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# Effect of different salinities of a dynamic water system on biofilm formation

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**AISI-1020 carbon steel coupons were fixed onto a water circulation loop in order to study the effect of varying NaCl concentrations on formation of biofilms by natural populations of microorganisms. Overall, we observed a reduction in the number of bacteria attached to the metal surfaces as NaCl levels increased. At 12.85 and 80 g/l NaCl, the respective bacterial counts were: $1.7\times10^9$ CFU/cm$^2$ and $7.5\times10^2$ CFU/cm$^2$ for aerobic species; $1.3\times10^4$ CFU/cm$^2$ and $2.1\times10$ CFU/cm$^2$ for anaerobic species; and $1.8\times10^3$ CFU/cm$^2$ and $4.6\times10$ CFU/cm$^2$ for sulfate-reducing species. However, the opposite trend was observed for the numbers of iron-reducing bacteria: $4.1\times10^6$ CFU/cm$^2$ at 12.85 g/l NaCl and 7.5 $10^8$ CFU/cm$^2$ at 80 g/l NaCl, respectively. Fungal counts remained constant throughout the experimental period. The salt concentration at which the maximum corrosion rate was observed was 35 g/l. In view of the marked loss of metal mass recorded at this salinity, AISI-1020 carbon steel proved to belong to the group of alloys less resistant to corrosion.** *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 45–48.

**Keywords:** AISI-1020 carbon steel; biocorrosion; biofilms; salinity

## Introduction

Coastal zones of the marine environment are commonly selected to station several industries, such as synthetic rubber plants, crude oil refineries and chemical plants. This is due not only to the facilitated transfer of cargoes by sea transport but also to the practically unlimited availability of sea water, which is used extensively in the cooling of both equipment and products.

According to Gentil [5], the corrosive potential of seawater can be determined initially by its salinity, a parameter that is practically constant in the oceans yet may vary in coastal seas. Along the Brazilian coastline, which is very long, such variation is well established.

The utilization of seawater as a coolant creates several problems for industrial equipment, which are related to both water salinity and the presence of microorganisms capable of speeding up corrosion of metal structures.

During microbially induced corrosion (MIC), which involves biofilm formation on solid surfaces and is a phenomenon directly linked to electrochemical corrosion, microorganisms have an active role, producing metabolites that accelerate electrochemical reactions [12].

The rate of crevice corrosion of different stainless steels increases due to an increase in the cathodic reaction brought about by seawater biofilms [13].

The corrosion rate of carbon steel in freshwater containing low amounts of sodium chloride or acidified by addition of different acids differs depending on the environmental conditions used [11].

Considering the varied aquatic environments that may favor growth of bacteria, microalgae and fungi, many industrial structures can undergo biocorrosion. On the other hand, it is well known that microorganisms differ in their susceptibility to NaCl concentrations. In view of this, the aim of the present work was to analyze how different seawater salinities affected the attachment of microorganisms onto AISI-1020 carbon steel metal surfaces.

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## Materials and methods

### Metal probes

Coupons made of AISI-1020 carbon steel (3.8 cm×10 cm; mean area 4.68 cm$^2$) were used as probes. Although carbon steel is susceptible to corrosion, it was selected as the test alloy for this work because it is widely used in Brazilian petrochemical plants. The metal surfaces were jet sprayed with sand, isopropyl alcohol and acetone. After drying, the coupons were weighed with accuracy up to 0.1 mg.

### Fluid phase

Seawater from the Guanabara Bay, Rio de Janeiro, Brazil, was used. Its composition (in g/l) was: chloride, 12.85; sulfate, 2.5; bicarbonate, 0.4; magnesium, 1.2. The salinity levels were adjusted to test concentrations by addition of NaCl. Counts of the planktonic microorganisms were: aerobic bacteria (cel/ml): $3.2\pm1.4\times10^5$; anaerobic bacteria (cel/ml): $9.5\pm1.2\times10^6$; sulfate-reducing bacteria (cel/ml): $2.4\pm2.2\times10^2$; iron-reducing bacteria (cel/ml): $5.1 \pm 1.1 \times 10^4$; and fungi (CFU/ml): $2.7\pm1.5\times10^4$.

### Experimental

The experiments were carried out an experimental loop made of PVC and connected to a tank (capacity: 30 l) filled with running seawater in order to simulate field conditions. Water circulation was maintained at a constant flow rate by two 1/15 HP pumps.

The metal coupons were fixed onto plastic rods with plastic screws and set along the water circulation loops to simulate the conditions of exposure to circulating water within the pipes of a cooling system. During the experimental period, the flow rate was 0.48 $m^2$/h; salt concentrations ranged from 12.8 to 80 g/l and the temperature and pH values were 30±1°C and 8±1, respectively. Seawater with salt concentrations appropriately adjusted with NaCl was renewed every 2 weeks in order to ensure a supply of nutrients to the microorganisms. Coupons were withdrawn at 30-day intervals for analysis. For each condition studied, at least four experiments were carried out.

### *Preparation of microbial suspensions for analysis*

Once removed from the tank, the sampled probes were placed inside vessels filled with seawater from the experimental system and transferred to flasks containing 30 ml of sterile distilled water. Both sides of the metal coupons were then aseptically scraped with a sterile spatula and the resulting suspension was used for analysis. Tenfold dilutions were prepared. Distilled water containing NaCl (12.85, 35, 50, or 80 g/l, depending on the experiment) was used as diluent for aerobic microorganisms; anaerobes were suspended in a reducing solution as described previously [9].

### *Enumeration of microorganisms*

Aerobic bacteria were quantified by counting the number of colony-forming units (CFU) on Petri dishes containing nutrient agar (Merck no. 5443). Incubation was carried out at 30±1°C for 48 h.

Anaerobic bacteria were quantified by most probable number (MPN) [6,8] in antibiotic-type flasks containing thioglycolate medium (Difco) purged with $N_2$. Incubation was at 30±1°C for 28 days.

Sulfate-reducing bacteria (SRB) were quantified by the MPN method using modified Postgate medium [10] purged with $N_2$. Incubation was 30±1°C for 28 days.

Iron-reducing bacteria were quantified by enumerating the number of typical colonies (bacterial colonies that precipitate iron) in petri dishes containing ammonium ferrous sulfate medium (Merck), using a Quebec counter. Incubation was at 20±1°C for 10 days.

Fungi were quantified by counting the number of CFU on Petri dishes containing Sabouraud agar (Merck). Incubation was at 30±1°C for 7 days.

All media were prepared with distilled water to which NaCl had been added to make up the desired salinity for testing.

### *Sulfide content*

Total sulfide content was determined by a colorimetric method using *N*,*N*-dimethyl-*p*-phenylenodiamine and ferric chloride after treating the biofilm suspensions with conc. HCl, in order to measure the amount of hydrogen sulfide produced. The standard curve used for reference was obtained with $Na_2S$-monohydrate P.A. (Merck). Readings were taken at 670 nm [1,7].

### *Loss of metal mass and corrosion rates*

The metal coupons were weighed before the start of the experiment and after removal of the attached biofilm, and were treated to determine weight loss due to corrosion. This treatment consists of removing the deposits by immersion of the metal in 26% HCl, followed by washing them in running water, neutralization in 10% NaOH and further washing in running water. The metal surfaces were dehydrated with isopropylic acid

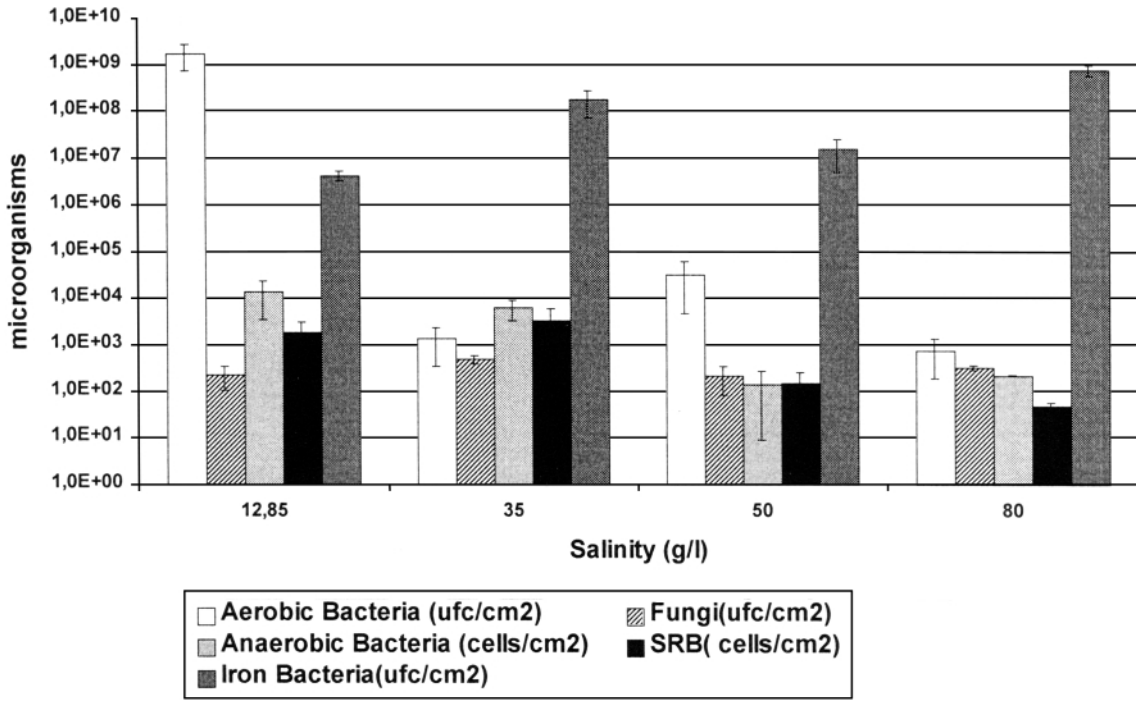


**Figure 1** Number of microorganisms detected in biofilms on the surface of carbon steel samples exposed at different salt concentrations after 30 days incubation. Mean value of four experiments.

and acetone for 5 s. After drying the treated coupons the material was placed in a desiccator and weighed to 0.01 mg. [3]. The corrosion rate was determined by the Rabald index, which is an indicator of weight loss $\Delta m$ ($g/m^2/day$).

### *Control experiments*

In order to verify the influence of seawater alone on metal corrosion, especially with respect to the presence of NaCl, samples of carbon steel were placed inside flasks containing 100 ml of seawater, with salt concentrations adjusted to test levels by addition of NaCl. The flasks were then sterilized at 121°C for 20 min and kept for 30 days prior to evaluation.

## Results and discussion

After 30 days of exposure of AISI-1020 carbon steel coupons to seawater, biofilms were formed at all salinity levels tested. However, increasing NaCl concentrations led to a reduction in the number of bacteria attached to the metal surfaces, except for iron-reducing bacteria and fungi. The counts of microorganisms recorded at each salt concentration studied are presented in Figure 1. The number of fungi remained constant irrespective of seawater salinity; thus, fungal species were not directly responsible for the differences in biocorrosion observed as salt concentration changed.

The percentage of iron-reducing bacteria attached to the coupons was proportional to seawater salinity; this may be linked to the corrosive property of the chloride ion which, by producing high levels of $Fe^{2+}$, can provide large amounts of substrate for this type of bacterium. With respect to anaerobic species, the percentage of adhered cells was inversely proportional to increasing salt concentrations, possibly indicating that a hypertonic medium causes damage to the bacterial cell wall and thus reduces viability. Moreover, when high numbers of iron-reducing bacteria are present, large amounts of $Fe(OH)_3$ can be generated and once precipitated, may create an anaerobic environment. However, this same condition may impose a constraint on microbial growth by hampering the diffusion of other nutrients. Different parts of the biofilm may contain insufficient levels of some components or lack them altogether, depending on their relative rate of diffusion [2].

Data on total sulfide content indicated the presence of metabolically active SRB. The levels of total sulfides were 14.3, 105.3, 192.3 and 0.8 $\mu g/cm^2$, respectively, for salinities of 12.85, 35, 50 and 80 g/l. These species, by reducing sulfur compounds, lead to the formation of sulfide and consequently promote cathodic depolarization of metal surfaces, accelerating the corrosion process. However, despite a continuous and marked growth of microorganisms, the concentration of dosed sulfide increased up to 50 g/l NaCl and then fell abruptly at still higher NaCl levels. A lack of correlation between sulfide content and numbers of SRB has been reported previously [4] and was found to relate to the detachment of the most outward layer of the biofilm or even the whole biofilm due to water turbulence.

Table 1 shows the Rabald indices, which estimates weight loss in grams per square meter per day, and permits the ranking of alloys, except aluminum alloys, into resistant to corrosion ($\leq$2.4 $g/m^2/day$), fairly resistant (>2.4–24 $g/m^2/day$), non-resistant (between >24 and 72 $g/m^2$ $g/m^2/day$) and not recommended (>72 $g/m^2/day$) [5]. AISI-1020 carbon steel exposed to seawater was resistant to corrosion in the absence of viable microorganisms. However, the behavior of the metal alloy varied in the presence of attached biofilms, and the differences related to the salt levels tested. At 12.85, 50 and 80 g/l NaCl, the metal coupons proved to be fairly resistant yet were susceptible to biocorrosion at 35 g/l NaCl. The reduction in corrosion rates between 50 and 80 g/l NaCl may possibly be explained by the fact that oxygen solubility decreases continually as NaCl levels go up.

**Table 1** The Rabald indices determined on carbon steel coupons exposed at different salt concentrations of seawater in the absence of microorganisms. Mean value of four experiments

| Salinity (g/l) | $\Delta m$ ($g/m^2/day$), seawater | $\Delta m$ ($g/m^2/day$), sterile seawater |
|---|---|---|
| 13 | 15.7±2.9 | 1.7±0.4 |
| 35 | 46.7±5.7 | 1.8±0.1 |
| 50 | 20.5±4.3 | 1.5±0.7 |
| 80 | 25.8±0.7 | 1.6±0.5 |

The NaCl concentration at which the maximum corrosion rate of carbon steel took place in this study was 35 g/l, which suggests that most ocean waters (characterized by a NaCl content of 35 g/l and normally harboring many different microorganisms) have a high corrosive potential for this type of alloy. Considering the role of microorganisms in biofilms, it is worth noting that at the critical NaCl concentration of 35 g/l, we observed a greater attachment of the consortium of SRB and iron-reducing bacteria which, according to the literature, are the greatest accelerators of metal corrosion by promoting cathodic depolarization and oxidation of iron.

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