sensible and can be irreversibly deactivated by changes in the temperature. Another important factor is the pH, which affects the cellular components sensitive to acidic and basic pH; for example, DNA and many proteins can be destroyed by an acid pH, while the RNA and the phospholipids react with an alkaline pH. On the other hand, changes in the chemical composition of the environment can promote corrosion. For example, oxygen is capable of forming different toxic substances even when it is the vital substance for breathing organisms. MIC also has been characterized using EN techniques (Dubey and Upadhyay, 1999) and EPS (Stadler *et al.*, 2008).

The bacterial attack to a surface is the first step in the formation of a bacterial biofilm (Jayaraman *et al.*, 1998, 1999; Zuo, 2007). After bacteria adhere to the surface, they multiply and form micro-colonies. However, this bond is weak and little organized, and it requires intercellular adhesion (Mack *et al.*, 1996). Adhesion of the microorganisms to the substrate is due to membrane proteins codified by specific genes of the bacterial chromosome, like at 1E. The biofilm formation requires that the microorganism grows in an homogeneous population and the need of the biochemical processes to accumulate exopolysaccharide has been established (Fernández-Delgadillo *et al.*, 2003). Biofilms modify the interaction between the metal surface and the environment, in particular protecting the surface from biocorrosion as is shown by *Pseudomonas fragi*, *Escherichia coli* and *Bacillus brevis* (Jayaraman *et al.*, 1998, 1999) or by *Actinomyces* (Valdez *et al.*, 2008). Changes at the interface due to the colonization of the microorganisms produce oxygen concentrations cells, and in consequence the surface under the colonies is anodic, whereas the non-affected areas are cathodic (Fernández-Delgadillo *et al.*, 2003). Microorganism colonies reduce the oxygen diffusion speed to the metal and, if the oxygen diffuses through the colony, the organisms may consume it. The concentration cell produces corrosion underneath the colonies. This phenomenon is responsible for corrosion of the metal, and may generate colony accumulations that can be responsible for the obstruction of tubes such as water pipes in nuclear reactors, submarines, chemical reactors or domestic waste disposal systems (Askeland, 2004; Marcó, 2004).

The goal of the present work was to determine the influence of bacterial organisms on the corrosion in copper electrodes and to find out if a biofilm can be formed that can induce or inhibit corrosion. To do this, isolated bacteria strains from copper surfaces of potable water pipes and from oxidation lagoons were evaluated using EN and SEM.

2. Materials and methods

The isolation of copper microorganisms was achieved on green copper surfaces of potable water pipes and from an oxidation lagoon. To obtain the microorganism from the metal surface, the pipe was scratched with a dissection needle and the isolated porous coat was placed in tubes of 1.5-ml diameter. In the laboratory, the samples were hydrated in an 8 percent NaCl buffer and were homogenized in a shaker for 20 min. Then, 100 μl of this homogenized mixture was placed in a R2A medium and incubated at 25°C for 24 h. Three replicate tests were prepared per sample (Greicy and Gaylarde, 2005). In order to obtain axenic strains, the colonies were placed in a new medium.

2.1 Strain identification

To make the bacteria genus identification from the isolated strain that showed the capability to reduce Cu^{+2}, an amplification of the gene 16S rDNA was made for each of them. DNA used as template for PCR was isolated and purified according to Sambrook *et al.* (1989). The oligos used for such amplifications were (Invitrogen) (Uhlig, 2000):

FD1:5′ – CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG

RD1:5′ – CCC GGG ATC CAA GCT TAA GGA GGT GAT CCA GCC

with an amplification of the gene segment of 1,500 bp. The PCR reaction was developed in a total volume of 30 μl using the mixture Platinum PCR Supermix High Fidelity (Invitrogen) and 1 μl of each primer FD1 and RD1 (10 p moles). The template DNA was prepared using a representative sample of the isolated strain to identify it, again suspended in 50 μl of sterile distilled water, after which it was shaken for 1 min in a vortex. Then, 1 μl of the bacteria suspension was taken for each PCR reaction.

The program was undertaken in a C1000 Thermal Cycler Bio-Rad under the following conditions: 94°C for 2 min, 30 cycles of 94°C for 30 s, 45°C for 40 s, 72°C for 2 min, and a final extension of 5 min at 72°C.

For the PCR product purification, a DNA Clean Concentrator-5 kit from Zymo Research was used. The purified product was sent to the Sequenciation Laboratory of the CINVESTAV, Irapuato, Mexico. The obtained sequences were compared with the database of the Ribosomal Database Project (RDP) in the classifier section (Wang *et al.*, 2007) with the goal to determine the bacteria genus corresponding to the sequences. Moreover, the sequences were compared with the data bank of the NCBI using the Blast program (http://ncbi.nlm.nih.gov/BLAST/) (Zhang *et al.*, 2000) looking for homology with the reported sequences of known organisms. From the database of the GenBank (Benson *et al.*, 2008), the complete sequence of the 16S ribosomal gene for the RDP identified genus was obtained (Weisburg *et al.*, 1991). The sequences of the obtained isolated strain, together with the sequences of the 16S genes

of the complete genome, were multiple-aligned using the ClustalX2 method (Larkin *et al.*, 2007).

2.2 Corrosion reaction evolution stimulated by the bacteria

The EN technique was used to measure the corrosion activity stimulated by the microorganisms. The EN signal was obtained through a single channel potentiometer "ACM Instruments" model Gill AC, which included the software for different electrochemical techniques, and a power booster interface that gave a maximum current of ± 2 A and a maximum voltage of ± 20 V. The electrodes were made of 1/4″ diameter rod and a 6″ length of copper in accordance to the preparation suggested by García-Hernández *et al.* (2009). The three electrodes were mechanically supported by No. 12 gauge electrical wire. A hole was drilled at one of the ends in order to connect them to the potentiostat. All of the samples were polished using sand paper of 250, 500, 1000 and 1500 grade, especially the free end. The electrodes were mounted on the electrode holder and the free ends were polished using alumina powder (0.3 μm) until they had a mirror finish. The electrodes were cleaned with ethanol and sterilized for 3 h at a pressure of £15 per square inch. They were then supported in an Erlen Meyer flask in a Luria broth (LB) and sterilized again for 2 h. To make the inoculate preparation, bacteria were isolated in an R2A culture medium and incubated at 40°C for 24 h. Inoculation into the flask was made with colonies of each axenic culture during six weeks at 40°C. They were three repetitions for the control test and the inoculated test. Each week, corrosion measurements were taken by EN and from these the corrosion resistance (resistance noise) values were evaluated.

To evaluate the corrosion resistance noise value (R_n) (Brusamarello *et al.*, 2000) the following expression was used:

$$R_n = \frac{\sigma_V}{\sigma_I} \quad (1)$$

where σ_V is the standard deviation of the EN potential signal and σ_I is the standard deviation of the EN current signal.

2.3 Scanning electron microscopy

Optical characterization was undertaken each month using SEM with a JEOL JSM-606 LV microscope. The samples were processed in accordance with the SEM laboratory protocol of Centro de Física Aplicada y Tecnología Avanzada of Universidad Nacional Autónoma de México Campus Juriquilla. The electrode probes of 1 cm were fixed in a 25 percent glutaraldehide solution for 1 h. Water removal was achieved through series alcohol dilution (80, 90 and 100 percent) followed by xylene; xylene removal was through free air drying.

3. Results

Microorganism isolation was undertaken at five sampling locations; one was an oxidation lagoon that had in its surface brown yellowish mucilage. The other four were in copper potable water pipes with a blue greenish surface, with easy scab detachment.

By means of the gene 16S sequencing technique for strain identification, seven bacteria strains were identified: *Pantoea agglomerans*, *Alcaligenes faecalis*, *Bacillus cereus*, *Brucellaceae bacterium*, *Enterobacter cloacae*, *Delftia tsuruhatensis*, and *Pseudochrobactrum asaccharolyticum*. These bacteria strains have been deposited and are available at the Microbiology Laboratory of the Natural Science Faculty of Universidad Autónoma de Querétaro, México, under the identification numbers presented in Table I, where also is shown the BLAST of the strain identification for all of the samples studied, their origin, and the percentage to the closest relative of the isolated strain. The genetic sequences for each strain were determined. All of the strains had high similarity (from 97 up to 98 percent) with their closest identified strain. *Pantoea agglomerans*, *Bacillus cereus*, and *Pseudochrobactrum asaccharolyticum* were isolated from the potable water pipes, while *Alcaligenes faecalis*, *Brucellaceae bacterium*, *Enterobacter cloacae*, and *Delftia tsuruhatensis* were isolated from the oxidation lagoons.

In order to check the resistance of the isolated strains to Cu^{+2}, a test was performed that consisted of growing them inside a rich media (LB) containing from 0.05 up to 0.25 μg/l of $CuSO_4$. The results showed that all of the strains were capable of growth in the whole range of media.

EN current measurements of the copper electrode corrosion for a mixture of bacteria strains and control samples (without bacteria) are shown in Figure 1. The initial values of current (week 1) were small in both cases (almost zero), with a little higher current in the control sample. After incubation progressed over several weeks, the current increased. The control sample presented larger changes in current value, indicating that the control electrodes suffered greater corrosion than did electrodes exposed to bacteria.

EN potential measurements of the copper electrode corrosion with a mixture of bacteria strains and control (without bacteria) are shown in Figure 2. The initial values of potential were small (see week 1), but then the potential difference increased substantially (by orders of magnitude) with longer time of exposure.

Using equation (1), the corrosion resistance, R_n, was evaluated as a function of time (Figure 3). From these values, the rate of corrosion was evaluated using the Stern and Geary equation [24]. The results are shown in Figure 3.

The power spectrum densities (PSD) of potential noise and current noise could be interpolated by a power function $f^{-\alpha}$ function (a straight line with a slope $-\alpha$ on a log vs log scale). The parameter α sometimes can be used to distinguish between the different types of corrosion (Brusamarello *et al.*, 2000; Legat and Dolecek, 1995). In uniform corrosion, the slope of the potential power spectrum density is close to zero (α from -2 to 7 dB V/decade), whereas in localized corrosion the values of the parameter α generally are much higher (from 20 to 30 dB V/decade). In mixed corrosion, the value of α is intermediate (from 10 to 15 dB V/decade). From the PSD analysis of the present samples (graphs shown in Figure 4), an indication of mixed corrosion was obtained in the control sample during first week ($\alpha = 16.7$) and this change to localized corrosion in the control samples (α between 20 and 30) according to the Legat classification (Legat and Dolecek, 1995), whereas in the bacteria strain samples it localized corrosion always was observed (α between 27 and 28). These results are presented in Table II, as are the statistical parameters of the EN measurements for the control and the bacteria strain samples for each week of observation.

Figure 5 shows the SEM images of bacteria adhering to the copper electrode. This confirmed the formation of a biofilm on the substrate surface. It was observed that the film

Table I Bacillus strain identification based on the 16S gene sequence analysis

Source/strain identification	Blast	Similarity (%)	GenBank 16S gene accession no.
Oxidation lagoon/CA 100	*Alcaligenes faecalis* strain WM2072	98	AY548384
Potable water tap/CA 101	*Bacillus cereus* strain INRA C43	97	AM747221
Oxidation lagoon/CA 102	*Brucellaceae bacterium* 47211606	97	AY353698
Oxidation lagoon/CA 103	*Delftia tsuruhatensis* strain BM90	97	EU779949.1
Oxidation lagoon/CA 104	*Enterobacter cloacae* strain XJU-PA-7	97	EU733519
Potable water pipe/CA 105	*Pantoea agglomerans* strain GIST-CPs11	98	EF428997
Potable water tap/CA 106	*Pseudochrobactrum asaccharolyticum* strain CCUG 46016	97	AM180485.1

Figure 1 Current EN of copper electrodes isolated, control samples (upper) and under bacteria strain (lower) in weeks 1, 3, and 6

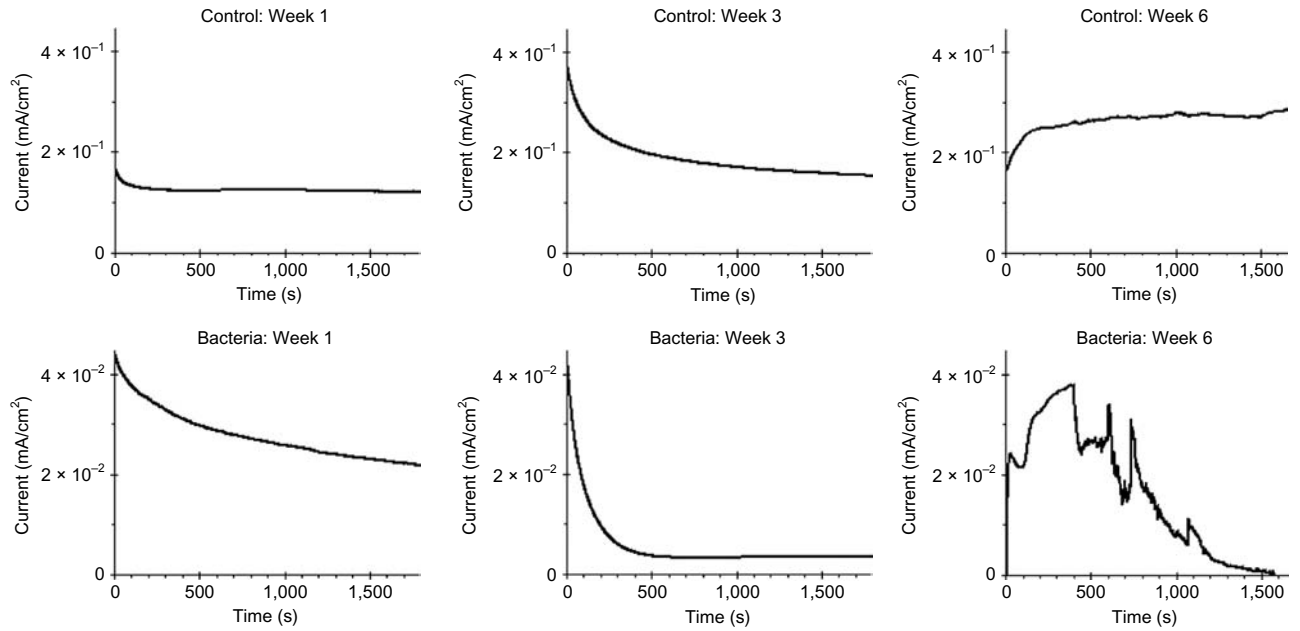


Figure 2 Potential EN of copper electrodes isolated, control samples (upper) and under bacteria strain (lower) in weeks 1, 3, and 6

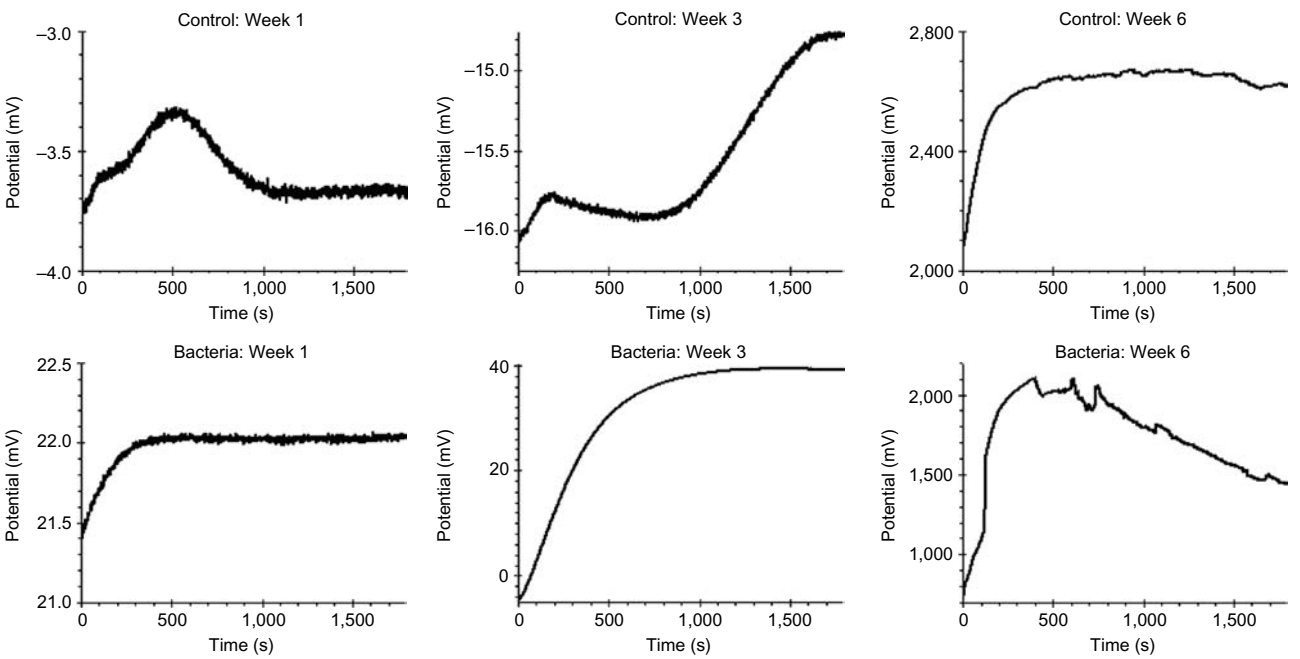


Figure 3 Corrosion noise resistance, R_n, in Ω cm^2 (left) and corrosion rate in mm/year (right) as function of time (in weeks) for control and under bacteria strain electrodes, obtained from EN data

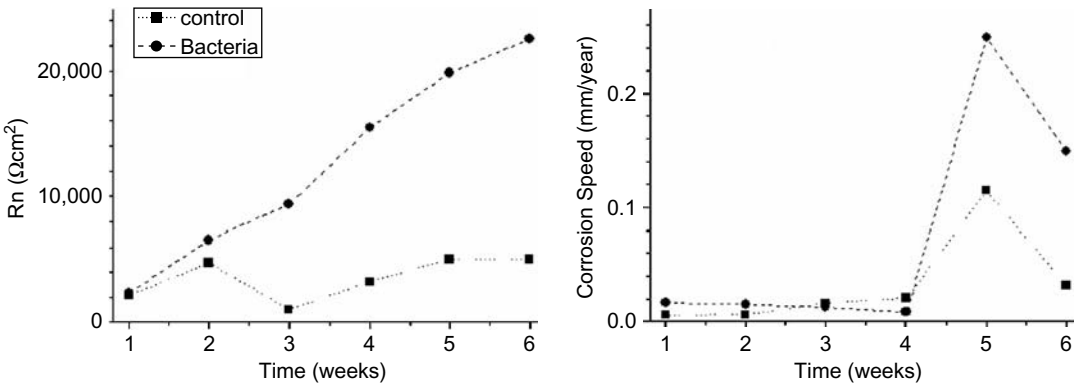


Figure 4 PSD plots (in dB) as function of the log of frequency (Hz) for control (upper) and under bacteria strain (lower) electrodes for weeks 1, 3, and 6

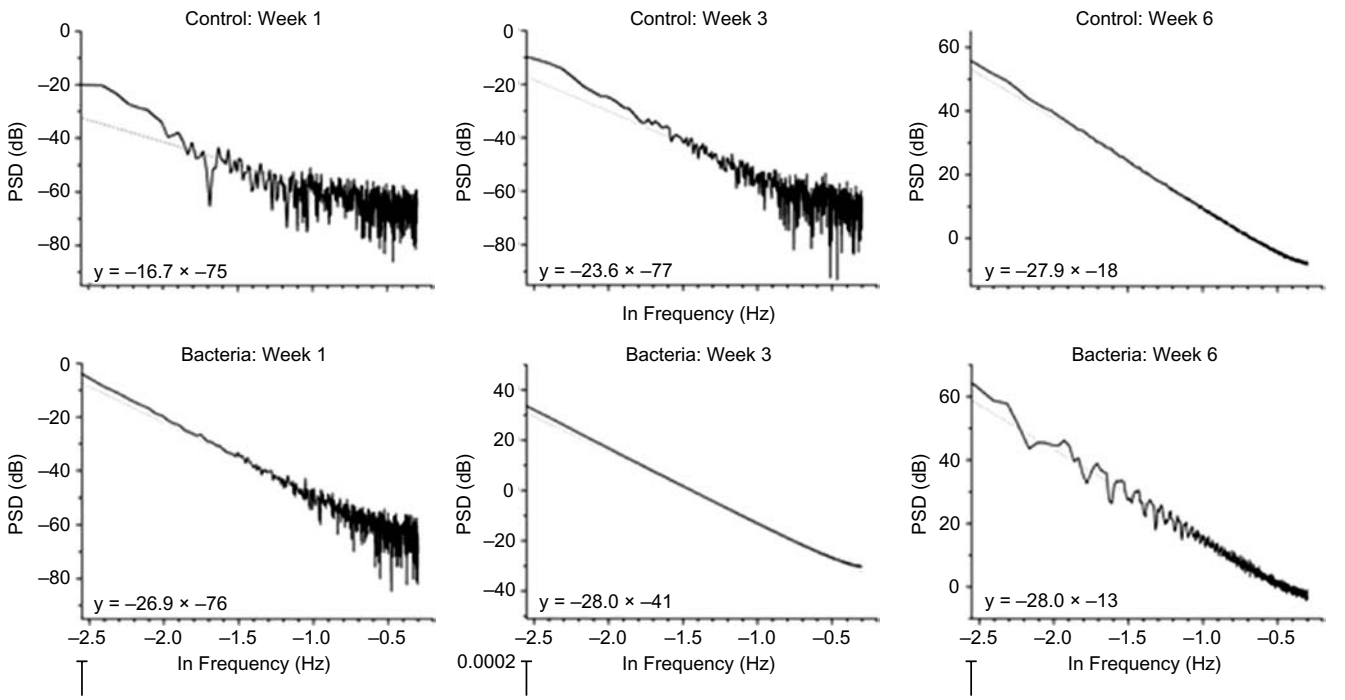
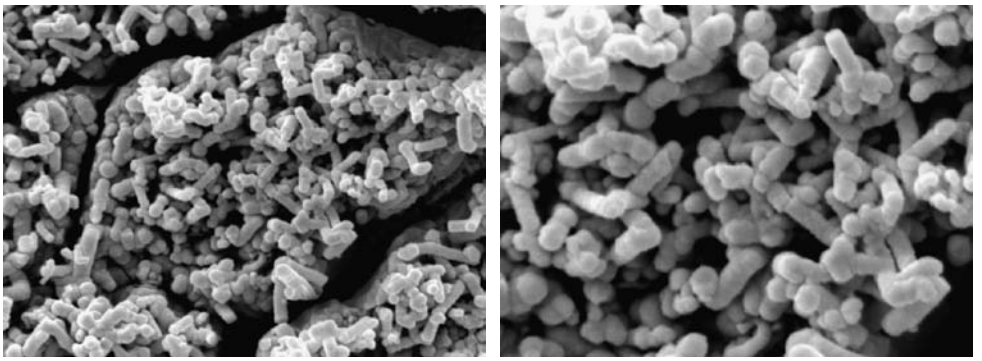


Table II EN results

Sample (week)	α (from PSD)	Corrosion rate (mm/year)	R_n (σ_v/σ_I)	Skew (potential)	Kurtosis (potential)	Corrosion type
Control (1)	16.7	0.059	2,149	0.89	0.63	Localized (almost mixed)
Control (2)	20.8	0.006	4,740	1.23	0.31	Localized
Control (3)	23.6	0.016	1,036	0.81	0.96	Localized
Control (4)	27.9	0.021	3,236	0.83	0.40	Localized
Control (5)	27.9	0.115	5,040	2.56	8.05	Localized
Control (6)	28.0	0.032	5,038	3.26	11.00	Localized
Bacteria (1)	26.9	0.017	2,336	2.99	8.59	Localized
Bacteria (2)	27.0	0.015	6,547	1.27	0.79	Localized
Bacteria (3)	28.0	0.013	9,393	1.63	1.47	Localized
Bacteria (4)	27.8	0.008	15,460	0.84	0.095	Localized
Bacteria (5)	27.7	0.249	19,840	0.14	1.08	Localized
Bacteria (6)	28.0	0.149	22,573	1.18	1.42	Localized

Figure 5 SEM images showing the adhesion of the bacteria strain to the copper electrode at (a) 5,000X and (b) 10,000X



(a) **(b)**

consisted of a homogeneous bacteria population fixed with exopolysacharids.

4. Discussion

The corrosion resistance noise values obtained were high, indicating that the bacteria strain protected the electrodes from corrosion. The α parameters obtained indicated that the bacteria strain prevented transition of the corrosion morphology to generalized attack because the values of PSD implied more localized corrosion with extended time of exposure.

The coexistence of different strains of microorganism in this specific environment leads to the formation of mixed culture biofilms. Additionally, these ecosystems also provide an ideal environment for microbial residents to coordinate metabolic activities and share tent5Sic elements encoding beneficial traits with each other. This might explain the production of exopolysaccharides within the biofilm, resulting in the observed protection of the copper against surface corrosion. In the past, this has been considered as a corrosion inhibition mechanism (Jayaraman *et al.*, 1998, 1999; Zuo, 2007).

It must be realized that population analysis based on culture-dependent techniques usually underestimates the actual microbial heterogeneity (von Wintzingerode *et al.*, 2002). However, in the present study, even if some microorganisms could not be recovered from the ecosystem by conventional culturing methods, their DNA was successfully extracted and amplified. Thus, the microorganism's unique DNA sequences in the clone libraries truly represented their presence in the ecosystem and enabled apparently dissimilar bacterial genes to be detected, organized within a biofilm community.

Horizontal gene transfer events are known occur in any natural ecosystems and are likely to be more efficient in biofilms than in suspended cells (Molin and Talker-Nielsen, 2003). Therefore, cells in biofilm communities may be able to adapt to the specific environment despite initially unfavorable conditions, forming a heterogeneous population.

The structure of the biofilms, when exposed to flowing liquids, has been described by a heterogeneous model in which the microorganisms form a dense, planar and homogeneous biofilm (Nyvad and Fejerskov, 1997). The present results, as summarized in Figure 5, clearly describe such a structure.

5. Conclusion

In this investigation, MIC of copper due to the presence of bacteria strains isolated from potable water pipes and oxidation lagoons were studied using EN and SEM. Using 16S rDNA gene sequence analysis for bacterial strain identification in biofilms on the surfaces of copper potable water pipes and from oxidation lagoons, seven bacteria strains were found: *Pantoea agglomerans*, *Alcaligenes faecalis*, *Bacillus cereus*, *Brucellaceae bacterium*, *Enterobacter cloacae*, *Delftia tsuruhatensis*, and *Pseudochrobactrum asaccharolyticum*. Corrosion studies were undertaken over a period of six weeks, during which copper electrodes were placed inside a LB culture media with and without bacteria. It was concluded that bacteria strains isolated from the surfaces of the copper electrodes prevented corrosion, as demonstrated by the SEM examinations and EN analyses, due to the formation of a biofilm that can act as an anticorrosive coating. This biofilm maybe is provoked by the production of the exopolysacharides from the bacteria strain that restricts the corrosion activity because it prevents interaction between the metallic surface and the surrounding environment.

It should be noted that these findings from relatively short-term tests should be treated with caution as it is known that bacterial infestations have resulted in substantial damage in copper potable water piping. Quite apart from any possible health risk associated with the presence of bacterial growths in potable water systems, it would be risky to hope that the presence of biofilms on the internal surfaces of copper piping might provide any degree of reliable long-term corrosion protection.

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