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# Influence of microbial adherence on corrosion of UNS 1008 carbon steel and hybrid nano-structured coatings

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## Abstract

**Purpose** – Microbes that are able to grow on different surfaces can cause the deterioration of the underlying layers because of their metabolic activity. The purpose of this study is report the ability of fungi-bacteria consortium (FBC) in anaerobic media, and marine strain bacteria, to attach onto UNS 1008 carbon steel and zinc epoxy coats.

**Design/methodology/approach** – Impedance analysis, scanning electronic microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were used to evaluate the adherence, biofilm formation and corrosion effect of FBC and marine bacteria onto UNS1008 carbon steel in anaerobic and aired conditions, respectively. In a similar way, the anticorrosive performance of hybrid coats on UNS 1008 carbon steel against marine bacteria.

**Findings** – In aerobic conditions, the outer layer shows a micro-crack appearance and several semi-sphere products that could be because of spore formation. In anaerobic conditions, evidence of iron sulfide surrounded by a mixture of sulfur-containing extracellular polymer substance was observed by SEM images and EDS analysis. The presence of hybrid coats (zinc epoxy with carbon nanotubes CNT content) affected the level of microbial adherence and the concentration of corrosion products ( $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}(\text{OH})_2$  and  $\text{FeS}$ ); the cell attachment was lower when the steel surface was coated with Zn/CNTs.

**Originality/value** – This study opens a window for further evaluations of CNTs associated with metals as active materials to assess the corrosion on extreme corrosive environments, like in oil and gas industries the microorganisms play an important role either to increase or reduce the corrosion processes.

**Keywords** Carbon nanotubes, Marine microorganisms, Sulphate-reducing microorganisms, Zinc epoxy coats

**Paper type** Research paper

## Introduction

Biocorrosion or microbial influenced corrosion (MIC) is the damage caused on metal surfaces by microorganisms because of their metabolic activities. Some organisms associated with metals in terrestrial and aquatic habitats are sulphate, iron and  $\text{CO}_2$ -reducing bacteria, sulfur, iron and manganese oxidizing bacteria (Abdolahi *et al.*, 2014). Among them, sulphate-reducing bacteria (SRB) are recognized as a major group of microorganisms associated to anaerobic corrosion. These microorganisms can coexist in naturally occurring biofilms with a wide bacterial community including fermentative bacteria, often forming synergistic communities (consortia) that affect

electrochemical processes through cooperative metabolism (Enning and Garrelfs, 2014).

The biocorrosion process may be recognized by a combination of events: corrosion, presence of microbial slime masses, presence of hydrogen sulfide and ferrous or ferric hydroxide, mainly in anaerobic systems (Enning and Garrelfs, 2014). A wide variety of bacteria have been isolated or detected in the petroleum industry; however, because of their detrimental effects, SRB have been the most commonly studied group. Concerning the mechanisms of action, most of the basic

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theories on electrochemical corrosion are relevant to biocorrosion and could be used to understand the acceleration of the corrosion in different media under anaerobic or aerobic conditions. Most coats in the industry are used for the control of the microbiologically influenced corrosion (MIC), and they are designed to provide an effective barrier against corrosion processes and biocidal effect to inhibit either or both conditions. They have been synthesized in organic, inorganic or hybrid schemes; however, some of them have been degraded when exposed to MIC because of the microbial attack, either as a consortium or specific bacteria culture, specially under anoxic conditions, like marine water or in oil and gas pipelines (Gu et al., 1998). Recently, different researchers have reported studies on the action of SRB or fungi-bacterial consortium (FBC), which are exposed to epoxy coats (Wang et al., 2012; Tambe et al., 2016). SRB or FBC-induced biocorrosion associated to anoxic sulphate rich environments are recognized to cause severe corrosion damage. Nowadays, environmentally friendly coats have improved the physicochemical and geometric properties, especially for resistance to microbiological corrosion attack. New generation of coatings denominated "hybrid nano architected sacrificial coatings" has emerged in a context of double control protection mechanism: the cathodic protection, by the incorporation of sacrificial particles into the coating, and a barrier effect because of the presence of the polymeric composite itself (Cubides and Castaneda, 2016). Inclusion of additives can improve both properties (Praveen et al., 2007; Cubides and Castaneda, 2016). Such an additive, like carbon nanotubes (CNTs), influences the interconnectivity of active particles and synergistically fills the voids during the production process of the coatings within the polymeric matrix, as shown by Cubides and Castaneda when using zinc rich epoxy coats with contents of CTNs (Cubides and Castaneda, 2016). In the present study, impedance analysis, scanning electronic microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were used to evaluate the adherence, biofilm formation and early corrosion effect of FBC and marine bacteria onto UNS 1008 carbon steel under anaerobic and aerobic conditions, respectively and the anticorrosive performance of hybrid coats on UNS 1008 coupons against marine bacteria.

## Materials and methods

### Cultivation of microorganisms

The FBC were isolated from samples collected with instruments from the interior of pipelines. The consortium was inoculated in a culture medium used to grow *Desulfovibrio desulfuricans* ATCC 1249 from the American Type Culture Collection (Liu et al., 2015). The pH value was adjusted to 7.2 after de-aeration using nitrogen. The medium was autoclaved at 120°C for 15 min. The FBC consortium was incubated at 37°C.

The marine strain was isolated from samples collected from the Mexican Gulf at a depth of 1500 m. Sediment sample was inoculated in nutrient broth with 0.8 per cent NaCl (NB-NaCl) for 24 h and the strain was isolated in nutrient broth agar plates for isolation of single colony. The strain was maintained in glycerol 15 per cent at -80°C. The cells were cultivated on NB-NaCl for 24 h at 21°C for all the tests performed. All reagents used were from Sigma-Aldrich®.

### Electrochemical analysis

Experiments were performed by using a 50 mL three-electrode electrochemical glass cell. The UNS 1008 carbon steel samples and coated carbon steel (zinc epoxy base coats) were considered as the working electrodes of 8 × 5mm rectangular dimensions mounted in a 3 mm width resin case. Composition of carbon steel UNS 10080 is 99.31–99.7 per cent Fe, 0.30–0.50 per cent Mn, 0.10 per cent C, 0.05 per cent S and 0.04 per cent P. The reference electrode is a saturated calomel electrode, and a platinum screen was used as a counter electrode. External and internal surfaces of the glass cells were autoclaved. Working electrodes, reference and auxiliary electrode were sterilized with 70 per cent ethanol and acetone and set under UV light and laminar flow for 30 min.

The electrochemical testing procedure consisted of a measurement of an open circuit potential (OCP) and electrochemical impedance spectroscopy for a period of 28 days. OCP was measured during 10 min prior electrochemical impedance spectroscopy measurements. Impedance measurement was performed at OCP in a frequency range from 100 KHz to 10 mHz with 10 mV amplitude. All electrochemical experiments were performed in duplicate to ensure reproducibility. All tests were performed at 25°C. Experiments using sulphate reducing microorganisms were performed in anaerobic conditions, whereas the studies with marine bacteria were done in aerobic conditions. The electrochemical set up was performed on a potentiostat/galvanostat Biologic VSP 300.

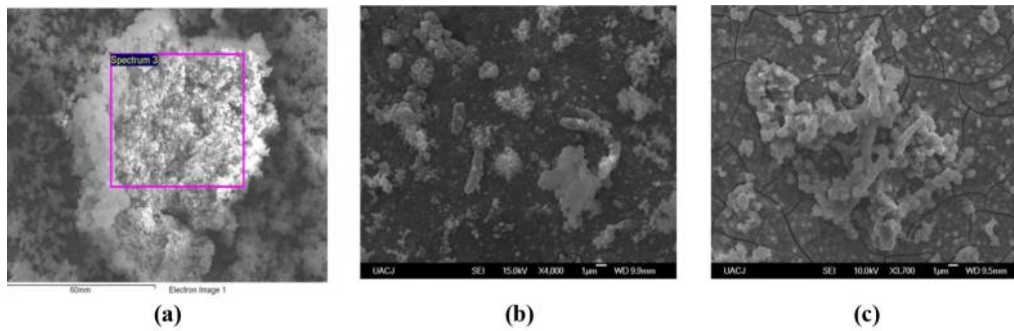
### Scanning electron microscopy sample preparation

Carbon steel coupons (UNS, 1008) and hybrid coats with adhered cells were rinsed with phosphate-buffered saline (PBS) 1X (8.0 g of NaCl, 0.2 g of KCl, 1.4 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and 0.2 g of KH<sub>2</sub>PO<sub>4</sub> per liter, pH 7.2). Steel coupons (UNS1008) with adhered cells were rinsed with PBS 1X (8.0 g of NaCl, 0.2g of KCl, 1.4 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and 0.2 g of KH<sub>2</sub>PO<sub>4</sub> per liter at pH 7.2) and fixed with four different methods (after washing): glutaraldehyde (GA) 2.5 per cent w/v in PBS for 2 h (washing every 30 min), paraformaldehyde 4 per cent w/v in PBS (washing every 30 min) and ethanol/acetic acid (3:1) for 10 min. All fixations were conducted at 25°C. After fixation, the cells were washed twice in PBS and then resuspended in sterilized ultrapure water to avoid salts crystallizing during the drying process and subsequent influence on SEM measurement. The samples were covered with gold and observed on JEOL JSM-700F field emission SEM for analysis. All reagents used were from Sigma-Aldrich®.

## Results and discussion

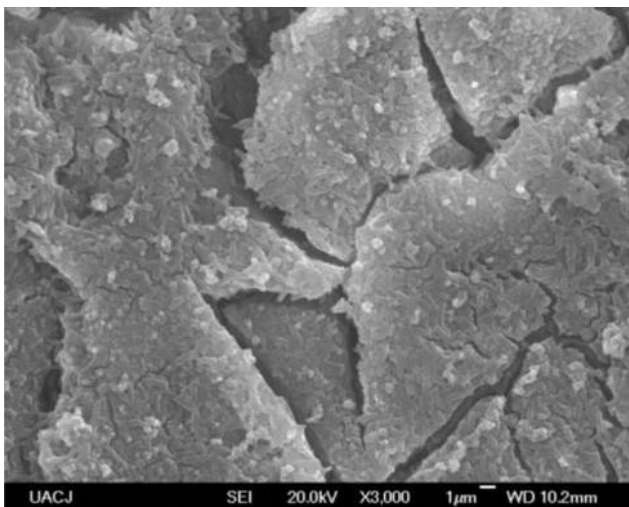
### Effect of fixation methods on sample preparation for SEM analysis

Figure 1 shows the different cellular structures from the FBC consortium, detected by SEM after 38 incubation days. In the figure the extracellular polymeric substance (EPS) under different fixation treatments is showed, and the corresponding EDS results show the corrosion products. The results suggest that the fixation methods could significantly affect the morphology of bacterial cell as well as the surface ultrastructure. The fixation methods containing alcohols such as ethanol/acetic acid [Figure 1(A)] are less suitable than those

**Figure 1** SEM images of microorganisms over UNS 1008 steel surfaces

**Notes:** Coupons of FBC consortium cells fixation with ethanol/acetic acid (A-Bar 60 µm), paraformaldehyde 4% w/v (B-Bar 1 µm) or glutaraldehyde 2.5% w/v (C-Bar 1 µm)

containing aldehydes [2.5 per cent GA, and 4 per cent paraformaldehyde; **Figures 1(B) and (C)**] for evaluating the cell morphology during corrosion processes. The cells fixed by 2.5 per cent GA and 4 per cent paraformaldehyde were preserved better than those treated with ethanol/acetic acid. This could be caused by the alcohols in the fixation solutions, which could dissolve the membrane lipids, form large pores in the cell, causing corrosion (Vekemans *et al.*, 2004; Ziółkowska and Wardzińska, 2015). The fixation methods applying aldehydes showed medium preservation ability for cell morphology judging from the images in **Figure 2**. It is postulated that aldehydes fixed cells by forming covalent chemical bonds between proteins and therefore could maintain the integrity of membrane lipids, as well as the surface macromolecules (Dapson, 2007). In the present study, 2.5 per cent GA showed the best performance for fixation with 4 per cent paraformaldehyde. Paraformaldehyde, the polymerized form of formaldehyde, would be depolymerized to formaldehyde when dissolved; therefore, the 4 per cent paraformaldehyde solution contained pure formaldehyde.

**Figure 2** SEM image of marine bacteria biofilm formed over UNS 1008 carbon steel

**Note:** Cells were fixed with glutaraldehyde 2.5% w/v (Bar 1 µm)

Compared with formaldehyde, GA could fix samples more tightly, as it has a longer molecule and two aldehyde groups which has potential to link more distant protein molecules (Kiernan, 2000). This might explain the superior performance of GA in fixing the bacterial filaments among the applied fixation methods.

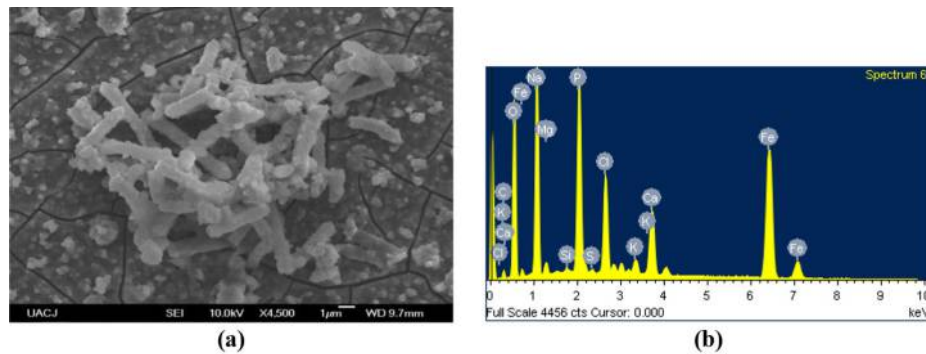
#### Fungi-bacteria consortium adherence on UNS 1008 steel coupons

**Figure 3(a)** shows SEM and EDS results for FBC consortium. Two kinds of the corrosion product layers were observed on the surface of the steel in the presence of FBC. The outer layer presented a micro-crack appearance. The inner layer under the detached film was a compact layer including many semi-sphere products which might be sulfur-free carbonates surrounded by a mixture of sulfur-containing extracellular polymer substances (Xu *et al.*, 2002). FBC were observed on the surface of the steel. As can be seen in **Figure 3(b)**, the corrosion products were mainly iron oxides and the element O should be ascribed to the iron oxides produced by the oxidation of sulfide when small quantities of oxygen enter inside of the anaerobic incubator. These results are similar to those of Sheng *et al.* (2002); they have concluded that the morphology of cells adhered to steel coupons has a significant influence on the corrosion, and when they are settled in a compact way they could act as a protective film on the metal surface.

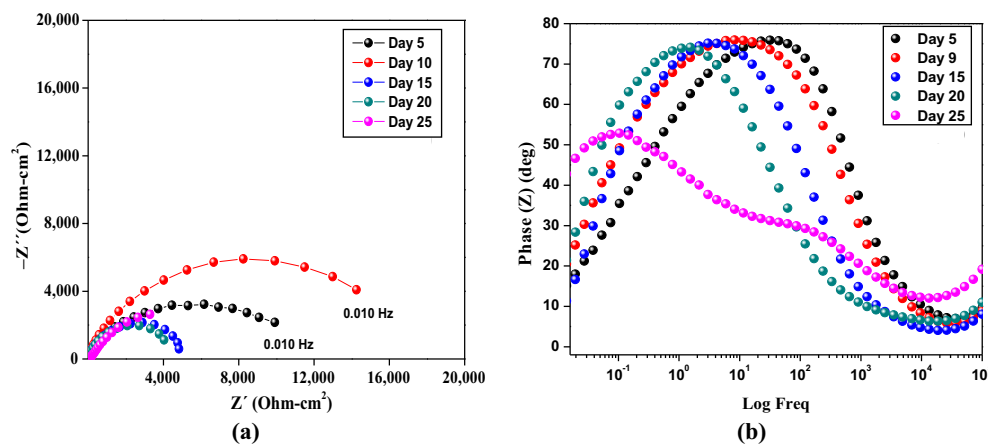
#### Impedance analysis

**Figure 4** shows the Nyquist diagram and the phase angle representation for the corrosion mechanisms of carbon steel UNS 1008 exposed to electrolyte with FBC. **Figure 4(a)** shows the phase angle representation, where there is one maximum point, at medium frequencies, which represents the corrosion resistance during the process of diffusion of the electrolyte into the steel. The time constant at medium frequencies at day 25 indicates the contribution of an extra layer formed on the steel surface, which can be attributed to the formation of a biofilm by the FBC consortium and the presence of corrosion products. The results suggest that the biofilm was formed during the first 5 days and continued increasing up to day 15. After 15 days, only one-time constant keeps increasing in the phase angle representation until day 20, as shown in **Figure 4(b)**.

SEM images in **Figure 3** show the presence of a porous polysaccharide layer after 28 days of exposure. The

**Figure 3** SEM and EDS analysis of FBC consortium on UNS 1008 steel surfaces

**Notes:** (a) SEM images of FBC treated with glutaraldehyde 2.5% w/v (Bar 1  $\mu\text{m}$ ); (b) EDS results corresponding to the corresponding treatment

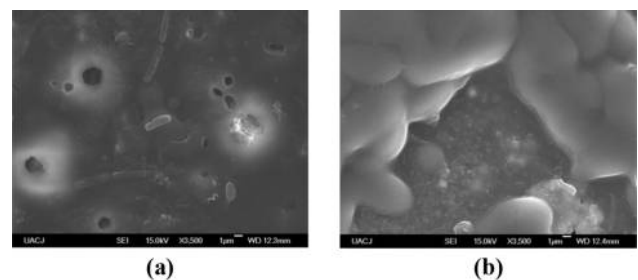
**Figure 4** Nyquist and bode diagram of FBC on UNS 1008 carbon steel

low-frequency maximum point increased from approximately 20 to 60° and shifted toward low-medium frequencies during the same time. This can be attributed to a decrease in the active area because of the electrical contact between particles and their distribution, while the charge transfer enables the electrochemical activity.

#### Marine bacteria adherence on Zn hybrid coats with carbon nanotubes

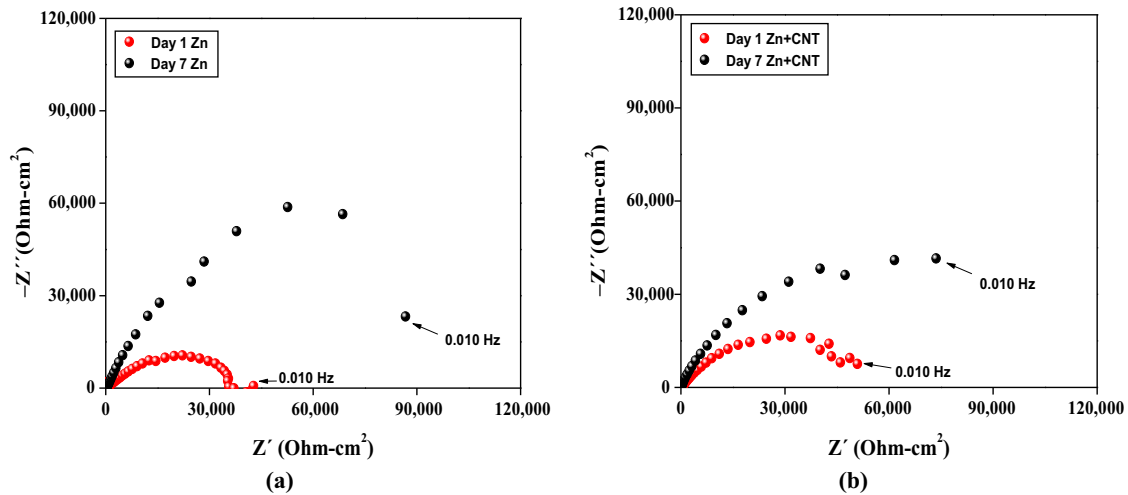
Zinc base coats on steel surface are widely used to enhance their life time (Praveen *et al.*, 2007). Zinc composite coats are part of the field which interest have been increased among the oil industry. In these cases the zinc metal have been used mainly with polymers and metal oxides, and their properties depend on electrochemical parameters that will confer functional properties like corrosion resistance (Benea *et al.*, 2002). To make these composites more attractive, they have been combined with nanoparticles because of their increasing availability, and particularly the CNTs have attracted interest in different areas including anticorrosive investigations (Show and Itabashi, 2008). Nevertheless, in corrosion studies there are few studies using CNTs in combination with metals, specifically zinc. In the present study, the effect of microbial

adherence on zinc and zinc-CNTs surfaces and their corrosion products were evaluated. Figure 5 shows marine strain adherences on the steel UNS 1008 coated with zinc (A) and Zn-CNTs (B) after 7 days of incubation under aerobic conditions (the trend was the same after 28 days of incubation).

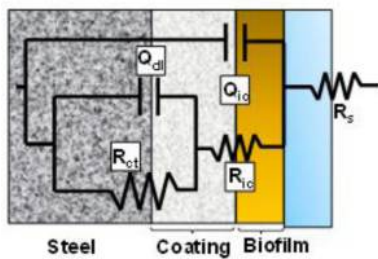
**Figure 5** SEM Images for marine bacteria biofilm on UNS 1008 steel surfaces

**Notes:** (a-Bar 1  $\mu\text{m}$ ) SEM images of marine bacteria on Zn coating; (b-Bar 1  $\mu\text{m}$ ) SEM images of marine bacteria biofilm on Zn-CNTs coating

**Figure 6** Electrochemical impedance spectra in Nyquist diagram obtained during 7 days of immersion with (a) Zn hybrid coating and (b) Zn + CNTs hybrid coating onto UNS 1008 carbon steel in presence of marine bacteria culture



**Figure 7** Electrochemical analogs proposed during 7 days of immersion with Zn hybrid coating and Zn + CNTs hybrid coating onto UNS 1008 carbon steel in presence of marine bacteria consortium



When the cells are in contact with the Zn base coats, the resistance to the adherence is low, the cells are deposited on the steel on and under the coating layer and the corrosion products are in higher concentrations. However, when the cells are in contact with the Zn-CNT coating the resistance to the adherence is higher and in consequence, the attachment is low as it is showed in the [Figure 5\(B\)](#). In this case, the corrosion products are at low concentration. [Show and Itabashi \(2008\)](#) have shown the effectiveness of CNTs additives on coats for anti-corrosion studies using weight loss measurements, salt spray tests and electrochemical techniques, all of them performed in acidic solutions. Finally, when the microbial adherence is evaluated, the results are similar, the attachment is lower when the surface is coated with Zn-CNTs, likely because of the ability of CNTs of acting as physical barrier to the corrosion process by filling in crevices, gaps, and micro holes on

the surface of deposit. During corrosion processes, the zinc is washed away, leaving the CNTs on the metal surface, reducing the corrosion rates and metal loss. Further studies in electrochemistry (impedance measurements) are necessary to confirm that the resistance of Zn-CNTs are higher than the zinc only when used as inhibitor.

#### Impedance analysis

The complex diagram showed in [Figure 6\(a\)](#) for Zn epoxy coats shows a loop with a finite semicircle, and an equivalent circuit represented in [Figure 7](#) can describe the characteristics of this system by using electrical elements (fitting parameters are shown in [Table I](#)). The  $R_{ic}$  magnitude represents the resistance of the Zn atoms reacting with the electrolyte. The magnitude or charge transfer decreases, meaning the reactive surface is increasing with time; more active particles are reacting while the electrolytic medium with microorganisms is getting inside of the coating. When the CNT is added onto the coats, the electrical connection between atoms produces a larger activation area; the charge transfer resistance prevails in this sample with a decreasing magnitude with time in comparison with the no-CNT sample as it is shown in [Figure 6\(b\)](#).

#### Conclusions

The strategy applied in the present study was to evaluate different fixation methods to identify corrosion mediated by different microorganisms on aerobic and anaerobic conditions using SEM. GA 2.5 per cent w/v was the more reliable method, either using marine consortiums or FBC. The artefacts were reduced and the different layers corresponding to corrosion

**Table I** Fitting parameters from equivalent circuit simulation for Zn and Zn + CNTs hybrid coating in presence of marine bacteria consortium

Marine bacteria consortium	Time (day)	$R_s$ (ohm $cm^2$ )	$R_{ic}$ ( $R_{biofilm}$ ) (ohm $cm^2$ )	$Q_{ic}$ ( $Q_{biofilm}$ ) ( $10^{-10}F cm^{-2}$ )	$n_b$ [1]	$R_{ct}$ (ohm $cm^2$ )	$Q_{dl}$ ( $10^{-09}F cm^{-2}$ )
Zn	7	$8.6 \times 10^{-4}$	350	48.3	0.7	49855.4	55214
ZnCNT	7	23.5	207	31.7	0.6	36432.2	13256

were clearly visible (cracking, microspheres, EPS and cell morphology). For further evaluation, it would be ideal to include the critical dry point along with the GA as a fixation method on steel, as well as on surface with different coats, to observe the effect of microorganisms on corrosion mechanisms from a wider point of view. The microbial adherence on USN1008 was dependent on its nature. The nature of the composite affected the microbial adhesion and as consequence the corrosion mechanism. The CNTs provided a barrier to the corrosion medium and the microbiological environment. This study opens a window for further evaluations of CNTs associated with metals as active materials to assess the corrosion on extreme corrosive environments, like in oil and gas industry the microorganisms play an important role either, to increase or reduce the corrosion processes.

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