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RESEARCH ARTICLE

Biofilms Formation and Microbiologically Influenced Corrosion (MIC) In Different Materials

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Abstract: ***Background***: Metal surface immersed in natural or industrial waters undergo a sequence of processes in time and space that lead to the formation of biological and inorganic (scaling) deposit adhesion of different microorganisms (bacteria, microalgae, fungi) on the metal surface through extracellular polymeric substances, causing microbiologically influenced corrosion. The biofilm formation and microbiologically influenced corrosion impact on economic interests and after inorganic corrosion they are the most important problems affecting different industries. The eradication of biofilm in the industry is difficult and costly. Technological importance in the thermoelectric industry by biofilm and biofouling formation lies in energy losses in heat exchanger systems. In the oil industry, problems derived from the presence of biofilms as filter plugging, corrosion in structures storage and distribution of fuel are presented. The aim of this work is to show the different industry cases of microbiologically influenced corrosion: jet aircraft fuel storage tanks and distribution plant, steel plant, thermoelectric industry. The evaluation of microbiologically influenced corrosion and the biofilm formation are investigated.

Methods: Microbial counts were performed by conventional techniques. The formation of the biofilm and the attack on the metal surface were studied through scanning electron microscopic techniques.

Results: Different results were obtained for each of the industrial environments studied.

Conclusion: Strategies to evaluate corrosion problems systems are proposed through microbiological and physical-chemical studies.

Keywords: Biofilms, biofouling, microbiologically influenced corrosion, MIC, materials, industries, environment.

1. INTRODUCTION

Clean metal surface immersed in natural or industrial processes waters undergoes a sequence of simultaneous processes leading to formation of biological deposits (bacteria, microalgae, fungi) and inorganic deposits (scaling) that adhere to the metal surface through extracellular polymeric substances (EPSs) causing biocorrosion or microbiologically influenced corrosion (MIC) [1, 2].

EPSs of high molecular weight are secreted by microorganisms into their environment and establish the functional and structural integrity of biofilms. EPSs are mostly composed of polysaccharides (exopolysaccharides) and proteins, but include other macro-molecules such as DNA, lipids and humic substances. These compounds are important in biofilm formation and cells attachment to surfaces. EPSs constitute 50% to 90% of a biofilm's total organic matter [3-6].

MIC associated with the presence of biofilms is characterized by pitting processes that are often related to bacterial or fungal colonies or complex microbial communities occurring at localized areas of attack.

In industrial facilities, biofilm prevention is highly convenient since once the biofilm is attached to the surface, it is difficult and costly to remove. Industrial plants comprise numerous places where corrosion and biofouling processes are potentially detrimental [7-9].

In the oil industry, the presence of biofilms causes severe problems such as filter plugging, manometer failure, corrosion and biocorrosion of metals and alloys used in the construction of storage, extraction and fuel distribution structures, destruction of protective coating of these systems and finally a marked alteration in the product quality with high economic losses [10-14]. Another oil sector affected by MIC, microfouling and macrofouling corresponds to offshore platforms, where severe losses are produced due to blockage of geological structures of oilfields, biofouling of pipes that notably reduce the maintenance of the tubes con-

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necting the oil platform. Besides, injection systems of natural water or water coproduced in the extraction of diverse products (water, gas, oil, fuels and others products) are also affected [15-17].

In the thermoelectric industry, technological importance of biofilms mainly lies in (1) costly energy losses caused by reduced heat transfer and increased resistance to liquid flow in the heat interchange systems and in (2) corrosion problems invariably associated with the presence of biofilms. Biofouling may affect economic issues and after corrosion, it is the most important problem affecting structures immersed in sea waters.

On the other hand, biocorrosion, corrosion and inorganic crust are the most detrimental effects caused by an inadequate chemical control. Moreover, these processes have a negative effect on the production of breakages, yield losses, material losses, accelerated degradation of elements and disequilibrium in industrial equipments.

The present study shows results from investigations on MIC in the oil industry, steel plants and thermoelectric industry. Different results were obtained for each of the industrial systems studied; the studies were conducted at the Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), La Plata, Buenos Aires, Argentina. The aim of the studies was to assess (i) the risk of MIC derived from the microbial activity and (ii) the role of biofilms in the corrosion processes. Microbial counts were performed by conventional techniques. The formation of the biofilm and the attack on the metal surface was studied through scanning electron microscopic (SEM) techniques. Different results were obtained for each of the industrial environments studied.

2. INDUSTRIAL ENVIRONMENTS AFFECTED BY MIC

2.1. Oil Industry

Corrosion and subsequent perforation of fuel oil storage tanks, especially those containing kerosene (JP_1) have caused important economic losses and serious problems of soil and underground water contamination. The corrosive attack is generally localized at the bottom of the tanks; where sediments or mud associated with small amounts of water contain active microbial populations of diverse fungal and microbial species. The action of fungal and bacterial species most frequently isolated in systematic samplings conducted in turbo fuel storage and distribution system was analyzed [18]. Aluminium and aluminium alloys are prone to biocorrosion and biofouling effects. The major part of the literature has been addressed to the biocorrosion of aluminium alloy aircraft fuel tanks by microbial contaminants of jet fuels. Minimal amounts of water in the fuel allow microbial growth and then, hydrocarbons are used as the main carbon source. Chemical contaminants and water provide nitrogen and the necessary trace elements for biological growth. Microorganisms isolated from contaminated fuels include different species of fungi, bacteria and yeast. The fungus *Hormoconis resinae*, and some species of *Trichosporon*, *Aspergillus*, *Penicillium* and *Fusarium* have been reported as the most aggressive species from the corrosion side. The breakdown of passive oxide films is due to the synergistic effects of aggressive anions chlorides aided by organic acids derived from hydrocarbon degradation, surfactant metabolites, and the adhesion effects developed at the fixation points on the fungal mycelium [18].

Fig. **1** shows the adherence of *Trichosporon sp*. 60 days after incubation of a 2024 aluminium alloy sample in mineral simplified medium with aviation fuel JP_1 (sterile) as the only source of carbon. Hyphae and elements of asexual reproduction were observed. Fig. **2** shows a 2024 aluminum alloy sample exposed to a *Trichosporon sp*. culture 60 days after incubation in a JP_1 (sterile) mineral medium simplified with aviation fuel as the only source of carbon and after biofilm removal. The impact of metabolites excreted by microorganisms on corrosion is intensified when they accumulate at the colony/metal interface. Organic acids result in the corrosion of aluminum alloys by fungal contamination of kerosene fuels [19]. The fungal mycelium adheres firmly to the metal surface in those areas where three phases are generally present: water/fuel/alloys. When the fungal mat is removed by physical methods, the metal attack is severe, reproducing the spores and hyphae contours and showing a preferential dissolution in the attachment areas (Fig. **2**).

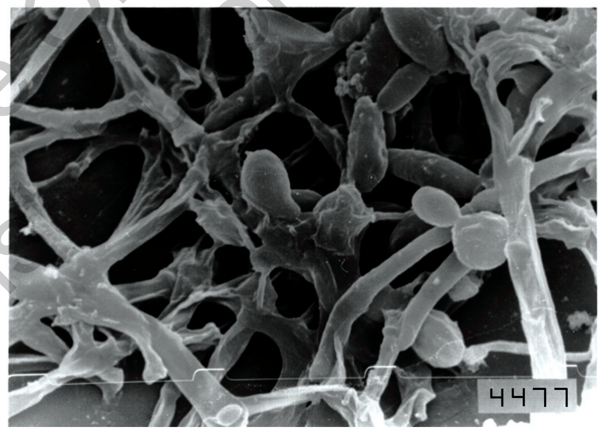

Fig. (1). SEM (Jeol JSM-T 100) micrograph showing a sample of 2024 aluminium alloy immersed. Biofilm by *Trichosporon sp*., hyphae and elements of asexual reproduction are observed (X 3500).

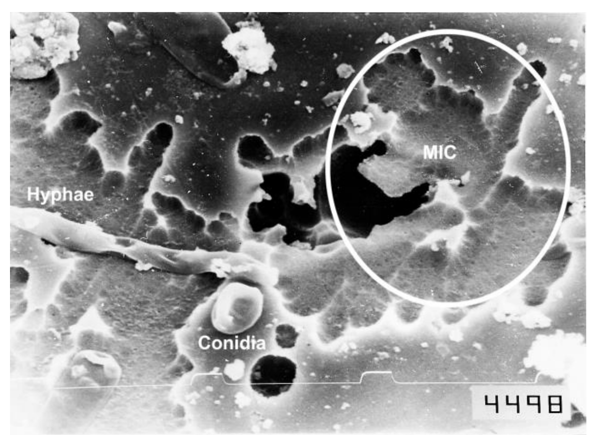

Fig. (2). SEM (Jeol JSM-T 100) micrograph showing a sample of 2024 aluminium alloy. *Trichosporon sp*. after biofilm removal. Pitting attack can be observed (X 3500).

2.2. Steel Plant

Three samples obtained in a steel plant were studied: E1 and E2 emulsions (the first liquid and oxygenated and the second black colour and muddy) and a third sample taken from a filter. Microbiological counts of total mesophilic heterotrophic aerobic bacteria, acid-producing bacteria, sulfate-reducing bacteria (SRB), iron-oxidizing bacteria, *Pseudomonas sp.*, fungi and yeasts were carried out [9]. Samples were observed under JSM 6360LV scanning electron microscope. Microfouling was observed under optical microscope (Olympus BX51). Table **1** shows microbiological counts performed with microbiological conventional techniques obtained from different samples [9].

Growth of microbial consortia formed by *Hormoconis resinae,* yeasts and bacteria (Fig. **3**) could be observed on E1 and E2 samples.

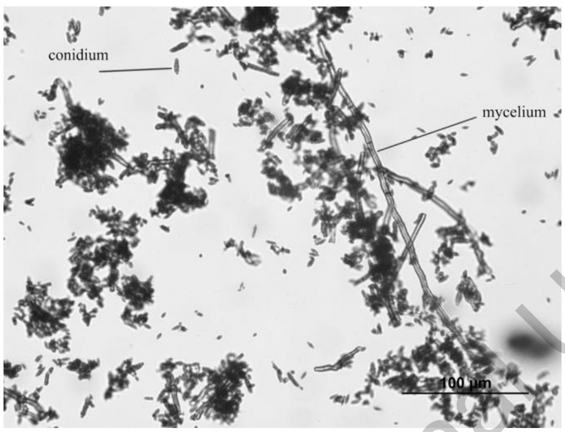

Fig. (**3**). *Hormoconis resinae* observed under optical microscope (40X), stained with blue lactophenol.

Growth and formation of biofilms of heterotrophic aerobic bacteria and anaerobic facultative bacteria develop microenvironments favorable to growth of iron oxidizing bacteria and yeasts that are incorporated to the biofilm forming microbial consortia. Through excretion of acid metabolites, the yeasts produce pH reduction encouraging *Hormoconis resinae* growth and subsequent risk of biocorrosion (Table **1**) [10-12].

The *Pseudomonas sp*. microorganism is able to grow at the expense of synthetic oil in 4% (oil) demineralized water by using and degrading compounds present in these emulsions (esters and emulsifiers) [12, 13] and adhere to the surfaces forming biofilms. These biofilms produce zones of different oxygen gradients that accelerate the biocorrosion processes [14, 15].

Microscopy observations of the filters revealed brilliant zones and the formation of ochre to orange tubercles together with pits irregularly distributed on the surface (Figs. **4** and **5**). This was confirmed by the growth of viable microorganisms indicated in Table **1**.

Microorganisms found in this system are involved in the MIC processes [10, 11, 16]. It should be noted that biocorrosion processes [12, 13] are clearly of electrochemical nature. The corrosion process was developed at a long term and was not controlled by workers at the plant.

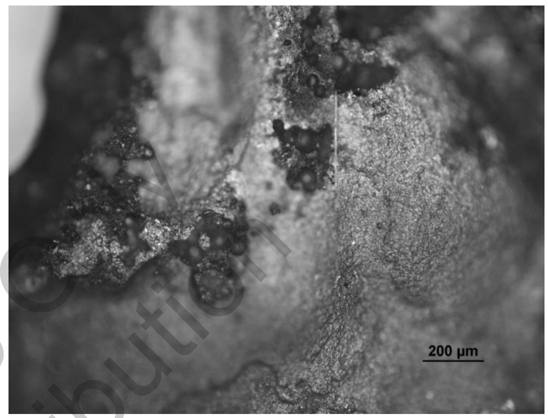

Fig. (4). Light microscope photograph showing the filter with ochre to orange and brilliant zones (10X).

Fig. (**5**)**.** Photograph showing filter with attack (pitting).

Table 1. Microbial counts (mo/mL).

Sample	Total Heterotrophic Aerobic Bacteria	Acid-Producing Bacteria	Sulfate-Reducing Bacteria (SRB)	Iron-Oxidizing Bacteria	Fungi and Yeasts
E 1	1000-10000	1000-10000	1-10	1-10	50
E2	100-1000	100-1000	10-100	10-100	(-)
Filter	(-)	(-)	(-)	(-)	(-)

(-) Without Growth

2.3. Thermoelectric Power Plants

One of the key factors affecting directly or indirectly a power plant is the low efficiency of its cooling system and mainly of its condensers. Low efficiency is caused by an increase in the resistance to heat transfer and corrosion problems. Adhesion of organisms inside the piping system results in the reduction of the pipe diameter and a subsequent decrease in the water flow. Cooling systems of electric power generating plants use sea water with no previous treatment. Thus, plankton and larvae of different organisms enter the system and find available food and an adequate place to settle inside the tubes. The biofouling developed in the cooling system of electric power plants is one of the most important causes affecting negatively the cooling system, functioning directly or indirectly. The type of biofouling adhered to the areas or sectors under high velocity flow is known as microfouling, (thickness below 500 μm). In low velocity areas, accumulation of biomaterial is greater and is known as macrofouling [20].

Experiments were conducted at a thermoelectric power plant located in the province of Buenos Aires, Argentina. Samples of 70/30 copper nickel (disks of 15 mm diameter) were located on acrylic panels (material of the power plant piping system) to study bioadherence and formation of biofouling. The composition of the alloys was Cu 67%, Ni 31.9%, Fe 1, 10%. Observation of biofouling and corrosion products were made using SEM (Jeol JSM-T 100).

It is well known that copper is used as antifouling in the marine environment due to its biocide properties. However, after different exposure times, diatoms and protozoa adhere to the metal surface forming biofilms. Toxic characteristics of the 70/30 copper-nickel alloy impose a pretreatment of the surface through the excretion of abundant EPS excreted by microorganisms [21].

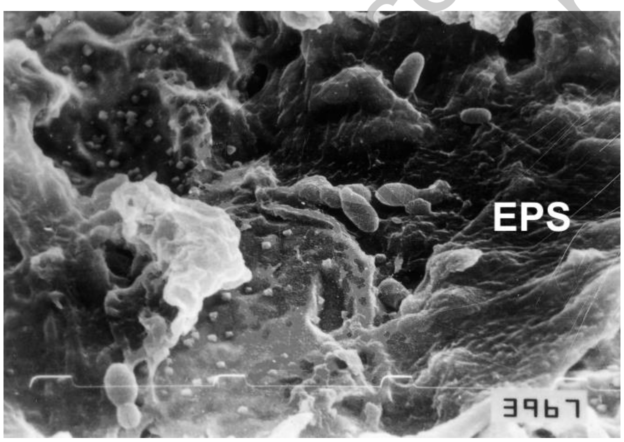

Fig. (6). SEM micrograph showing a sample of 70/30 copper-nickel 7 days after immersion in natural sea water. Bacteria, EPS and corrosion products can be observed (X 3500).

Only isolated bacteria adhered to the surface of 70/30 copper-nickel samples after 7 days in seawater exposure (Fig. **6**) shows an increase in number (CFU/cm^2) after 28 days. Diatoms and abundant *Zoothamnium sp*. colonies mixed with organic and inorganic material were observed 49 and 60 days after exposure. The sessil protozoan *Zoothamnium sp* (Fig. **7**) is a particularly important indicator of contamination in these systems due to its strong adhesion to the surfaces by means of a mucilage exudate radially by peduncle. Detachment of one of the peduncle leads to removal of the passive layer of the metal substrate giving rise to differential aeration effects and subsequent attack.

The corrosion products layers formed were not uniform and compact. This was mainly due to precipitation of copper salts, adsorption of organic, adherence of biological materials. Detachment of the protective layers was produced in certain areas; preferential dissolution of copper (probably Cu_2O) was observed in those areas where anion diffusion was restricted (such as under the external layer or between grains) [21].

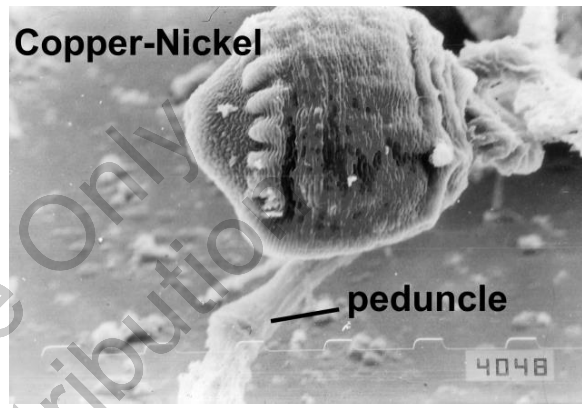

Fig. (7). SEM micrograph showing a sample of 70/30 copper-nickel 49 days after immersion in natural sea water. *Zoothamnium sp*. peduncle is adhered to the substrate (X 5000).

CONCLUSION

After analyzing and understanding the problems related to biofilm formation and MIC in the above mentioned systems, it is necessary to elaborate global proposals to avoid important economic losses. The proposals should include collection of qualitative-quantitative data of the different systems to be studied. The measurements of the variables and parameters are necessary to characterize the systems to eradicate formation of scaling, biofilms, biofouling, and problems related to biocorrosion. All the information regarding microorganisms present and the physicochemical characteristics of the industries affected should be collected, organized and assessed. Workers and staff responsible for the industrial plants should be trained to reduce errors to the maximum extent when making decisions regarding these problems. Theoretical/Practical courses should be provided and guidelines elaborated to train the personnel at the plants for different cases of biocorrosion.

CURRENT & FUTURE DEVELOPMENTS

The integrity of the infrastructure of different industrial systems affected by MIC caused by the formation of biofilms could be controlled through the development of microstructured and nanostructured coatings, materials having suitable surfaces finishes, low porosities, low surface roughness and use of new biocides which are environment friendly.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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