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Electrochemical Techniques as a Tool for the Evaluation of Biocide Effect upon Biofilm Generating Bacteria.

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Abstract

The microbial induced corrosion (MIC) is a very dangerous process, which affects the oil industry, particularly the hydrocarbons extraction, transport and storage. The activity and microorganism growth at the pipelines steel cause surface modifications, which can induced a more complex corrosion process. The biocide evaluation for the MIC decreasing has been normally based upon microbiological tests, and just a few references mention alternating methods which can be used as criteria for their evaluation.

In this work, a commercial biocide was tested, using different electrochemical laboratory techniques, in order to determine its effect upon a biofilm generating bacteria consortium, obtained from the Atasta – Nohoch gas transporting pipeline, in the southeast of Mexico.

The scanning electron microscope observation of APIXL52 steel coupons, exposed to the consortium action, revealed bacteria presence, as well as a damaged steel surface. A type of localized corrosion was observed on the steel surface, and it was associated to the bacteria effect.

Using microbiological techniques, the biocide lethal concentration was determined, and a concentration of 100 ppm was used to kill completely the consortium population in both, plancktonic and sessile parts.

The electrochemical techniques: Polarisation Resistance, Electrochemical Impedance Spectroscopy and Electrochemical Noise, allowed describing the corrosion process associated to the microbial consortium and the biocide effect upon it.

Keywords: Microbial induced corrosion, biocide, electrochemical techniques, biofilm.

1.0 Introduction

The microbial induced corrosion (MIC) is a very dangerous process, which affects the oil industry, particularly during the hydrocarbon extraction, transport and storage. The activity and microorganisms growth at the pipelines steel cause surface modifications, which can induced a more complex corrosion process. In general, the study of the MIC and the biocide evaluation for corrosion decreasing, have been normally based upon microbiological tests and gravimetric techniques, and just a few references mention alternating methods, which can be used as criteria for their evaluation.

At the same time, most of the research on MIC has been done considering the use of isolated strain bacteria. However, this situation may be wrong, as in natural conditions the microorganisms are found in consortiums, and the corrosion processes could be different. Moreover, and according to the measurements taken on the laboratory, different strains on the system could lead to different responses to biocide effect, and a specific chemical compound may have different responses to those expected with an isolated strain.

The use of electrochemical techniques for the evaluation of CIM, as well as the biocide selection and evaluation, have been recently studied, and this is an interesting area which requires more research, in order to implement and standardize their laboratory experiments and field application.

The polarisation resistance (PR) is the most common electrochemical technique used to evaluate corrosion rates on metals surface. This evaluation allows measuring instantaneous corrosion rates, and its monitoring can be continuous. Therefore, the technique could be useful when detecting corrosion rate changes.

Besides the PR, there are other electrochemical techniques, such as the Electrochemical Impedance Spectroscopy (EIS) and Electrochemical Noise (EN), which also can be used to obtain information and explain the corrosion processes occurring on the metal surface.

The EIS technique, apart from the corrosion rate values, could provide information about the controlling corrosion process and film formation on the metal surface. The technique also allows the calculation of the system components, such as the solution resistance, charge transfer resistance and double layer capacitance, by means of their electric representation in an equivalent circuit.

Electrochemical Noise data can provide an indication of the type of corrosion damage that is occurring; it is widely used to distinguish between general and localized attack. This is an important advantage over other electrochemical techniques. Further fundamental advantages include the ability to monitor corrosion in low conductivity environments (for example, thin film condensation) and the absence of "artificial" polarization effects. Noise measurements are made in the completely "natural" (freely corroding) state.

Additionally to the studies obtained from the electrochemical techniques, it is important to complement the results with an efficient surface analysis technique, such as the scanning electron microscopy (SEM) and the atomic force microscopy (ATM), among others. This surface evaluation could provide information and physical evidence about the metal surface damage caused by the microorganisms involved in the corrosion process.

2.0 Experimental

To evaluate the biocide effect upon biofilm generating bacteria, using the electrochemical techniques: PR, EIS and EN, the following activities were carried out:

2.1 Sampling. The consortium used in this work was taken from a gas pipeline, during the inner cleaning procedure. The collected samples were inoculated in a selective medium, following the recommendations for field biological sampling, stated on API-RP 38, 1990.

2.2 Microorganisms cultivation. The microorganisms were kept in a Posgate-C medium (Ronald, 1995) under anaerobic conditions, which was obtained by bubbling nitrogen gas. This atmosphere was used in all the experimental work.

2.3 Evaluation of the consortium growth. The consortium was inoculated in vials with 100 ml Posgate C medium. API XL52 steel coupons (polished and degreased), were placed into the vials, which were incubated at room temperature. These vials were used as "sacrificial" to determine the consortium kinetics growth. This was done following the Most Probable Number (MPN) method.

The plancktonic and sessile microorganisms growth was determined separately. The plancktonic microorganisms were taken from the bulk solution. The steel coupon, with the sessile microorganisms, was placed into a vial containing 10 ml saline solution and solid-glass beads, in order to remove the bacteria from the metal surface when shaking the vial; 1 ml from this solution was used for the MPN method.

2.4 Corrosion rate determination using the gravimetric technique. This was done according to the ASTM standard G1-90: "Standard practice for preparing, cleaning and evaluating corrosion test specimens".

2.5 Surface analysis of the coupons exposed to microorganisms. The biofilm was fixed to the steel surface for 1 hour with a 2% glutaraldehído solution, and then become dehydrated using 4 ethanol solutions (15 15 minutes each): 25, 50, 75 and 100%, successively. After that, the samples were taken to the SEM for their analysis. To observe the surface corrosion damage, the biofilm and corrosion products were removed from the steel, and the samples were taken to the SEM.

2.6 Lethal biocide concentration. After the most probable number was determined for the plancktonic and sessile microorganisms, different biocide concentrations were added, in order to determine the lethal biocide concentration, according to Eagar et al, 1988.

2.7 Corrosion rate determination using electrochemical techniques. These experiments were carried out in a 1 l standard cell, as shown in Figure 1, with a three electrode system: API XL52 steel as working electrode, a graphite rod as auxiliary and a saturated calomel electrode as reference. The electrolyte used was 800 ml Posgate C medium. Nitrogen gas was bubbled to remove all the oxygen and maintain anaerobic conditions. The electrochemical cell was connected to an ACM 772 potentiostat, and a PC was used for data recording. All experiments lasted approximately 1 month, and during this time, several PR, EIS and EN tests were carried out, at least once a day.

Figure 1. Electrochemical cell used in the experimental

2.7.1 Polarisation Resistance technique. A potentiodynamic method was used to obtain the potential-current (E-I) ratio, applying a ± 10 mV overpotential, with respect to the free corrosion potential, Ecorr. The Sequencer® and Core running® software programs were used for data management, along with the V4 Analysis® software for data analysis.

2.7.2 Electrochemical Impedance Spectroscopy technique. These experiments were carried out considering a frequency range of 1000 – 0.1 Hz, and ± 10 mV signal amplitude, recording 40 points per test. Data recording was made using the Sequencer® and Core running® software programs, along with the Zview® software for data analysis.

2.7.3 Electrochemical Noise technique. To perform these experiments, three identical steel electrodes were considered, using one of them as reference electrode. Measurements were taken with the Sequencer® and Core running® software programs, and the EN Analyse® program was used to analyse all data. Each experiment lasted approximately 2000 seconds, taking one point every second.

2.8 Biocide effect upon the microbial induced corrosion using electrochemical techniques. To evaluate the biocide effect upon the MIC using electrochemical techniques, an experimental set up, identical to that described in section 2.7, was arranged. 15 days after the experiment was started, it was considered that a biofilm was formed and 200 ppm commercial biocide concentration was added to the cell. Several PR, EIS and EN tests were carried out, as described previously.

3. Results and discussion

3.1 Evaluation of the consortium growth. The consortium growth was determined for both, plancktonic and sessile microorganisms. This was done in order to establish the experiments length in presence of microorganisms. Figure 2 shows the population growth for plancktonic and sessile bacteria.

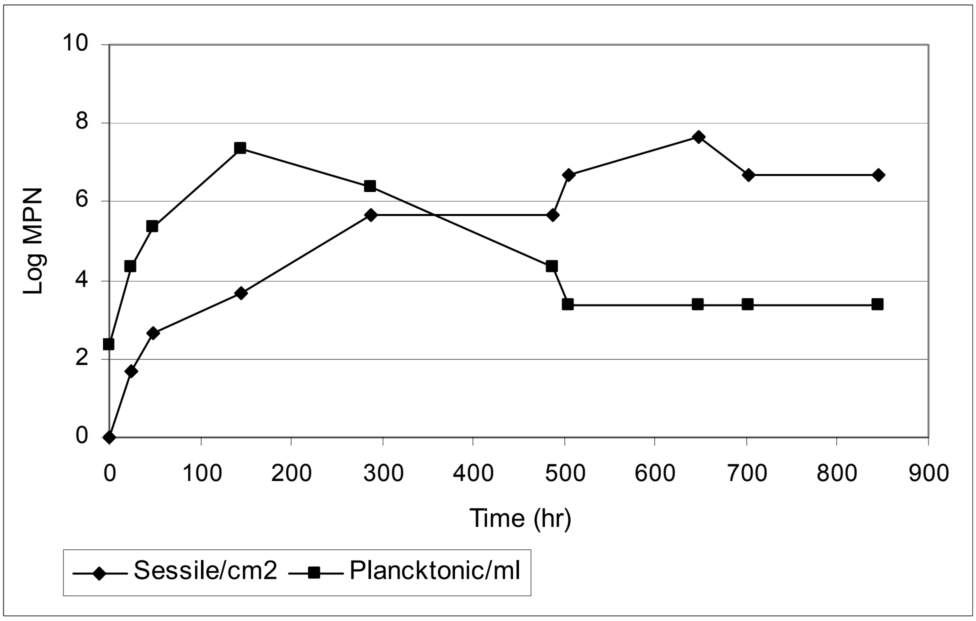

Figure 2. Population growth for sessile and plancktonic microorganisms. API XL52 steel in Posgate C medium.

As it can be seen in this Figure, the plancktonic bacteria did not show any "lag" phase, and there was an active growth up to 150 hr, reaching a population of about 10^7 bac ml^{-1}. After this point, the bacteria growth was stopped and the microorganisms number decreased until a steady state after 500 hr, with a plancktonic bacteria population around 10^3 bac ml^{-1}.

On the other hand, the sessile microorganisms population increased from the inoculation, but the growth was less accelerated than for the plancktonic bacteria. The maximum population on the coupons metal surface was found after 500 hr, and there were about 10^7 bac ml^{-1}. This value remained stable until the end of the experiments.

This behaviour is considered typical for sulfate reducing bacteria (SRB); the plancktonic microorganisms, when in contact with an enriched medium, increase their population until the environmental conditions become unfavourable (decreasing of nutriments, pH, etc.); for the sessile microorganisms, there is a continuous growth, which could be enhanced due to the conditions at the end of the kinetics (Tatnall & Pope, 1993).

There are some studies reported on the literature, but they just provide information about the plancktonic microorganisms growth (Mora Mendoza et al, 2003; Mendoza Flores et al, 2003). In these works, it is considered that the required time to observe damage on the metal surface depends on whether the plancktonic microorganisms reach a steady state, or a death stage. However, according to the results observed in this study (Figure 2), the above mentioned could not be right, since the sessile microorganisms need longer incubation time than the plancktonic bacteria, in order to reach their maximum growth. It is expected a relationship between the corrosion damage extent and the bacteria population on the metal surface. This situation indicates that the incubation time should be long enough to allow full sessile microorganisms evolution.

3.2 Corrosion rate determination using the gravimetric technique. The average corrosion rate obtained from the sterile conditions, using only the Posgate C medium, was 1.2 mpy. On the other hand, the average weight loss shown by the corrosion coupons exposed to the consortium action was 0.029 g, which corresponds to 2.97 mpy, assuming uniform corrosion. The differences between the coupons under sterile conditions and those exposed to the consortium, made evident that the microorganisms presence on the metal surface increased the corrosion rate.

This technique provides average corrosion rates in a specific time, and it is not possible to establish any relationship between the microorganisms growth and the corrosion rates. Therefore, the results obtained with this technique should be complemented with other techniques, in order to get all the required information to establish the corrosion process kinetics, when in presence of microorganisms.

3.3 Surface analysis of the coupons exposed to microorganisms. API XL52 steel samples were exposed to the consortium action, and then taken to the SEM. Due to the microbial action, the coupons were covered by a biofilm, which was thicker at the lower part of the sample, as seen in Figures 3 and 4. This behaviour was observed in most of the experiments, causing a pronounced effect on the corrosion occurring at the metal surface.

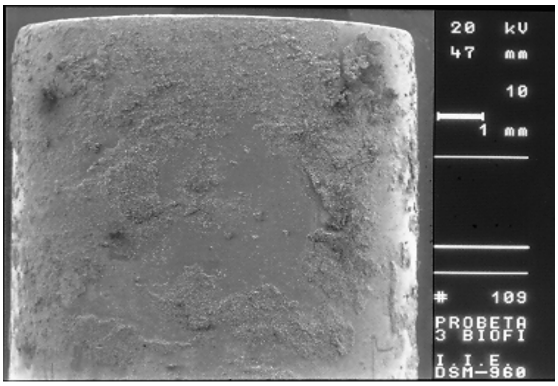

Figure 3. API XL52 steel samples, after being exposed to consortium action. Lateral side view

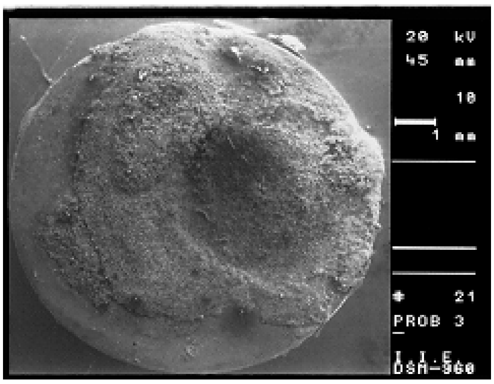

Figure 4. API XL52 steel samples, after being exposed to consortium action. Lower side view

The biofilm structure, shown in Figure 5, exhibited light and dark areas. During the SEM observation, a microanalysis of these areas was taken, finding that the dark areas were formed mainly by iron sulfide, which is a corrosion product, while the light areas were constituted, besides de corrosion products, by organic material. In Figures 6 and 7, some microorganisms immersed in the biofilm are presented, and approximately 2 µm bacillus shapes where observed.

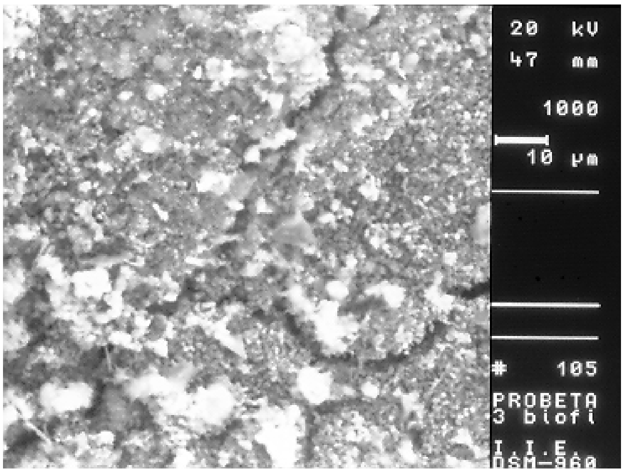

Figure 5. Biofilm formed on the metal surface after 1000 hours exposition to microbial consortium.

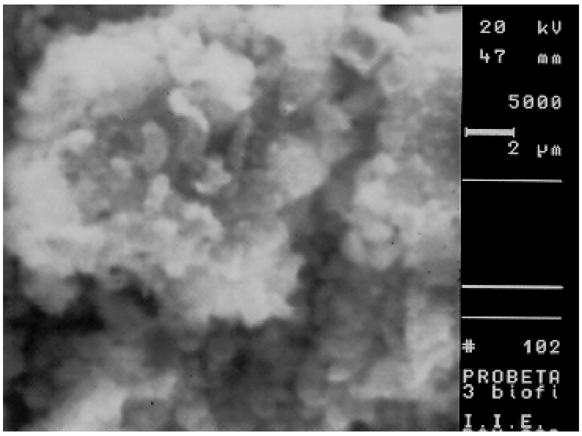

Figure 6. Microorganisms immersed in biofilm formed on the metal surface after 1000 hours exposition to microbial consortium.

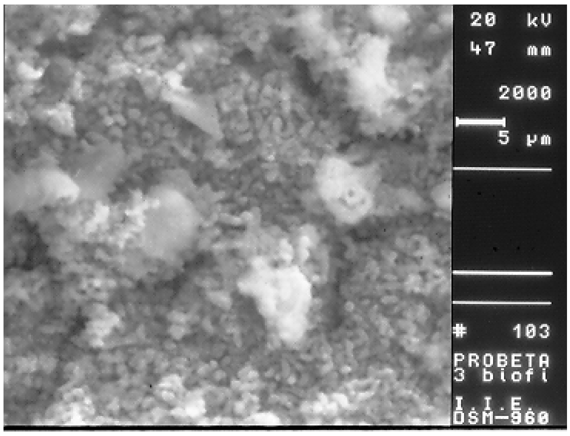

Figure 7. Microorganisms immersed in biofilm formed on the metal surface after 1000 hours exposition to microbial consortium.

3.4 Corrosion rate determination using electrochemical techniques. Three electrochemical techniques were used to evaluate de biocide effect upon the MIC processes: Polarization Resistance, PR, Electrochemical Impedance Spectroscopy, EIS, and Electrochemical Noise, EN.

At the beginning of this study, only the steel under sterile conditions, with no microorganisms into the solution, was considered in order to obtain initial reference values. The corrosion rates obtained with the three electrochemical techniques are presented in Figure 8. From this Figure, The behaviour observed was very similar when using the three techniques: values about 0.5 mpy were observed along the experiments, indicating low corrosion rate, and that under the conditions considered in this work, the posgate C medium is not aggressive to the metal surface.

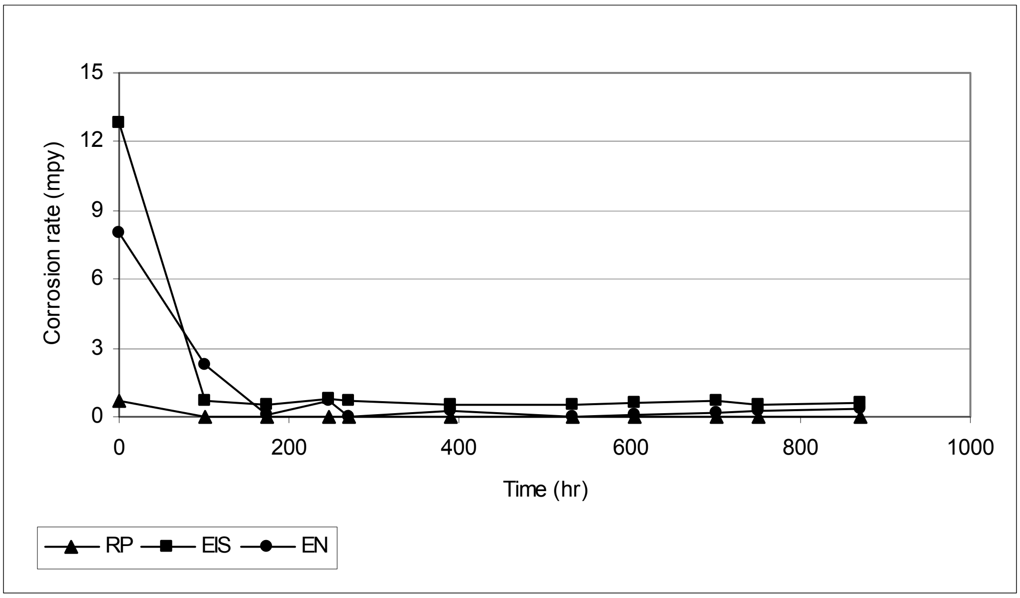

Figure 8. Corrosion rates with time, obtained under sterile conditions. API XL52 steel.

Regarding the biocide action, 100 ppm concentration was enough to kill both, sessile and plancktonic microorganisms. Some experiments considering only the biocide action, without any microorganism were performed, and the chemical compound was found to be no corrosive to the steel surface. Therefore, it was decided to add 200 ppm biocide concentration, in order to ensure the biocide action.

3.4.1 Polarisation Resistance technique. The corrosion rate values obtained when using the Polarisation Resistance technique, in presence of the microbial consortium and after the biocide was added are shown in Figure 9. From this graph, it is observed that initial corrosion rate values below 1 mpy were induced by the microbial consortium. After 200 hr of bacteria action during the experiments, the corrosion rate gradually increased until values about 20 mpy at approximately 500 hr. Later on, the biocide was added into the medium, decreasing the corrosion rate.

The above mentioned suggested that, for this system, de corrosion damage depends on the microorganisms population adhered to the metal surface. At the same time, when the microorganisms are killed due to the biocide action, there is a reduction on the corrosion rate values. However, some microbial metabolism products, which are aggressive to the steel, still remained on the metal surface. Therefore, even after the microorganisms are killed, the corrosion rate may be higher, compared to that observed at the beginning of the experiments.

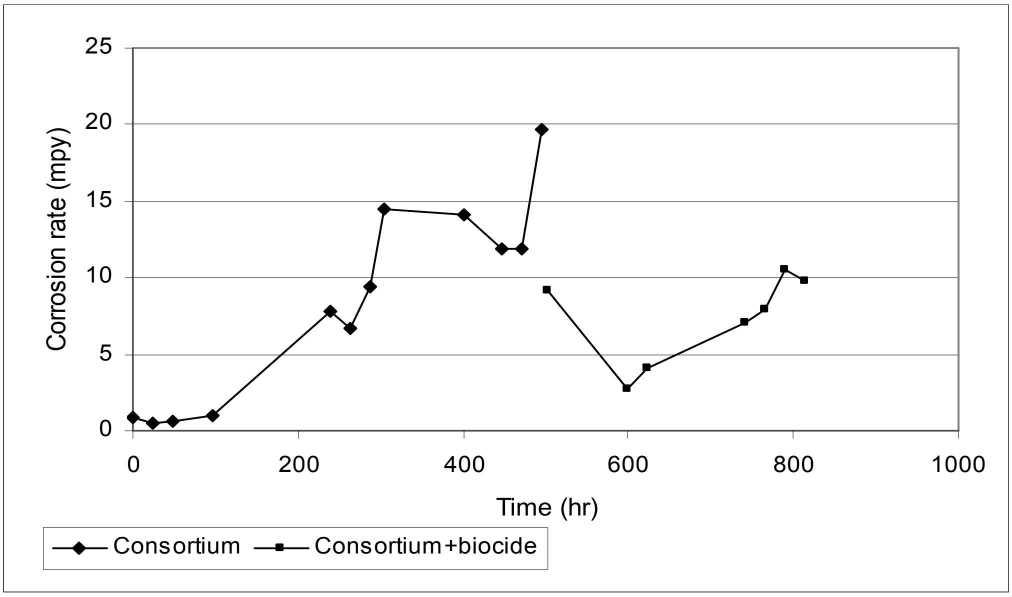

Figure 9. Corrosion rate values obtained with the Polarisation Resistance technique, in presence of the microbial consortium and after the biocide was added.

3.4.2 Electrochemical Impedance Spectroscopy technique. The corrosion rate values, obtained with the EIS technique, are shown in Figure 10, where initial corrosion rate values below 1 mpy were observed. As observed when using the PR technique, after 200 hr there was a gradual increment on the corrosion rate, up to around 12 mpy at 500 hr. When the biocide was added into the medium, the corrosion rate decreased in a similar way to that presented by the PR technique.

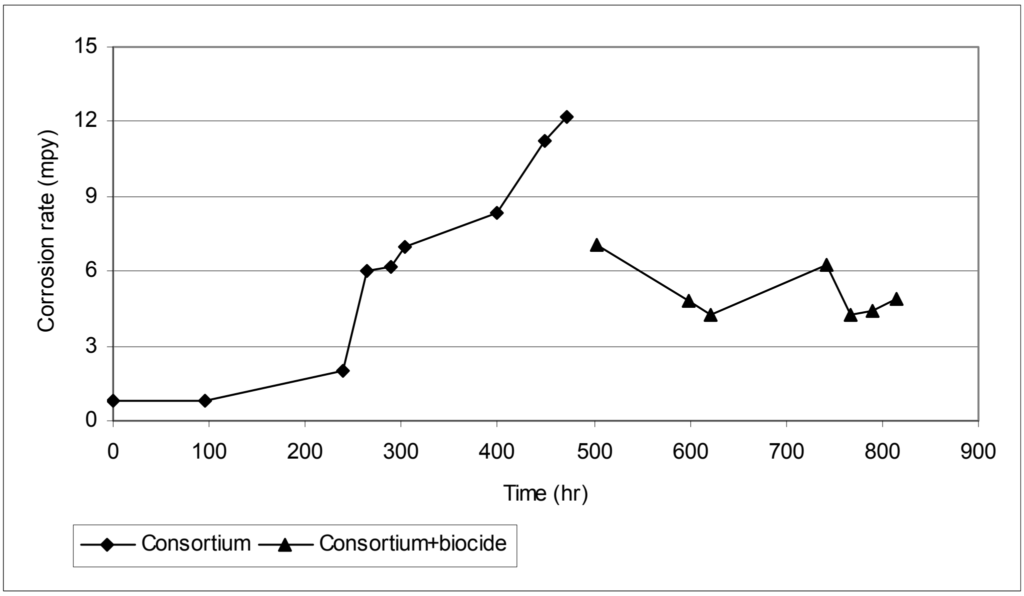

Figure 10. Corrosion rates obtained with the Electrochemical Impedance Spectroscopy technique, in presence of the microbial consortium and after the biocide addition.

3.4.3 Electrochemical Noise technique. The Electrochemical Noise measurements, presented in Figure 11, corroborate the behaviour shown by the other two techniques. Initially, values below 1 mpy were observed, and the corrosion rate increased above 12 mpy at 200 hr. Once the biocide was added, the corrosion rate values reported with this technique, decreased, showing the same behaviour, previously mentioned.

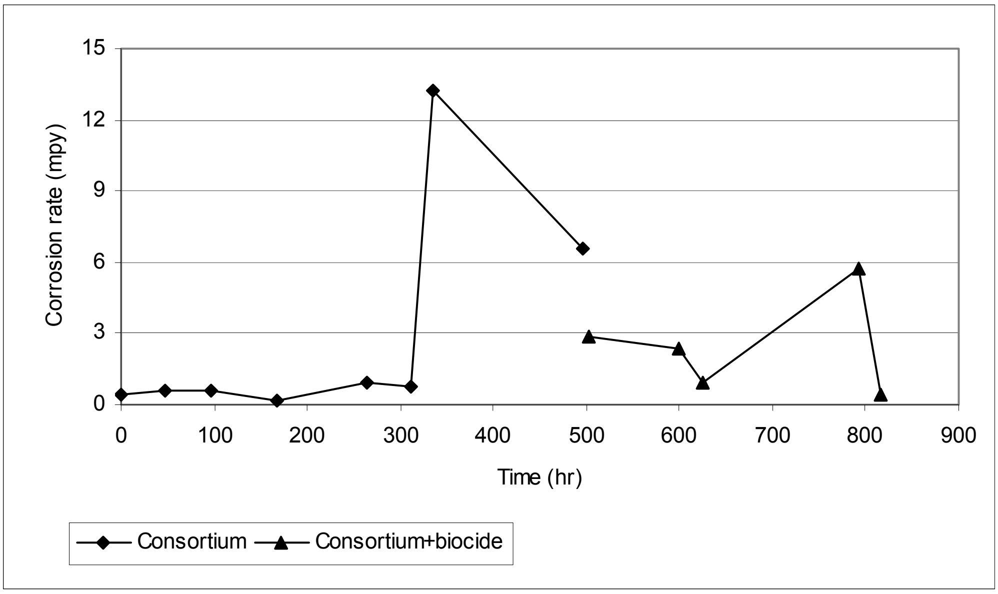

Figure 10. Corrosion rates obtained with the Electrochemical Noise technique, in presence of the microbial consortium and after the biocide addition.

The results obtained with the techniques: PR, EIS and EN, make evident the microbial consortium effect on the increment of corrosion rates for this system. At the same time, the biocide effect can be observed when, after the chemical addition, the steel corrosion rate decreased.

Taking in account this information, it is possible to conclude that the three techniques can be considered to determine the biocide influence upon the microbiologically influenced corrosion, even if these techniques are or not recommended to evaluate corrosion rates on systems similar to those used in this study. This is because these techniques are used mainly to evaluate uniform corrosion and the results obtained from the surface analysis indicated a type of localized corrosion.

4. Conclusions

According to the results obtained from this study, the following conclusions were reached:

- The bacteria population growth is different for sessile and plancktonic microorganisms. Moreover, long incubation times are required to reach the maximum concentration of sessile bacteria.

- The average corrosion rate, obtained with the gravimetric technique, increases in presence of the microbial consortium.
- The films formed on the metal surface are constituted of microorganisms, organic material and some corrosion products, such as iron sulphide.
- In general, the sessile microrganisms growth is related to a corrosion rate increase, and the process becomes localized.
- The results obtained with the three techniques: PR, EIS and EN, were very similar between them, and make evident the microbial consortium effect on the increment of corrosion rates for this system.
- The biocide addition decreased the corrosion rate. However the presence of aggressive products, formed because of the microbial action, was not affected by the biocide action and higher corrosion rate could be observed.
- The use of these three techniques allows determining the effect of the biocide upon the microbiologically induced corrosion.

5. Acknowledgments

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