Int. J. Electrochem. Sci., 11 **(2016)** 7750 – 7763, doi: 10.20064/2016.09.37

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Assessment of the Antifouling Effect of Exopolysaccharides Incorporated into Copper Oxide-Based Organic Paint

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Received: 31 May 2016 / *Accepted:* 11 July 2016 / *Published:* 7 August 2016

The anticorrosive and antifouling performance of copper oxide-based organic coatings incorporated with microbial exopolysaccharides (EPS) was studied using electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) after immersion in water produced by oil wells and *in natura* seawater. EPS incorporation in the paint did not influence the coating's final anticorrosive behavior. Moreover, no bacterial adhesion occurred during the immersion period in the presence of EPS, indicating the biopolymer as a promising biocide for the antifouling paint sector.

Keywords: EIS, microbiological corrosion, paint coatings, copper.

1. INTRODUCTION

Various factors may control the performance of anticorrosive paint for protection of metallic structures. The main function of such coatings is to act as a functional barrier between the metallic surface and the environment, controlling diffusion/migration of water and other chemical species to the coating/substrate interface [1,2]. When submerged metallic structures are subject not only to corrosion, but also to biological fouling or biofouling. This process can be defined as an undesirable accumulation of microorganisms, algae and animals on submerged substrates, with consequent deterioration of the surface [3]. First identified over 200 years ago, this phenomenon is a natural destructive process which affects metallic structures, causing major losses in the form of property damage and production downtime [4-5]. Problems related to biofouling started receiving greater attention by the scientific community in the 1960s, accompanying developments of the naval industry and offshore activities [6,7]. Systems based on copolymers with copper oxide (Cu_2O) or zinc oxide (ZnO) and organic biocides are commonly used to control biofouling [8]. However, the protection provided by these coatings does not last longer than 12 - 18 months, due to constant erosion [9]. The high concentration of heavy metals, like copper and zinc, in coastal areas also gives rise to great concern about biocides used in commercial antifouling protection systems [10]. The substitution of environmentally compatible substances for these biocides is thus a matter of great interest, and in this context, the use of natural products inhibiting the formation and growth of fouling organisms is an important technological advancement for the antifouling coating industry sector [11]. For this reason, exopolysaccharides (EPS) have been proposed as a promising antifouling compound [12].

This study reports the behavior of copper oxide (Cu_xO)-based anticorrosive coatings incorporated with EPS extracted from marine cyanobacteria as an auxiliary biocide for application as an antifouling compound.

2. MATERIALS AND METHODS

2.1 Production of paint with EPS

This study assessed the use of copper oxide (Cu_xO)-based paint (commercial paint) with and without incorporated EPS for protection of carbon steel against corrosion and biocorrosion. EPS was obtained according to the method described by Melo *et al.*[13] and was ground and incorporated to the paint in the solid state at a concentration of 30% (percentage in mass).

Coupons of AISI 1020 carbon steel with an area of 4.0 cm^2 were previously sandblasted and sanded (up to 600 grit sandpapers), degreased by immersion in acetone and dried with hot air jets. This material was used in experimental tests with and without EPS incorporated to coatings. Copper wire was welded into coupons in order to allow electrochemical measurements and monitoring of electrochemical parameters. Both coupons were embedded in epoxy resin, and the exposed carbon steel surfaces were coated with the paints produced.

2.2 Corrosive medium

Table 1. Constitution of water from oil wells of the post-salt and pre-salt regions. (Provided by Petrobras S.A.)

Composition	Concentration ($mol.L^{-1}$)	
	Pos-salt	Pre-salt
$C_2H_3O_2^-$	0.00896	-
HCO_3^-	-	0.005
Cl^-	1.653	3.082
$HCOO^-$	2.22×10^{-5}	-
SO_4^{2-}	6.45×10^{-4}	9.37×10^{-4}

Br^-	-	0.0058
Ba^{+2}	1.53×10^{-4}	2.99×10^{-4}
B^{3+}	-	0.0241
Ca^{2+}	0.106	0.084
Sr^{2+}	4.97×10^{-3}	0.026
Fe^{+2} and Fe^{+3}	1.97×10^{-4}	$< 1.8 \times 10^{-5}$
Mg^{2+}	0.0162	0.011
K^+	0.00987	0.0624
Na^+	1.536	0.29
Mn^{2+}	5.76×10^{-5}	-
Li^+	-	$8,56 \times 10^{-4}$

Water produced in oil reservoirs of the post-salt and pre-salt regions (Table 1) was provided by PETROBRAS S.A. and used for anticorrosion tests. The medium was previously sterilized by autoclaving at 120ºC for 15 minutes to avoid formation of bacterial biofilms.

2.3 Coating analysis by Electrochemical Impedance Spectroscopy (EIS)

To assess the performance of coating with EPS, EIS measurements were carried out with a sinusoidal voltage signal of 10 mV amplitude and it was immersed for 144 days in water produced by post-salt and pre-salt oil wells. Impedance was measured as a function of immersion time, with potentiostatic regulation on an open circuit potential (OCP). The experiment was performed in a conventional cell with three electrodes attached to an AUTOLAB PGSTAT 30 potentiostat/galvanostat in a Faraday cage, using a saturated calomel reference electrode, a platinum auxiliary electrode and a carbon steel electrode coated with the paint under analysis. The frequency range applied was from 100 kHz to 0.01 Hz. The obtained results are shown as Nyquist and Bode diagrams.

2.4 Assessment of Biofilm Formation through Scanning Electron Microscopy (SEM)

Coupons coated with paint with and without EPS were immersed in *in natura* seawater for a period of 7 days. These assays were carried out in the laboratory, using 18 L of unstirred *in natura* seawater in a 20 L container. Before the experiment, the container was disinfected by immersion in a 5g/L sodium metabisulfite solution and then washed five times with distilled water to completely remove the disinfectant. To assemble the experimental system, these coupons were suspended in a PVC outer structure and immersed in seawater. The container was maintained at room temperature, and 10 L of the experiment seawater were exchanged by fresh *in natura* seawater every 24h, in order to keep minimal nutritional conditions to ensure cell viability. At the end of the assay (7 days), SEM photographs of the different coupons subjected to each condition were taken with the aim of visualizing the colonized surfaces by the biofilms produced by the marine bacteria.

3. RESULTS AND DISCUSSION

3.1 EIS characteristics of coating/metal system

Various authors [14,15] have shown the correlation between electrochemical impedance spectra and corrosion rates in order to assess corrosion protection behavior. This relation is relevant, as this spectroscopic technique is a non-destructive electrochemical test which may be applied in real-time assessment of corrosion rates. Furthermore, direct measurement of corrosion current along with impedance allows determination of the proportionality coefficient associating corrosion current with the various resistances estimated from the EIS. Thus, EIS may be used to assess paint corrosion protection behavior, since it has the following advantages: (i) it provides information regarding the process kinetics in the form of corrosion rate; (ii) it is a precise and reproducible technique appropriate for high resistivity media; (iii) it provides data regarding the electrochemical control mechanism, indicating whether the corrosive process occurs by activation, concentration or diffusion; (iv) it is a non-destructive, non-perturbative method, since signals applied have low amplitude and do not alter corrosion potential; and (v) it allows monitoring of the time evolution of passive or active states.

For all assessed coatings, quality of the Nyquist and Bode diagrams at medium and low frequencies was initially poor due to the dispersion of results, which made it impossible to distinguish the behavior of the different systems [16]. For this reason, adjustments could only be done in the high frequency region. Coating capacitance was initially determined at 100 kHz, providing a parameter for monitoring electrolyte permeation throughout the experiment [17]. Coating capacitance was calculated using equation (1):

$$C_c = \frac{1}{2\pi f\,|Z|\,\sin\theta} \qquad (1)$$

Where f is the frequency of the applied AC voltage; $|Z|$ is the absolute value (modulus) of impedance; and θ is the phase angle. Capacitance evolution towards higher values is associated with the penetration of water, oxygen and electrolytes. EPS-incorporated coatings had the higher initial capacitance values, indicating higher electrolyte permeation at the beginning of the experiment and consequently lower paint resistance (Table 2).

Table 2. Capacitances in Faradays (F) of different coatings at 100 kHz.

	Pos-salt		Pre-salt	
	Coating without EPS	Coating with EPS	Coating without EPS	Coating with EPS
$Cc_{t=0}$	1.63×10^{-12}	1.11×10^{-10}	9.67×10^{-12}	1.70×10^{-11}
$Cc_{t=144}$	1.63×10^{-9}	3.70×10^{-8}	3.54×10^{-9}	4.02×10^{-9}

Impedance values at high frequency indicate the coating's capacitive behavior and, consequently, may be used to assess paint porosity at the beginning of immersion time. Paints with high resistivity would show as straight lines parallel to the imaginary axis in Nyquist's diagram. As electrolyte permeation occurs, such straight lines are gradually converted into semicircles. Therefore, higher impedance values, and more parallel lines to the imaginary axis, correspond to lower coating porosities. Fig. 1 shows that the coating with EPS immersed in post-salt produced water has initially higher porosity, while other coatings have greater resistivity. This behavior may be explained by an increase in porosity due to EPS presence and higher permeation of post-salt water and associated electrolytes due to a lower concentration of their constituent ions and, consequently, a lower impediment to enter the pores of this material.

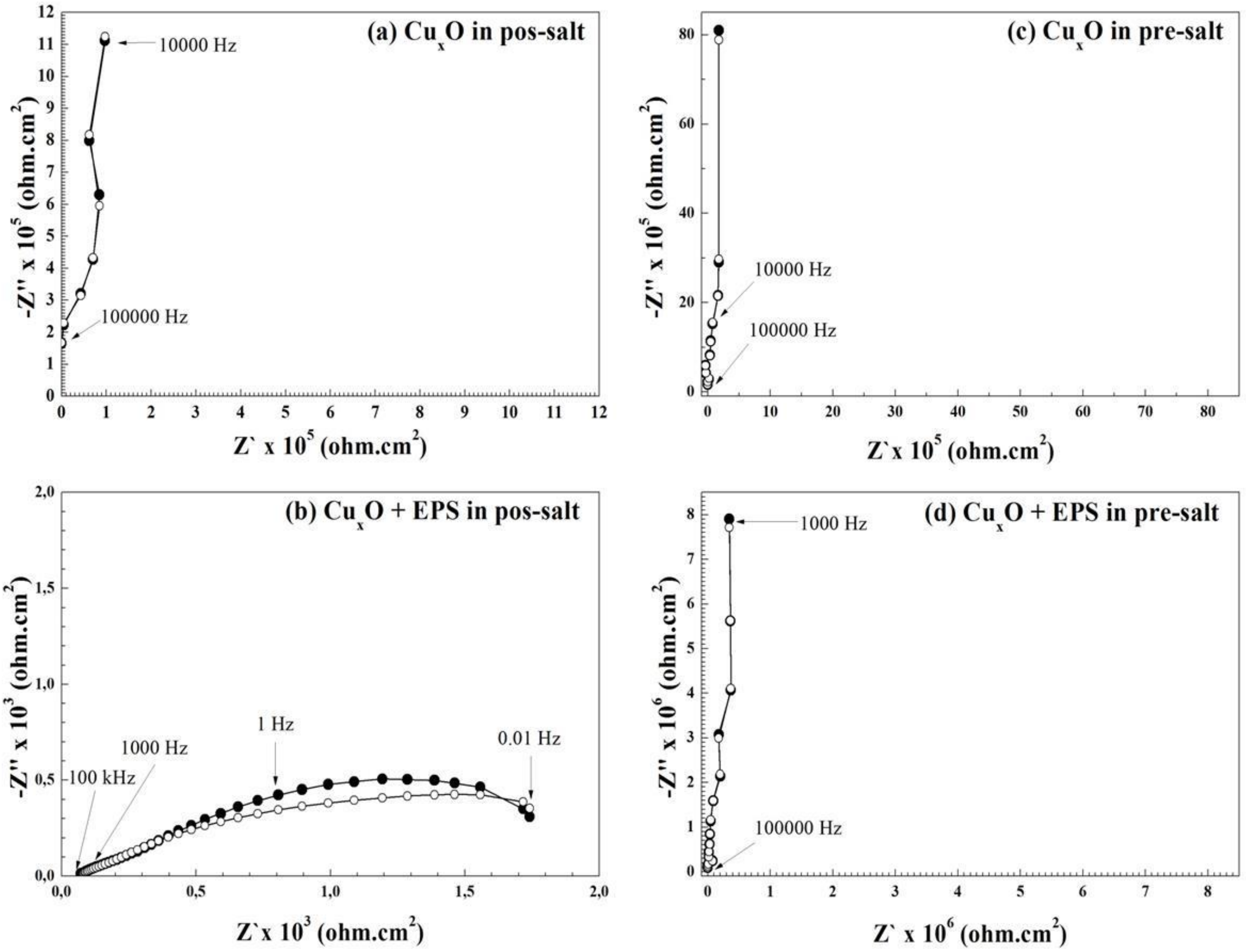


Figure 1. Nyquist diagrams (t = 0) of copper oxide-based organic paint in the absence and presence of EPS immersed in water produced by oil wells. The figure shows experimental results (●) and the respective fitted values (○).

After 144 days of immersion, however, all coatings showed capacitances with the same order of magnitude regardless of EPS presence, indicating that all paints have similar water permeation. In this context, an equivalent electrical circuit (EEC) (Fig.2) was proposed to explain the behavior of coatings with and without EPS after this period of immersion in water produced in post-salt and pre-salt oil wells. This circuit was also used to analyze the values obtained with the impedance spectrum through the Kramers-Kroning (KK) relation [18]. The KK relation provides a link between the real and imaginary parts of a complex number.

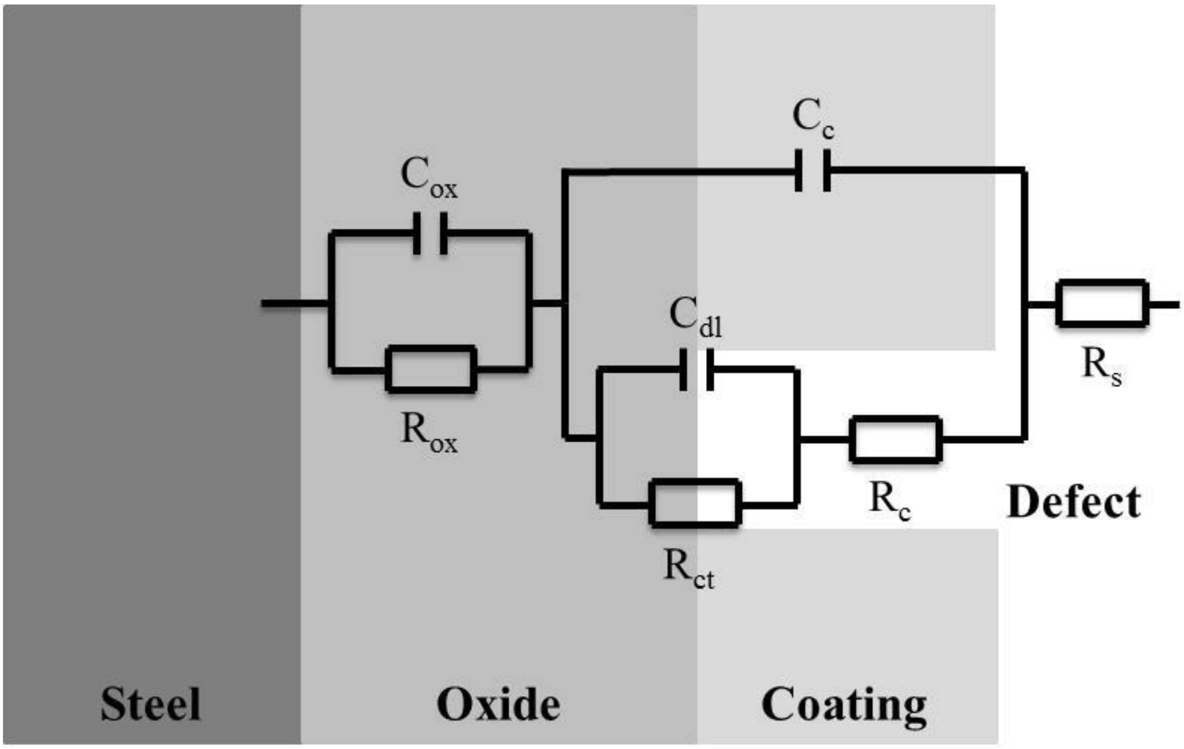


Figure 2. Equivalent electrical circuit proposed for copper oxide-based organic coatings in the absence and presence of EPS after 144 days of immersion in water produced by oil wells; Rs: solution resistance, Cc: paint capacitance, Rc: paint resistance, Cdl: bilayer capacitance, Rct: charge transfer resistance, Cox: oxide layer capacitance and Rox: oxide layer resistance.

Table 3. Parameters and error values in the EIS analysis of coating after 144 days of immersion.

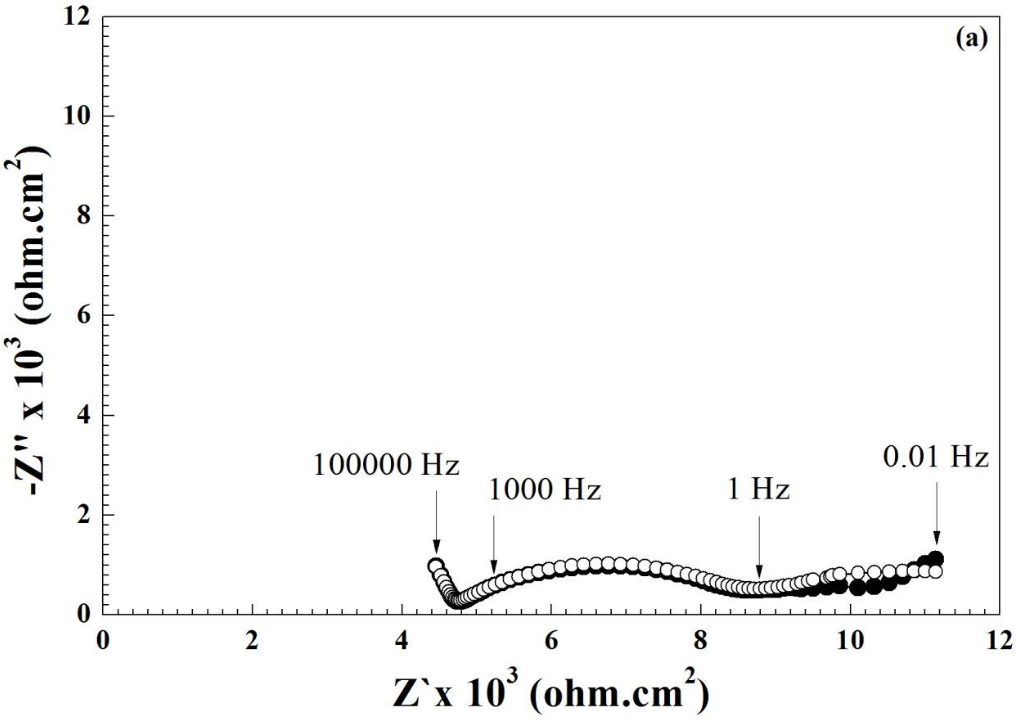
		Coating without EPS		Coating with EPS	
		Pos-salt	Pre-salt	Pos-salt	Pre-salt
R_s	Ω	4060	904	1360	880
	error %	0.45	1.21	0.24	1.02
C_c	F	6.0×10^{-6}	5.64×10^{-5}	1.19×10^{-7}	1.70×10^{-5}
	error %	3.79	2.22	1.04	2.87
n_1	value	0.581	0.459	0.646	0.677
	error	0.89	0.78	0.13	0.66
R_{ct}	Ω	4250	4590	4890	3220
	error %	3.84	1.49	0.58	2.01
C_{dl}	F	7.61×01^{-4}	4.79×10^{-4}	1.87×10^{-4}	7.38×10^{-5}
	error %	2.70	1.49	0.54	1.46
n_2	value	0.476	0.498	0.347	0.432
	error %	2.46	1.15	0.38	1.46
R_{ox}	value	4360	1950	95200	1760
	error %	0.19	0.23	4.42	0.26
C_{ox}	F	7.98×10^{-11}	1.80×10^{-10}	1.16×10^{-6}	1.93×10^{-10}
	error %	3.22	4.37	3.51	4.32

In other words, this test relates the invariance of linearity, time and causality of the equivalent electrical circuit proposed with experimental data [19]. A KK test with values $<10^{-6}$ means that the proposed EEC is the system that is the most likely to explain the phenomenon observed. KK test values between 10^{-5} and 10^{-6} are considered reasonable, while values between 10^{-4} and 10^{-5} are considered marginal and those $>10^{-4}$ indicate an invalid EEC. KK test results for the equivalent electrical circuit [R([R(RQ)]Q)(RQ)] indicate that the circuit proposed may be used to explain coating behavior with and without EPS (Table 3). Furthermore, it can be seen in Table 4 that the error of values obtained with the elements composing the EEC do not exceed 5.0%, validating the proposed circuit and its corresponding results.

Table 4. Kramers-Kronig test of the equivalent electrical circuit [R([R(RQ)]Q)(RQ)] for coatings in the absence and presence of EPS after 144 days of immersion; Z: total impedance, Z`: real impedance e –Z": imaginary impedance.

Kramers-Kronig	Coating without EPS		Coating with EPS	
	Pos-salt	Pre-salt	Pos-salt	Pre-salt
Z	6.08×10^{-9}	8.63×10^{-7}	2.75×10^{-8}	2.92×10^{-8}
Z`	2.69×10^{-9}	4.26×10^{-7}	1.32×10^{-8}	1.16×10^{-8}
-Z"	3.39×10^{-9}	4.37×10^{-7}	1.43×10^{-8}	1.76×10^{-8}

According to Lee *et al* [20], coating performance based on the results of the logarithm of absolute impedance values (log | Z | / ohm.cm^2) may be evaluated as follows: (i) excellent protection when log | Z |> 10, (ii) good protection when log | Z |> 8 and (iii) low protection when log | Z | ≤ 4. Moreover, the phase angle value shown in the Bode diagram at the high frequency region, usually 100 kHz, is used in different studies to assess coating degradation, even when measurements are done in the whole frequency domain (10^5 a 10^{-2} Hz) [21]. According to Touzain [21], a low phase angle value in high frequencies represents an ineffectual coating. The results shown in Bode phase diagrams of copper oxide-based organic coatings immersed in water produced by oil wells from the post-salt and pre-salt regions indicate their low corrosion resistance, regardless of EPS presence.



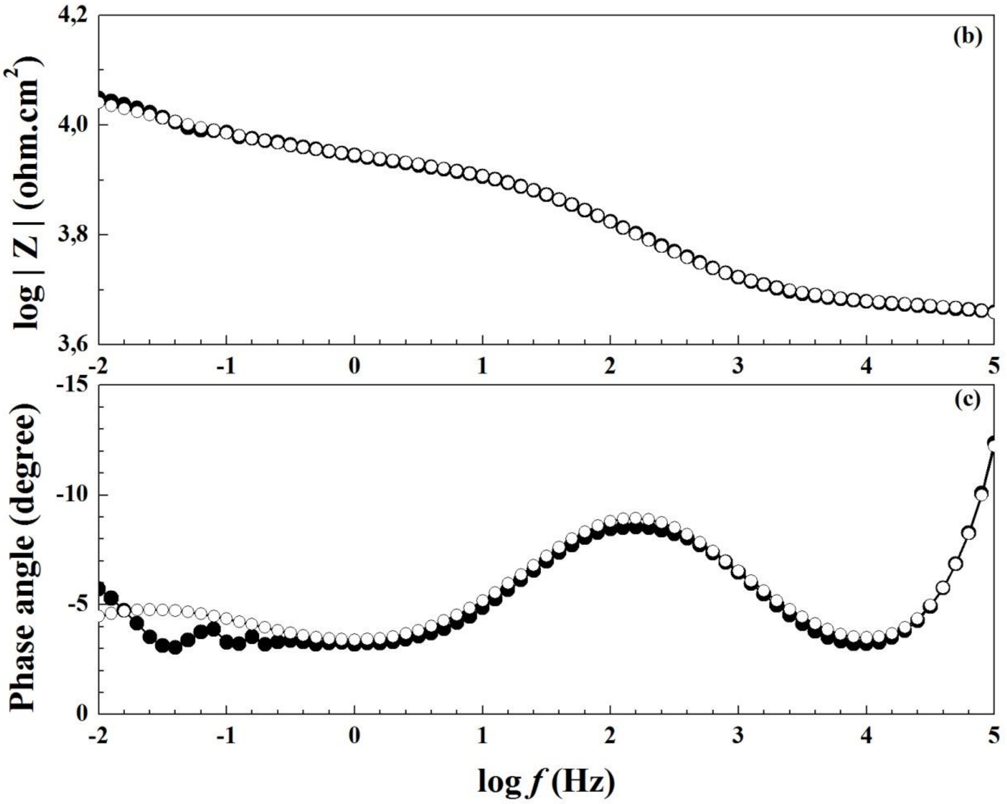
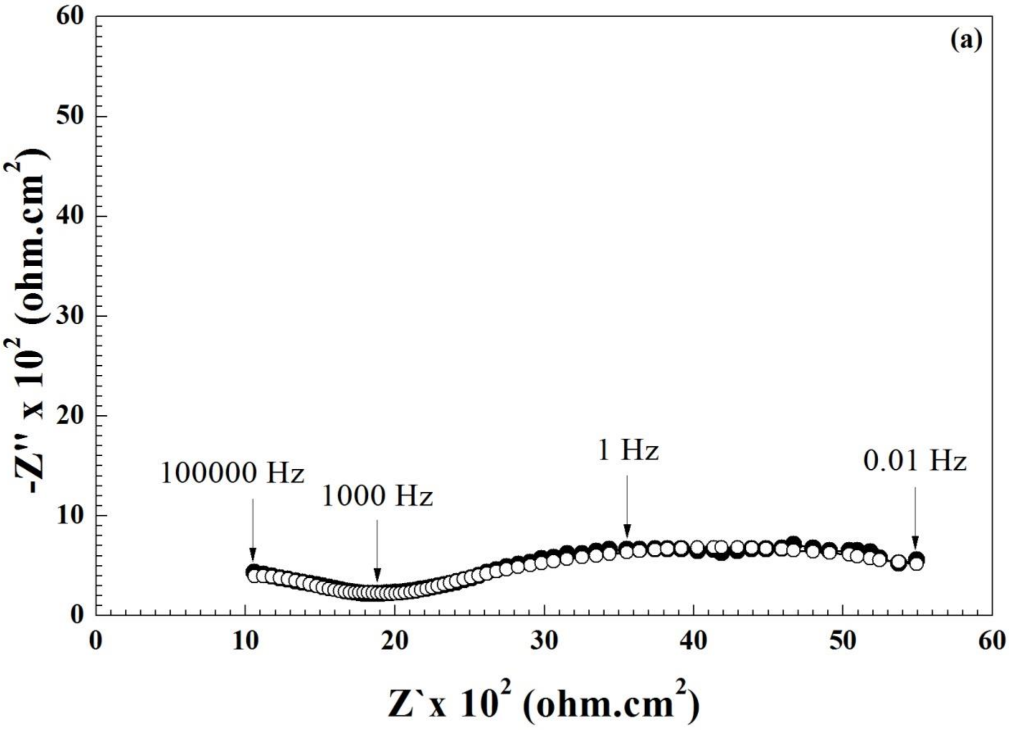


Figure 3. Nyquist diagrams (a), Bode Modulus plot (b) and Bode Phase plot (c) of the copper oxide-based organic coating in the absence of EPS after 144 days of immersion in water from post-salt production. The figure shows experimental results (●) and the respective fitted values (○).



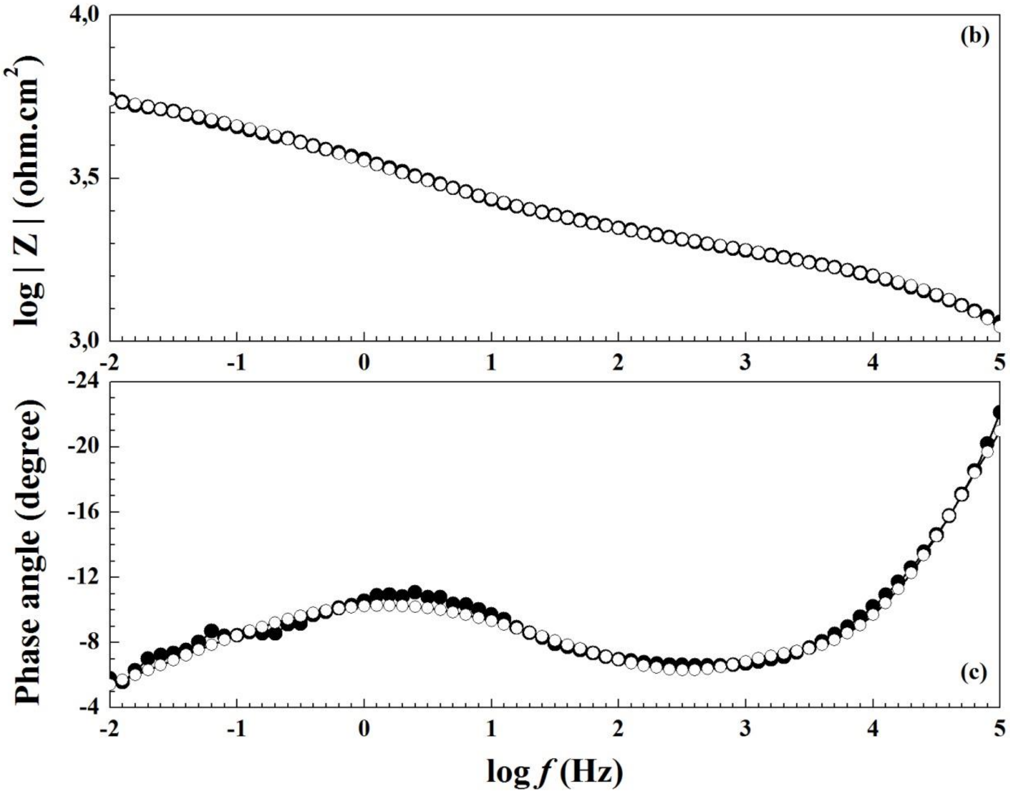


Figure 4. Nyquist diagrams (a), Bode Modulus plot (b) and Bode Phase plot (c) of the copper oxide-based organic coating in the presence of EPS after 144 days of immersion in water from post-salt production. The figure shows experimental results (●) and the respective fitted values (○).

Paint impedance diagrams in the absence and presence of EPS after 144 days of immersion in post-salt water are shown in Fig. 3 and Fig. 4, respectively. The Figures show higher impedance values for the coating without EPS, indicating greater coating degradation when associated to EPS. The Bode modulus diagram (Fig. 3b and 4b) shows the same profile in the different analyzed frequencies, and the Bode phase diagram (Fig. 3c and 4c) has higher values of phase angle in intermediate frequencies in the presence of EPS, indicating the existence of corrosion products in the inner part of the coating.

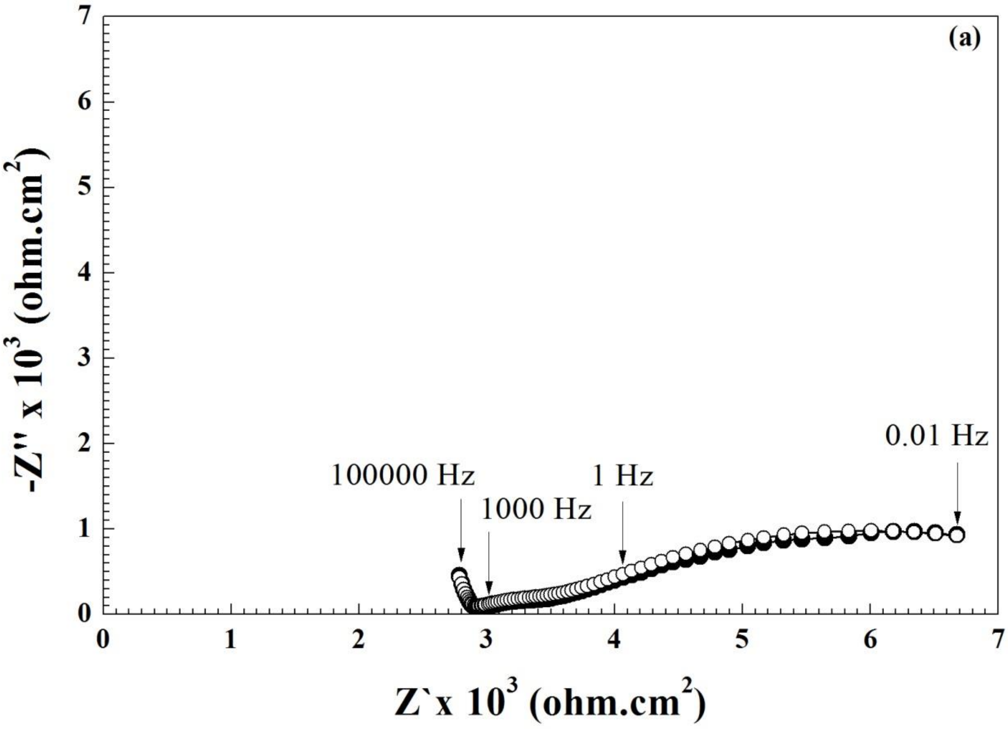
Paint impedance diagrams in the absence and presence of EPS after 144 days of immersion in pre-salt water are shown in Fig. 5 and Fig.6, respectively. The Figures show similar impedance values for both coating types (Fig 5a and 6a). While the Bode modulus diagram (Fig. 5b and 6b) shows the same profile in the different analyzed frequencies, the Bode phase diagram (Fig 5c and 6c) has higher phase angle values at low frequencies regardless of EPS presence, indicating the existence of corrosion products in the external layer of the coating.

The EEC proposed for these coatings considered that water and oxygen molecules reach the surface of the substrate through pore formation, originating electrochemical reactions at the paint-metal interface. The variable C_{dl} is associated to the bilayer capacitance, while R_{ct} represents charge transfer resistance. The proposed EEC also includes the variables R_{ox} and C_{ox}, respectively representing the resistance and capacitance of the corrosion products layer accumulated at the surface of the electrochemically active site.

3.2. Assay of bacterial adhesion in in natura seawater

Coated metallic structures are not immune to different types of corrosive processes. Corrosion occurs more easily in saline environments due to the presence of aggressive ions such as chlorides [22]. However, the presence of microorganisms on surfaces may stimulate an increase of localized corrosion processes through reactions at the material interface caused by bacterial metabolic activity [23]. To evaluate this type of process, scanning electron microscopy (SEM) was used to assess the adhesion of marine biofilm forming bacteria on coupons coated with copper oxide-based organic paint in the presence and absence of EPS in *in natura* seawater (Fig. 7).

Copper-based organic coatings without (Fig. 7a) and with (Fig. 7b) EPS show no bacterial presence before the beginning of the experiment. After EPS incorporation, the formation of pores can be recognized in the material, suggesting an alteration of initial anti-corrosion performance, as indicated in EIS results. The first stage of the process of microbial corrosion and formation of marine fouling is associated with the formation of bacterial biofilms. According to their composition, these polymers may promote an increase in the quantity of water in coating pores [24]. For this reason, biofilms may receive electrons coming from the pores of coated metals, favoring the flow of electrons from the metal (anode) to different electron acceptors like oxygen (cathode), accelerating the corrosion process [25]. When coatings are placed in *in natura* seawater they immediately undergo complex physical, chemical and biological interactions, resulting in absorption /adsorption of dissolved organic materials, electrostatic changes and other substrate-specific characteristics [26]. Fig. 7c shows the adhesion of marine bacteria, indicating initial formation of a marine bacterial biofilm, due to formation of a conditioning polymeric matrix. Formation of the conditioning biofilm is visible to naked eye as a thin rough layer adsorbed to the coating.



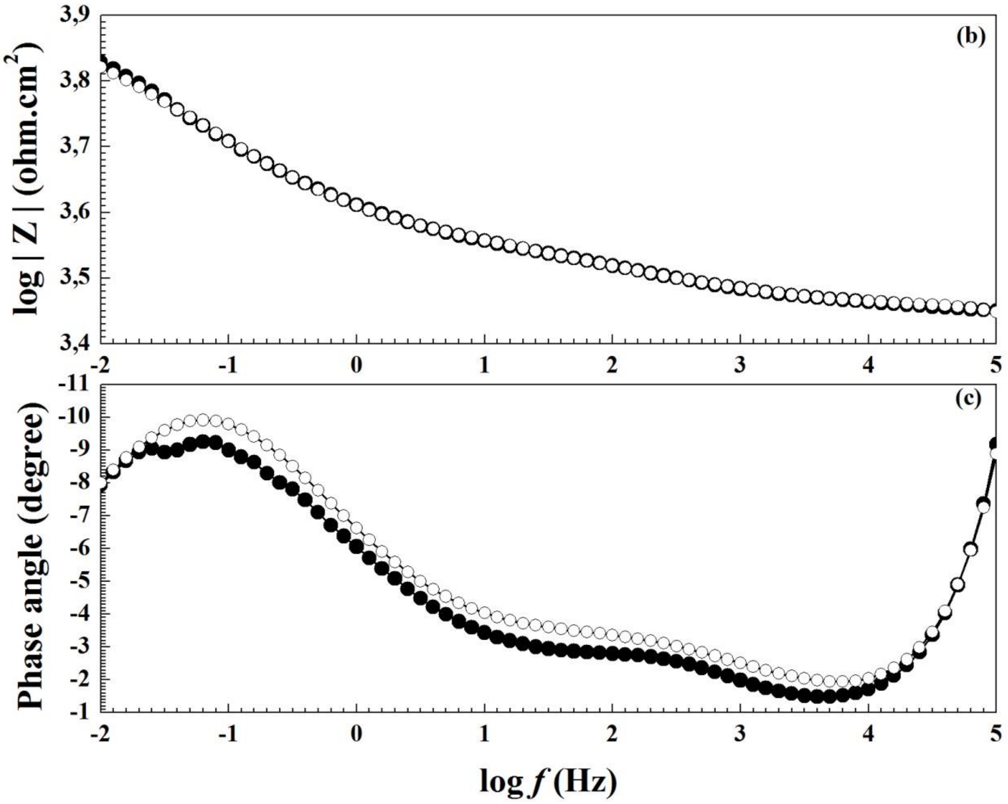
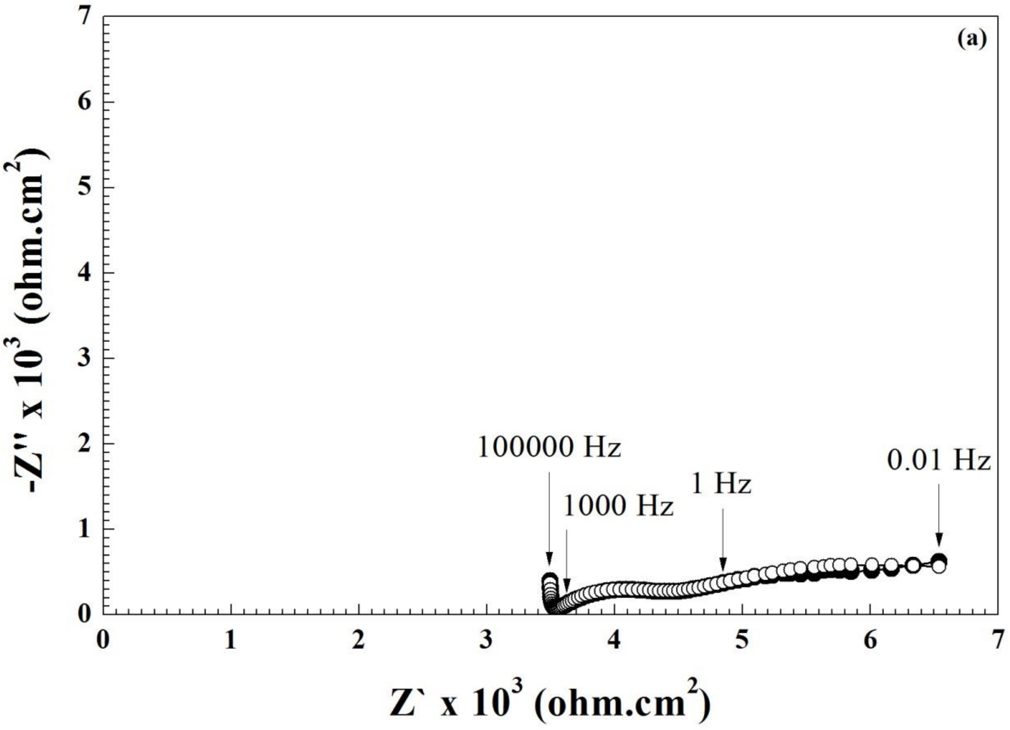


Figure 5. Nyquist diagrams (a), Bode Modulus plot (b) and Bode Phase plot (c) of the copper oxide-based organic coating in the absence of EPS after 144 days of immersion in water from pre-salt production. The figure shows experimental results (●) and the respective fitted values (○).



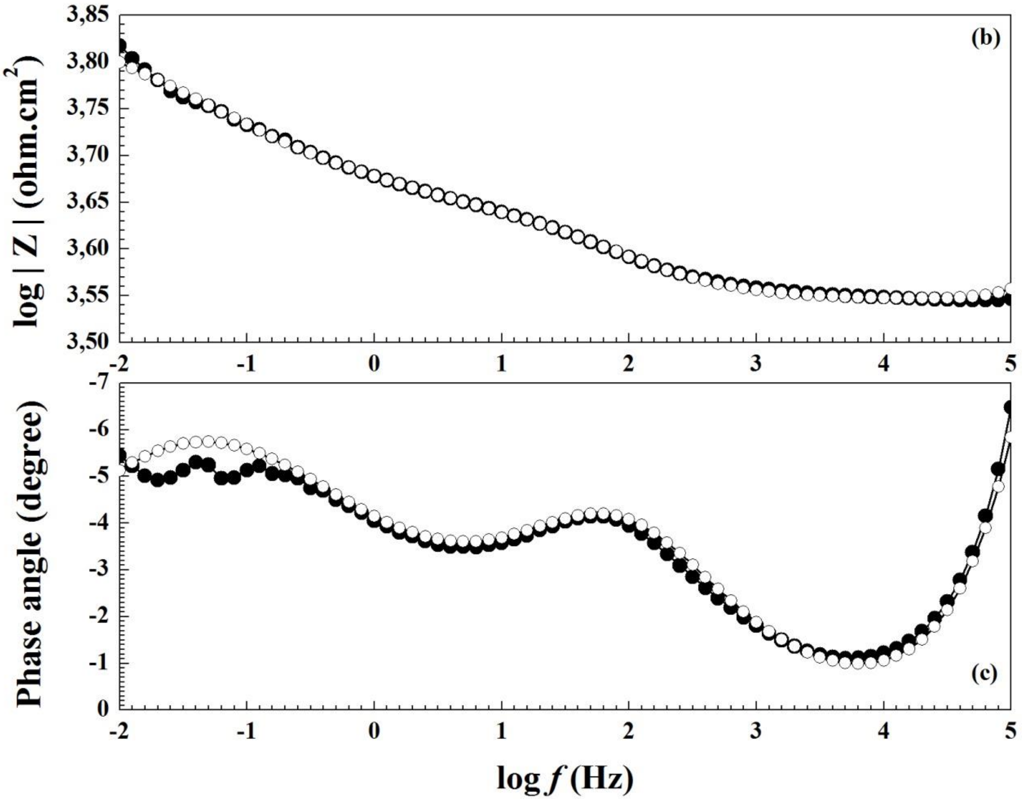


Figure 6. Nyquist diagrams (a), Bode Modulus plot (b) and Bode Phase plot (c) of the copper oxide-based organic coating in the presence of EPS after 144 days of immersion in water from pre-salt production. The figure shows experimental results (●) and the respective fitted values (○).

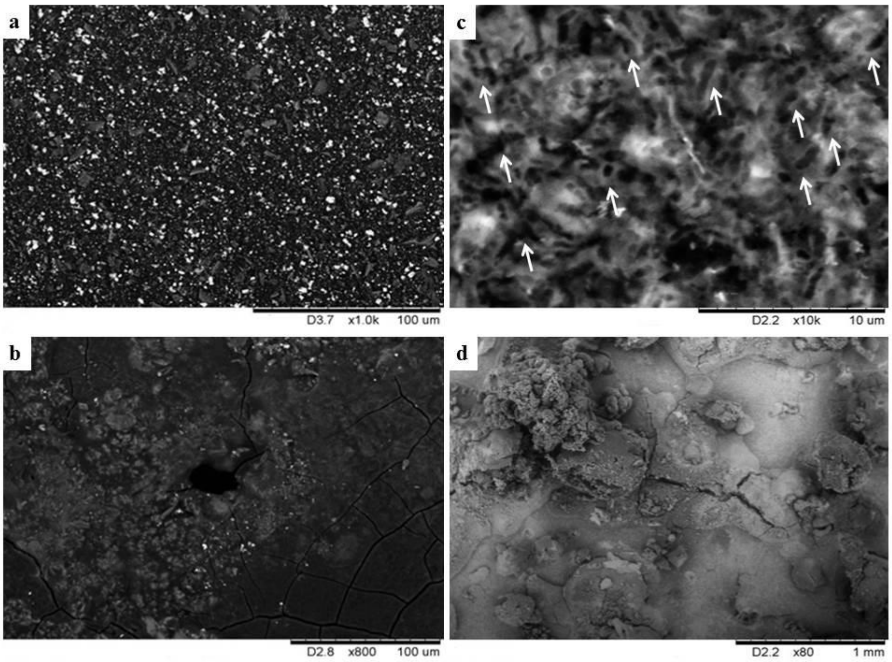


Figure 7. Scanning electron microscopy of copper oxide-based organic coatings immersed in *in natura* seawater; (a) initial coating without EPS; (b) initial coating with EPS; (c) final coating without EPS; (d) final coating with EPS.

When EPS was incorporated to the coating (Fig. 7d), corrosion products at the coating surface could be seen after 7 days of immersion in *in natura* seawater, but no marine bacteria were observed. EPS incorporation therefore inhibited bacterial establishment, indicating a delay in the coating colonization process.

4. CONCLUSION

One of the aims of this study was to characterize corrosion protection behavior of copper oxide-based organic paint with and without the association of biocide EPS immersed in water produced in pre- and post-salt region oil wells, using the EIS method. The equivalent electrical circuit used to interpret EIS spectra had very good adjustment to experimental data. Copper oxide-based coating showed no EPS-related changes in its corrosion protection property, since its behavior in the presence and absence of the biopolymer was the same after 144 days of immersion. Therefore, EPS did not alter the coating's anticorrosive properties during the studied period.

Furthermore, this study assessed the antifouling action of biopolymers associated to anticorrosive paint applied on metallic surfaces exposed to *in natura* seawater. Laboratory assays to evaluate the performance of modified paint regarding the formation of marine bacterial biofilm on carbon steel coupons coated with different paints with and without EPS clearly showed the biopolymer's antifouling effect under the conditions tested. The study's results establish that EPS is a promising biocide for the antifouling paint sector.

ACKNOWLEDGEMENTS
The authors thank the Brazilian Coordination for Improvement of Higher Education Personnel (CAPES) for their support in developing this study.

Competing Interests
The author(s) declare(s) that there is no conflict of interest regarding the publication of this manuscript.

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