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SRB-BIOFILM GROWTH AND INFLUENCE IN CORROSION MONITORING BY ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY

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ABSTRACT

A mixed culture of *D. gabonensis* and *D. capillatus,* in synthetic seawater supplemented with nutrients formed a biofilm combined with ferrous sulfide corrosion products that largely covered carbon steel electrodes (SAE-1020). Electrochemical impedance spectroscopy (EIS) and open circuit potential (OCP) measurements were carried out for this study. Electrochemical impedance results indicated the formation of three films: an inner corrosion products film, an outer corrosion products film and the biofilm or organic film in abiotic media. After one month of batch culture, it was observed that during biofilm formation there are two different controlled processes. During the first period activation-diffusion controlled corrosion was observed. The second period showed diffusion controlled corrosion due to biofilm establishment. Impedance spectra denote an increase in R_{ct} (charge transfer resistance) with time in the presence of biofilm. Magnitudes of the phase angle contributions are indicative of biofilm and corrosion products interphases heterogeneity during primary formation stages.

Keywords: sulfate-reducing bacteria, heterogeneous biofilm, heterogeneous interphases, impedance monitoring.

INTRODUCTION

Aqueous environment promote metals surface oxidation. Furthermore, under bacterial presence, biofilm formation also occurs, which might influence corrosion phenomena. Biofilms have been identified as heterogeneous aggregates, which are built in communities over materials surfaces or they are associated with interphases by a self-produced extrapolymeric matrix [1-5]. Biofilms might affect corrosion by changing the nature of the physicochemical interactions between metallic materials and their environment or the rate controlling step, thus accelerating or inhibiting the corrosion process [6, 7].

Sulfate-reducing bacteria (SRB) are able to form biofilms over carbon steel surfaces in poorly oxygenated areas. Thus, these bacteria are frequently involved in corrosion under anaerobic conditions and they act to transform sulfurs in hydrogen sulfide which, in the presence of ferrous ionic compounds tend to form ferrous sulfides [8, 9]. Different species of SRB have different influence on the corrosion process [9]; therefore experiments were conducted to study the effects of lately discovered SRB strains. *D. gabonensis* [10] and *D. capillatus* [11] are halophilic, hydrogenase positive sulfate-reducing bacteria, recently isolated from oil industry marine installations affected by corrosion. The role of these bacteria in corrosion phenomena has not been entirely documented.

Complexity of biocorrosión processes requires the utilization of an extensive variety of techniques for various analyses. Since biofilm development, metabolic activity and corrosion can be approached as electrochemical-nature based processes [7], electrochemical measurements can be used to study them. When open circuit potential (OCP) measurements have been carried out, an increase of this value through time has been shown due to iron protecting biofilms [12]; however, when iron passivation occurs this behavior is also exhibited, even in the presence of SRB, which can lead to localized corrosion phenomena [13]. Electrochemical impedance spectroscopy (EIS) has been used in the study of biocorrosion [14, 15], for monitoring corrosion rates [12], SRB influence in corrosion of buried pipelines [16], SRB influence in corrosion of reinforced concrete [17], bacterial corrosion inhibition [18], biofilm formation and influence in corrosion [19, 20, 21], etc. EIS provides quantitative and mechanistic information for biocorrosion processes [22].

An increase in capacitance values due to the presence of sulfate-reducing bacteria biofilm has been demonstrated [23, 20]. Polarization resistance changes are also indicative of this phenomenon [23]. Likewise, it was established that biocorrosión process can not be evident by EIS; on the other hand this technique supplied information about the metal surface and formation of biological interphase [23].

However, data obtained by EIS have not been extensively associated to biofilm formation during corrosion phenomena. Therefore, in this study we propose that marine SRB-biofilm growth and influence on corrosion can be monitored by EIS analyzing phase angle variations and Nyquist diagrams.

MATERIALS AND METHODS

Experimental system

The experiments consisted of growing biofilms in glass tubes of 2.5 cm diameter and 17.5 cm length, filled with 65 ml of culture media, covered anaerobically with neoprene rubber stoppers (where the electrodes were pressure-fixed and interstices sealed with silicone), then closed with a twisted plastic cap. Rectangular SAE-1020 carbon steel coupons with a surface of 9.6 cm^2 polished with 300-grit polishing paper were used as working electrodes. Titanium electrodes of 1.2 cm and 0.5 cm diameter were used as counter and reference electrodes respectively. Only one face of the electrodes was exposed to the culture media, the other faces were covered with polystyrene resin and the electrical connection was made using an insulated copper wire and coated with polystyrene resin. Prior to immersion, coupons were rinsed with distilled water, degreased with acetone, air-dried, sterilized in 70% ethanol solution for 30 min. and maintained air-drying in sterile conditions until introduced in the described system, which was purged with N_2 flux and filled with an appropriate culture medium previously autoclaved at 121°C/15lb for 15 min.

Media and biofilm culture conditions

Desulfovibrio gabonensis (DSM 10636) and *Desulfovibrio capillatus* (DSM 14982) strains were collected from pure strain cultures (same medium as described subsequently) during exponential growth phase, and 1.5 ml of each was used as inoculum into the culture media of the experimental system in order to form biofilms over carbon steel surface. Bacteria were grown in supplemented artificial seawater, as culture media, prepared with the following components (per liter of distilled water): Na_2SO_4 (3.0 g), K_2HPO_4 (0.2 g), NH_4Cl (0.2 g), KCl (0.2 g), $MgCl_2$, 2H2O (0.3 g), NaCl (30 g), $CaCl_2$, $2H_2O$ (0.1 g), sodium acetate (0.5 g), Difco yeast extract (0.5 g), Difco tryptone peptone (0.1 g), trace mineral element solution (10 ml), cysteine-HCl (0.5 g), resazurin (1mg), $NaHCO_3$ 2% (w/v) and final 20 mM concentration of sodium lactate. The pH value was adjusted to 7.2 with 10 M KOH solution and the medium was boiled and cooled to room temperature under O_2-free N_2 gas stream. The systems were incubated at 30 °C with no stirring. Uncovered working electrode surfaces were but inclined upwards 40° with respect to the horizontal position of the flat surface.

Electrochemical techniques

OCP and EIS measurements were carried out as a sequence of metal-electrolyte interphase experiments under biotic and abiotic conditions for 30 days in duplicate experiments, by using a potentiostat hooked with an FRA unit. The measured potential values on this work refer to Ti/TiO_2 electrode (+0.073 V versus SCE). Acquisition time for OCP measurements was 1 point/sec during half hour periods during the month. For EIS measurements (obtained at OCP) the amplitude of the applied potential was 10 mV over the frequency range 10 kHz to 10 mHz.

RESULTS AND DISCUSSION

OCP measurements

The open circuit potential was followed under sterile culture media and under SRB presence. In the former case, the values were modified from -0.65 V to -0.3 V (vs. Ti/TiO_2 electrode) average and in the latter case were modified from -0.643 V to -0-203 V (vs. Ti/TiO_2 electrode) average (Figure 1). Bacterial presence, in mixed biofilm and planctonic growth, depolarized the electrode by approximately 70-120 mV more than the depolarization caused by pure culture media (Figure 2). This magnitude can be explained by bacterial influence within the environment.

Corrosion potential increases gradually during the first 7 days (168 hours), remaining constant for the rest of the culture period, when SRB maintain 70-120 mV of depolarization with respect to abiotic media (Figure 2). This increased OCP can be due to cathodic depolarization caused by interfacial mechanisms, first proposed in 1934, where adsorbed hydrogen is consumed by hydrogenase positive SRB which induce local acidity [24] controlling corrosion process under anaerobic conditions. Consequently, impedance spectra show phase shift magnitudes at the 7th day or 168 hours of the experiment (Figure 5).

Electrochemical impedance measurements

Sterile culture media. The impedance measurements for the system without biological activity are presented in Figures 3 and 5A for 7 different times during one month of supplemented artificial seawater electrolyte batch exposition. Figure 3 shows three incomplete-like semicircles in Nyquist diagrams. At high frequencies (Figure 3C) semicircle shape appears but only after 668 hours. At initial times, the contribution of this high frequency region exhibits *quasi*-linear tendency, but as the time of abiotic media exposure increases, definition of a semicircle shape showing activation behavior exists, which controls the process at high frequencies due to an external layer. The appearance of this small semicircle might be attributed to the formation of an external layer of organic compounds in culture media (including electron donor lactate) in mixture with semiconducting poorly-adherent iron sulfide and/or other corrosion products, thus contributing to activation control of the charge transfer process with time. In Figure 5A we can verify this behavior at high frequencies, between 10 kHz and 1 kHz, which indicates the contribution of the external organic-inorganic "semiconducting layer" that contributes to the charging of the interface.

At middle frequencies (between 1 and 200 Hz) another incomplete like semicircle is shown (Figure 3B), and another third semicircle appears at the lowest frequencies (Figure 3A). The combination of these two last semicircles, exhibits Nernst layer like behavior [25] where a diffusion layer of finite thickness is manifested. These two semicircles are related to tightly adherent semiconductor corrosion products formation, first an inner heterogeneous layer that interacts with the metallic structure and then an outer homogeneous layer, where the predominant process that can be seen for this abiotic system is activation-controlled corrosion of the metal mainly by the inner heterogeneous layer.

In Figure 3A, we can observe an increasing tendency on the impedance values of the semicircles, which indicate that dissolution process (activation) rate is lower, in this case due to the growth of the inner layer among which can be a passive layer of iron sulfides. While organic and/or external corrosion products layer increase slowly, the inner heterogeneous corrosion products layer shows variations from 25 to 70 degrees which indicates great contribution of this internal porous layer in activation process (Figure 5A).

As observed in OCP measurements for abiotic system, after 7 days of culture (approximately 168 h) there is a change in the behavior of the system, in impedance results this is shown as an important shift to lower frequencies of maximum phase angle contribution (Figure 5A), from 100-10 Hz to 10-1 Hz as shown in Figure 5A, which is indicative of influence of charge transfer activation-control through time due to external heterogeneous layer.

Biotic conditions. Under the presence of halophilic hydrogenotrophic SRB, impedance measurements are presented for 7 different times during one month of batch culture in Figures 4 and 5B. Nyquist diagrams presented in Figure 3, and, as under sterile culture media exposure, three incomplete-like semicircles are shown. At high frequencies in Figure 4D, small semicircle formation is manifested. However, if observing phase angle plot for this system (Figure 5B) at high frequencies (between 10000 and 10 Hz) a magnitude in a different quadrant is observed, this is related to an outer layer that is not completely formed or detached from the surface of the inner layer. Under abiotic conditions the increase on phase angle magnitude was attributed to the formation of an organic-inorganic "semiconducting" layer, then, under bacterial presence this layer disappears, attributed to lactate and organic compounds consumption by bacteria increasing the conductivity of that layer.

At high and middle frequencies incomplete semicircle-like are also observed for initial exposure time, also related to inner and outer corrosion products layers (Figure 4). Described early for abiotic media, outer layer shows specific tendency (Figure 5B). However, there is a difference on inner corrosion products layer formation due to bacterial presence. Under sterile conditions we observed a gradual increase through time of this layer (observed at lower frequencies on Figure 5A), but under SRB-biofilm presence this layer is rapidly formed in comparison to the one formed under sterile conditions. Under sterile conditions the layer reaches its maximum value in 668 hours, while under bacterial presence high values can be observed since 27 hours of culture (Figure 5B), but this layer also shows non-stable growth since phase angle values at lower frequencies associated to its formation randomly oscillate in magnitude.

In Figure (5B), at high frequencies, an increase in phase angle values is observed, this contribution increases attributed to the semiconductor heterogeneous phase formation. This can be due to corrosion products embedded in extrapolymeric biofilm matrix, which first were tightly adhered to metal surface but as the corrosion products layer increases in thickness lose adherence but are not completely released to electrolyte because of the sticky network.

At intial time, under biotic conditions, an adsorption behavior is observed (Figure 4B), which can be attributed to the rapid adsorption of bacteria to the metal surface, but in subsequent times this behavior doesn't appear, probably due to the occupied sites over metal surface by the inner and outer corrosion products heterogeneous layers. As seen in OCP measurements for biotic (Figure 1) system there is a change in the behavior of the system also at approximately the 7th day, detected as maximum phase angle influence shift to lower frequencies, but in this case shift reaches frequencies around 0.1-1 Hz (Figure 5B) indicating higher charge transfer resistance under biofilm presence, or depolarization phenomena.

Under bacterial presence, after the 7th day, we can also see other phenomena: diffusion controlled process appears (Figure 4A). The linear part in Nyquist diagrams with a slope of more than 45° corresponds to the regular behavior of a system with an infinite diffusion layer [25]. Slope higher than 45°, as observed in Nyquist diagrams under bacterial presence, indicates not only a diffusional behavior, but also that there is present over metal surface a porous film which tends to be non homogeneous in pore distribution as seen in values around 382 h, but at 600 h, slope gets closer to 45° which indicates that the formed film reaches a more homogeneous pore distribution. At 600 h, in Figure 5B, at lower frequencies we can see that the values associated to inner corrosion products layer exhibit a depression which can be indicating that bacterial presence is controlling the reactions at the metal surface and not the inorganic corrosion product inner layer.

Cathodic depolarization theory assumes that hydrogen elimination is the kinetic limiting step for anaerobic microbial influenced corrosion (MIC) by SRB in ferrous metals. As observed in Nyquist plots (Figure 4A), this assumption can be valid for this system just when biofilm is developing, but once that is established over the metal surface, even if the mechanism is the same, the rate at which the whole corrosion reaction will progress is determined or limited by diffusion through biofilm mixed with corrosion products layers.

CONCLUSIONS

Biofilm formation and biocorrosión process contributions caused by halophilic-hydrogenotrophic SRB under supplemented artificial seawater can be qualitatively monitored by impedance and phase angle results,

Experimental results obtained with EIS measurements, for batch culture of mixed *D. gabonensis* and *D. capillatus* strains, exhibit that diffusion-controlled corrosion is induced by biofilm formation, where biofilm combined with corrosion products is acting as an infinite diffusion layer. Also, a shift to lower frequencies in phase angle magnitudes and the increase in semicircles magnitudes (Nyquist diagrams) indicates a depolarization mechanism represented by an increased charge transfer resistance.

Cathodic depolarization mechanism can be one of the various mechanisms describing how chemical transformation are carried out at the interphase and metal surface, however the proposal of cathodic hydrogen consumption as the limiting step in biocorrosion by hydrogenase positive SRB is limited to activation conditions, because once that biofilm is established, the limiting step is diffusion across the mixed biofilm and corrosion products heterogeneous layer. Thus, in the present conditions, abiotic corrosion is an active degradation process, while biotic participation is a diffusional phenomenon.

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FIGURES AND TABLES

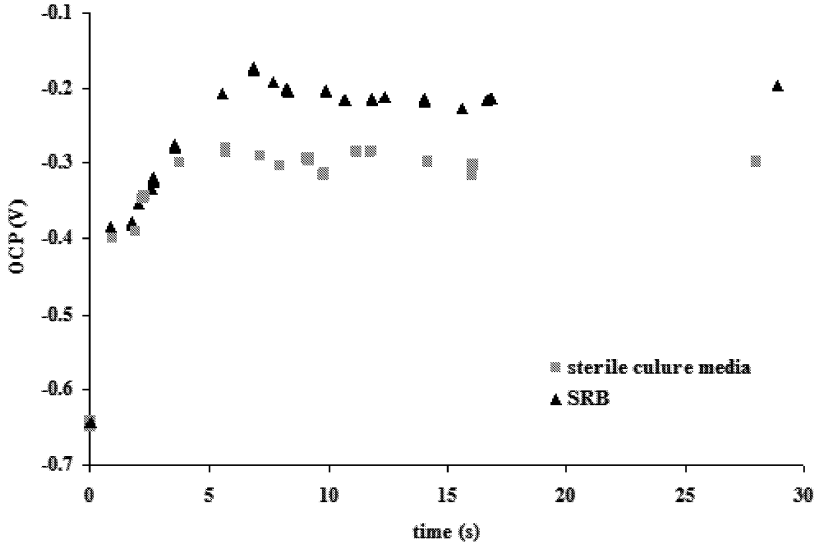


Figure 1. Time dependence of OCP variations influenced by *D. capillatus* and *D. gabonensis* mixed culture and sterile culture media.

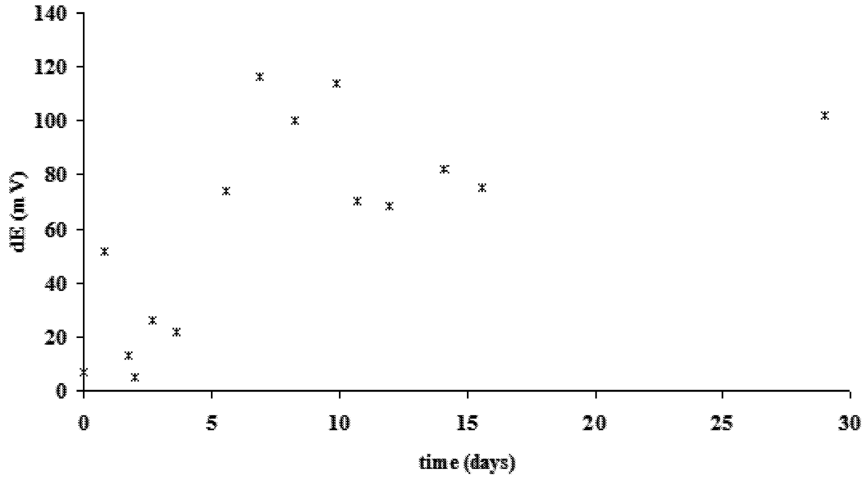
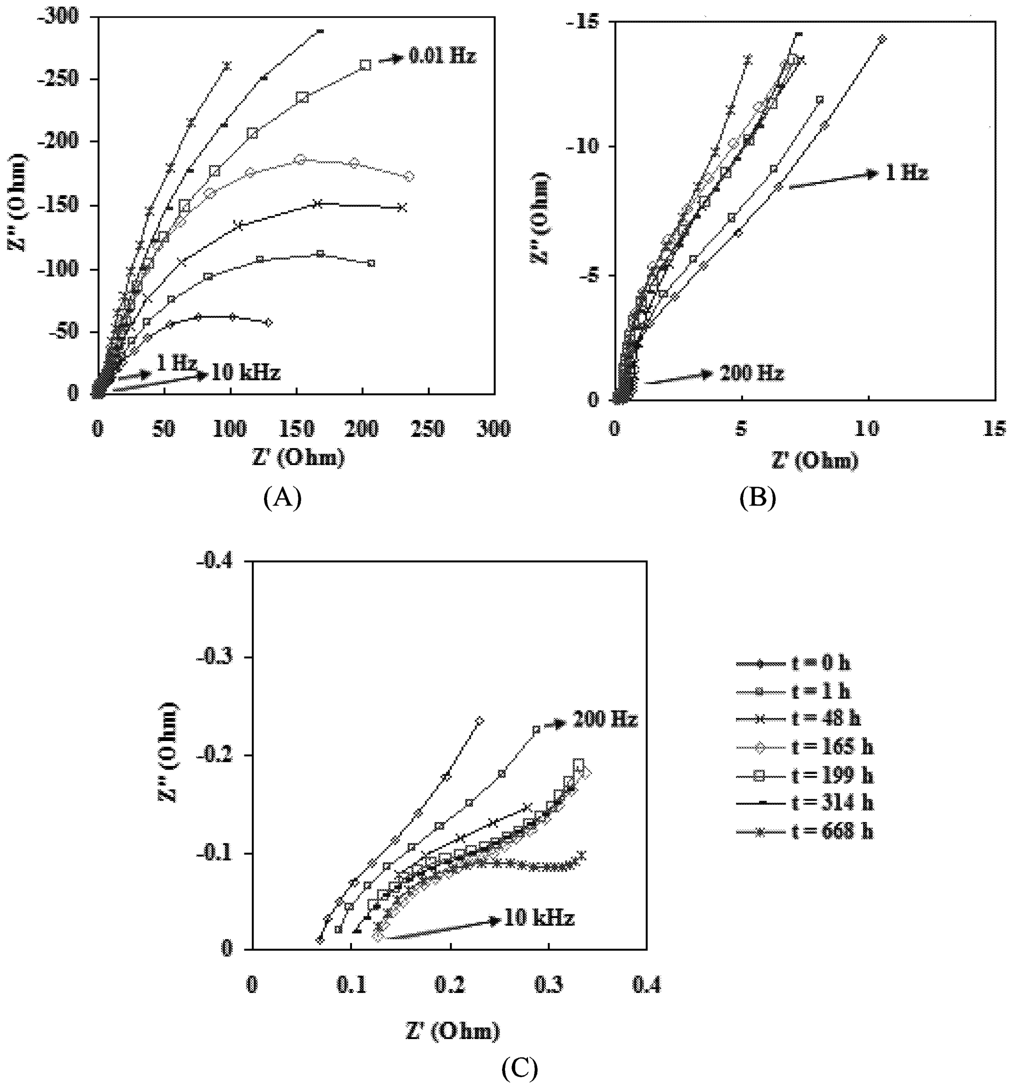


Figure 2. Time dependence of ΔE (OCP) influenced by *D. capillatus* and *D. gabonensis* mixed culture respect to sterile culture media.

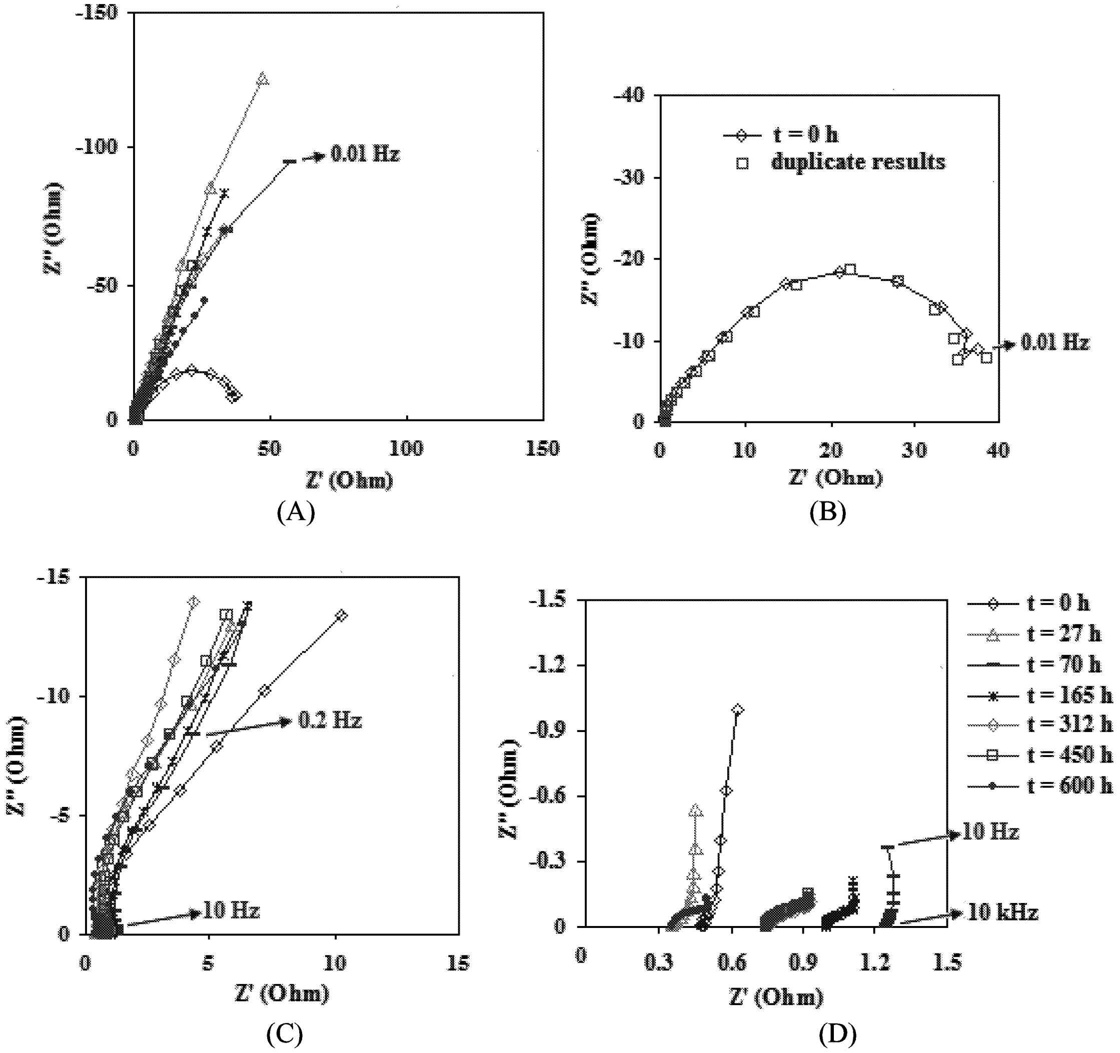


(A)

(B)

(C)

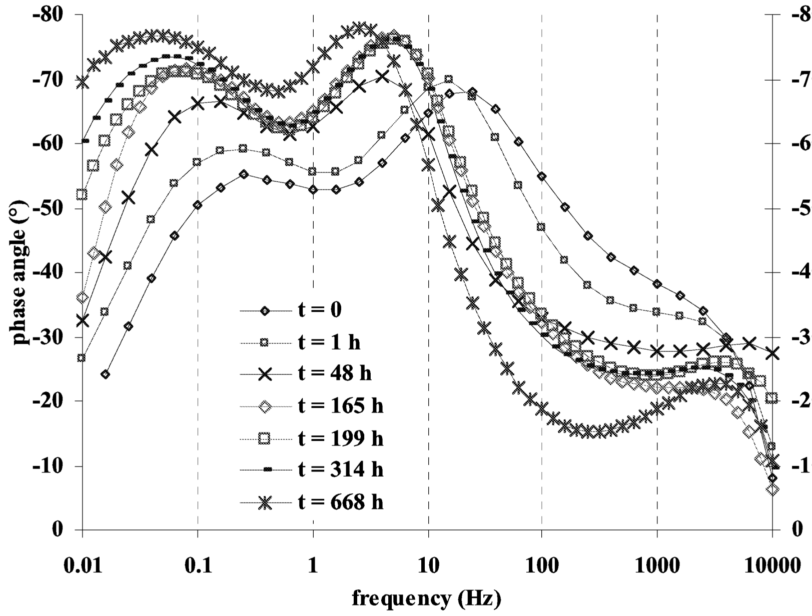
Figure 3. Time dependence of impedance results for sterile culture media:
(A) All frequencies, (B) Detail of middle frequencies (C) Detail of high frequencies.



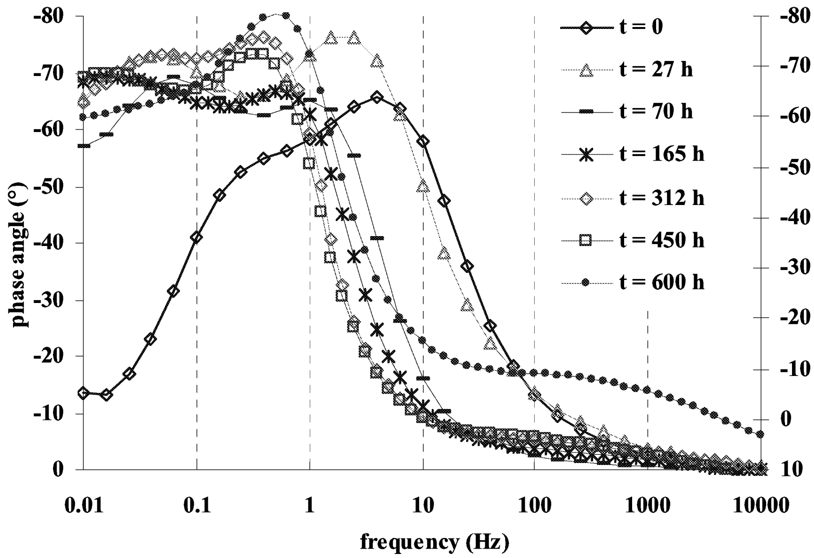
(A) (B)

(C) (D)

Figure 4. Time dependence of impedance results for sterile culture media: (A) All frequencies, (B) Zero time impedance, (C) Detail of middle frequencies (D) Detail of high frequencies.



(A)



(B)

Figure 5. Time dependence of phase angle results for:
(A) Sterile culture media exposure, (B) SRB-biofilm exposure.