

Electrochemical Characterization of Microbiologically Influenced Corrosion on Linepipe Steel Exposed to Facultative Anaerobic *Desulfovibrio sp.*

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In-situ electrochemical techniques were used to investigate the microbiologically influenced corrosion (MIC) of API 5L X52 linepipe steel by *Desulfovibrio sp.* (sulfate reducing bacteria; SRB) cultivated from a sour oil well in Louisiana, USA. These techniques include electrochemical impedance spectroscopy (EIS), open circuit potential (OCP) and linear polarization resistance (LPR). OCP trend showed anodic polarization shift of 100 mV between the biotic medium with reference to abiotic medium (control). These positive polarization shifts have been attributed to complex deposits of bacterial cells, extra-cellular polymeric substances and associated structures that synergistically altered the electrochemical environment of the system and increased the corrosion rate. Through circuit modeling, EIS results were used to interpret the kinetics and real time interactions between the electrode, biofilm and solution interfaces. The results confirmed that extensive localized corrosion activity of SRB is due to a formed biofilm and a porous iron sulfide layer on the metal surface.

Keywords: Corrosion, biofilm, impedance, sulfate reducing bacteria, *Desulfovibrio species*, SRB

1. INTRODUCTION

Microorganisms that are present in oil reservoirs are able to induce localized changes in the aqueous environment (such as - alter the concentration of the electrolyte, components, pH and oxygen concentration) leading to localized corrosion known as microbiologically influenced corrosion (MIC). Microbial activities are responsible for approximately 20% of the total corrosion cost in the oil and gas industry, of which a significant part is due to anaerobic corrosion influenced by sulfate reducing bacteria (SRB) and aerobic corrosion influenced by iron-reducing and oxidizing bacteria (IRB/IOB) [1-4]. The metabolic by-products of these microorganisms found in biofilms on steel surfaces affect the kinetics of cathodic and/or anodic reactions. Moreover, these metabolic activities can considerably

modify the chemistry of any protective layers, leading to either acceleration or inhibition of localized corrosion [1-4].

One of the most damaging microorganisms in pipelines is sulfate reducing bacteria (SRB). SRB are anaerobic and do not need oxygen to survive; rather, they use sulfate ions as a terminal acceptor and produce hydrogen sulfide (H_2S). This type of bacteria has the ability to reduce nitrate, sulfite and thiosulfate [2-4]. SRB are facultative anaerobes and can manage to stay alive in an aerobic environment until the environment becomes suitably anaerobic for them to grow. SRB obtain their energy from organic nutrients. They can grow in a pH range from 4 to 9.5 and tolerate pressure up to 500 atmospheres. Most SRB exist in temperature ranges of 25 – 60°C [2-4]. SRB can be found everywhere in the oil and gas production facilities from deep inside a well, to all the way, to the treatment facilities. The environment inside the pipeline systems has anaerobic or low oxygen concentration, considering the sulfate reducing bacteria as the main contributor to bio-corrosion. The interaction between their metabolic products and ferrous metal produces aggressive corrosive environment such as hydrogen sulfide (H_2S) [2-4].

It is important to consider the impact of evaluation methodology on the viable microorganisms and biofilm during MIC investigations. Different nondestructive electrochemical technique such as open circuit potential (OCP), linear polarization potential (LPR) and electrochemical impedance spectroscopy (EIS) are among the evaluation methods that are widely used in MIC studies. These techniques are sensitive enough to measure very low corrosion rates eliminating the need for laboratory accelerating of corrosion processes [2,3].

The objective of this study is to investigate the impact of environmental *Desulfovibrio sp.* on the corrosion behavior of API 5L X52 linepipe steel by using nondestructive electrochemical techniques. The bacterial consortium used in this study was cultivated from a sour oil well in Louisiana, USA.

2. MATERIALS AND EXPERIMENTAL PROCEDURE

2.1. Organisms and culture

The *Desulfovibrio sp.* (SRB consortium) used in this study was cultivated from water samples obtained from a sour oil well located in Louisiana, USA. The water samples were collected and bottled at the wellhead from an approximate depth of 2200 ft. as described under the NACE Standard TM0194 [5]. The SRB were cultivated in modified Baar's medium (ATCC medium 1250). This growth medium was composed of magnesium sulfate (2.0 g), sodium citrate (5.0 g), calcium sulfate di-hydrate (1.0 g), ammonium chloride (1.0 g), sodium chloride (25.0 g), di-potassium hydrogen orthophosphate (0.5 g), sodium lactate 60% syrup (3.5 g), and yeast extract (1.0 g). All components were per liter of distilled water. The pH of the medium was adjusted to 7.5 using 5M sodium hydroxide. The growth medium was then sterilized in an autoclave at 121°C for 20 minutes. The SRB species were cultured in the growth medium with filter-sterilized 5% ferrous ammonium sulfate. The ferrous ammonium sulfate

was added to the medium at a ratio of 0.1ml to 5.0 ml respectively. The bacteria were incubated for 72 hours at 37 °C under an oxygen-free nitrogen headspace.

2.2. Material Preparation

The coupons were cut from a 30 inch API 5L X52 carbon steel pipe with a chemical composition (in weight %) as shown in Table 1. The coupons were machined to a size of 10 mm x 10 mm x 5 mm and embedded in a mold of non-conducting epoxy resin leaving an exposed area of 100 mm². For electrical connection, a copper wire was soldered at the rear of the coupons. The coupons were polished with a progressively finer sand grinding paper to uniform surface until a final grit size of 600 microns was obtained. After polishing, the coupons were rinsed with distilled water, ultrasonically degreased in acetone and sterilized by exposing to 100% ethanol for 24 h.

Table 1. The chemical Composition of API-5L X52 carbon steel coupons

C	Mn	Cr	Nb	Ti	S	V	Ni	Mo	Fe
0.08	1.12	0.14	0.023	0.002	0.001	0.06	0.1	0.08	Balance

2.3. Electrochemical Tests

Electrochemical impedance spectroscopy (EIS), open circuit potential (OCP) and linear polarization resistance (LPR) measurements were carried out simultaneously under both biotic and abiotic (control) conditions for 14 consecutive days at different time intervals. The measurements were made in a conventional three-electrode ASTM electrochemical cell coupled with a potentiostat (Gamry-600). The electrochemical cells were composed of a test coupon as a working electrode (WE), a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode as shown in Figure 1.



Figure 1. Electrochemical Cell

All glassware was autoclaved at 121°C for 20 minutes at 20 psi pressure and dried prior to experiment initiation. Graphite electrodes, purging tubes, rubber stoppers and needles were sterilized

by immersing in 70 vol. % ethanol for 24 hours followed by exposure to a UV lamp for 20 minutes. Two solutions were used in this experiment: a sterile (control) solution and an inoculated (experimental) solution. Using aseptic technique (in a laminar flow hood), the control cell was prepared with 600 ml of enriched Barr's growth medium (described above) and the experimental cell was prepared with 600 ml enriched Barr's growth medium and inoculated with 5 ml of SRB consortium at 10^8 cells/ml. The electrochemical cells were purged for one hour with pure nitrogen gas to establish an anaerobic environment. The EIS measurements were performed on the system at the open circuit potential for various time intervals from immersion upto 30 days. The frequency sweep was applied from 10^5 to 10^{-2} Hz with an AC amplitude of 10 mV. Polarization resistance (R_p) was measured under the linear polarization resistance technique at a scanning amplitude of +/- 10mV with reference to the open circuit potential for various time intervals.

2.4. Surface Analysis of the Coupons Exposed to SRB

At the conclusion of each test, the working electrodes were carefully removed from the system for examination with electron microscopy. To fix the biological samples, the coupons (with undisturbed biofilm) were immersed for 1 hour in a 2% glutaraldehyde solution, serially dehydrated in ethanol (15 minutes each in 25, 50, 75 and 100% ethanol), and then gold sputtered. Afterward, electron microscopy, using field emission scanning electron microscopy (FESEM) coupled with energy dispersive spectroscopy (EDS) techniques were used to evaluate the biofilm and corrosion morphology. The coupons were then cleaned according to the procedure described under the ASTM-G1-3 [6] and pit morphology and density were examined using FESEM.

3. RESULTS AND DISCUSSION

3.1. Surface/Biofilm morphology and compositional analysis

The morphology observations and elemental analysis of corrosion products of API X52 steel immersed in enriched growth medium containing SRB after 14 days by FESEM is shown in Figure 2. As shown in Figure 2, there are three main distinctive areas: A1, A2 and A3. The light regions (A1 and A2) are considered the outer layers. Quantitative EDS analysis shows they are composed of a higher amount of sulfides, sodium chloride salts, phosphates in addition to carbon-based compounds. The presence of di-potassium hydrogen orthophosphate and sodium chloride in the growth media might lead to the precipitation of phosphorous-based compounds and sodium chloride on the surface. A1 is considered the inner layer in which the iron species, in addition to sulfur and phosphorous-based compounds are the predominant. These results suggest the formation of an amorphous type of iron phosphide would be possible under these conditions. Moreover, the presence of iron and sulfur supported the formation of biologically-generated sulfides in the corrosion products. The SRB metabolic activities drive the formation of different biogenetic sulfide products in addition to iron oxides [7].

MIC process starts with the biofilm formation on the metal surface. SRB cells attach to the substrate, grow, reproduce and produce an extracellular polymeric substance (EPS), which result in biofilm formation. The biofilm and induced corrosion products have a heterogeneous morphology and thickness as shown in Figure 2. At the conclusion of the experiment, the substrate of the steel could hardly be seen, as it was covered with a porous black layer. Jelly-glue substance could be observed among the corrosion products, which was speculated to be the EPS. The comma shaped bacteria *vibrio* occupied a small volume fraction as compared to the precipitated corrosion products and EPS. The EPS and corrosion products usually occupied 75-95 % of biofilms volume, while 5-25% is occupied by the cells [2,7].

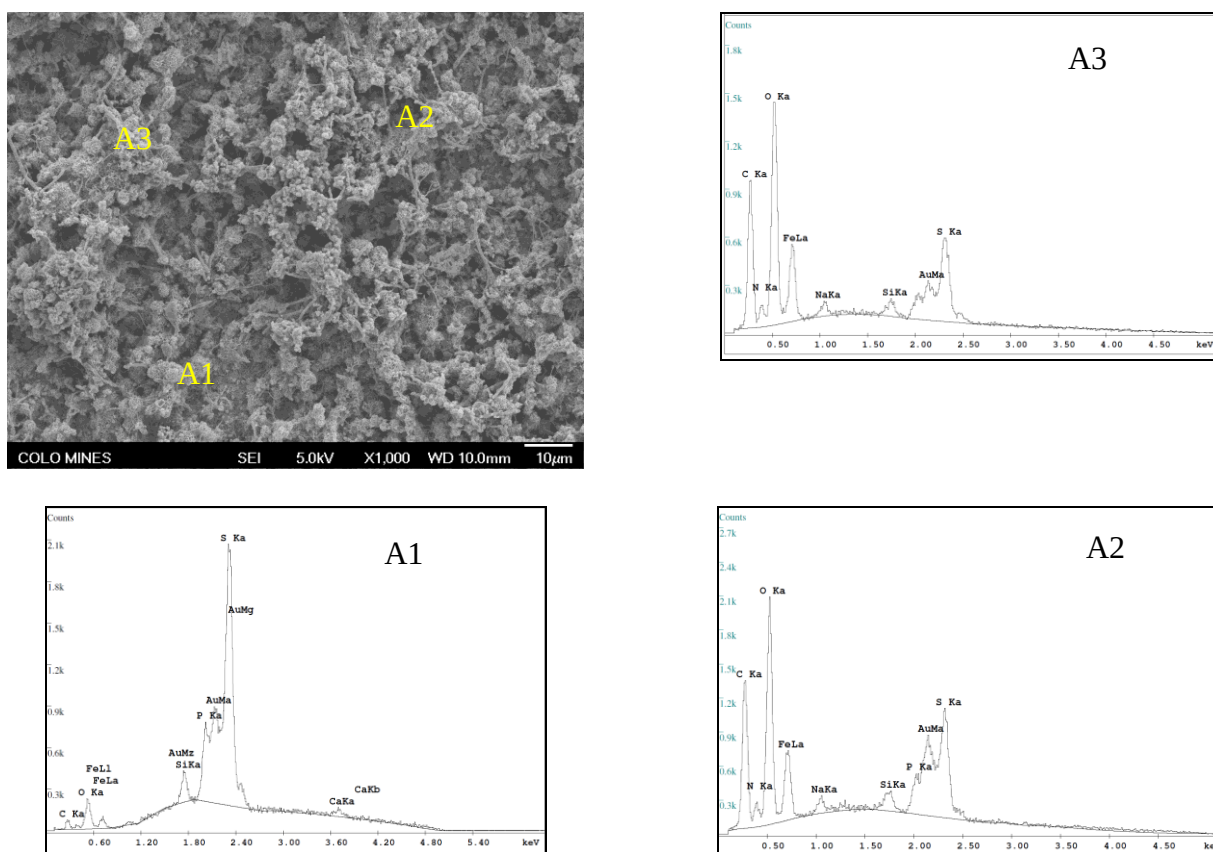


Figure 2. FESEM and EDS analysis for the biofilm developed on the API X52 exposed to the SRB containing medium after 14 days exposure.

The nature of SRB generated biofilm is shown in Figure 3. The 14 days old biofilm composed of comma shaped sulfate reducing bacteria cells, *vibrio*, embedded in the matrix of extracellular polymeric substances. The FESEM images of the surfaces after cleaning the biofilm and corrosion products of the carbon steel coupons exposed for 14 days in SRB-containing medium are presented in Figure 4. The results reveal extensive localized pitting corrosion on the surface with noticeable deeper pits along the grain boundaries and the boundary triple points.

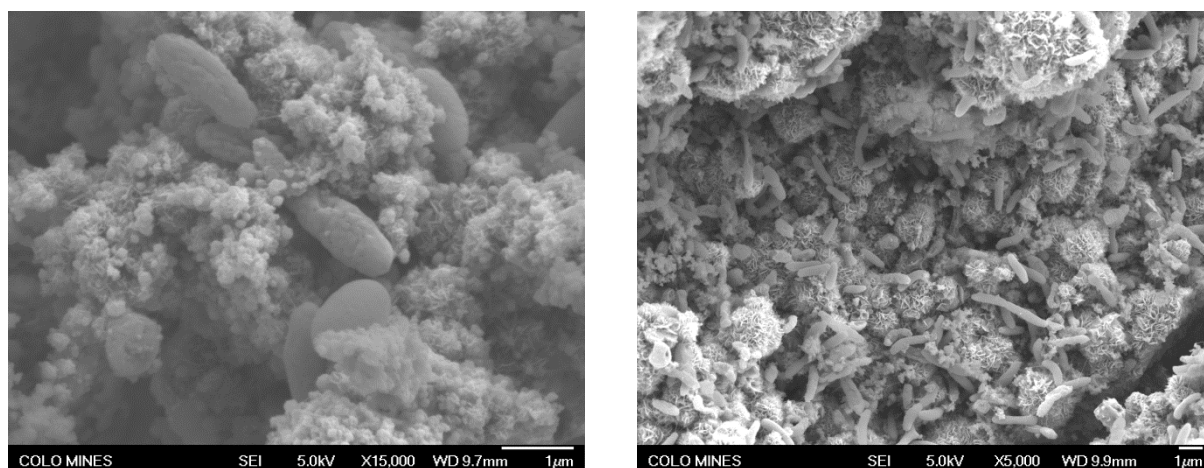


Figure 3. FESEM image for the biofilm developed on the carbon steel coupon exposed to the biotic media after 14 days exposure at 15000X and 5000X

The extensive attack on the grain boundaries has been related to the fact that bacterial initial attachment occurs on or near the grain boundaries, whereby the grain boundaries and triple points harbor more cells. This initial colonization influenced the subsequent growth, recruitment and biofilm formation. There are different reasons that explain why bacteria are favoring grain boundary: First, the elemental segregation occurs at the grain boundaries and bacteria are getting attracted towards it. Element such as a sulfur and phosphorus are reported to be segregated along the grain boundaries and both of these elements are favorable for bacteria and there are possibilities that these elements attract more bacterial cells. Second, the differential energy distribution between the grain boundaries and matrix could be another contributing factor [3,8]. As per literature, grain boundaries hold more energy than the surface of matrix. Bacteria could be considered as negatively charged structures, with chances of their being attracted towards these energy holding grain boundaries [8].

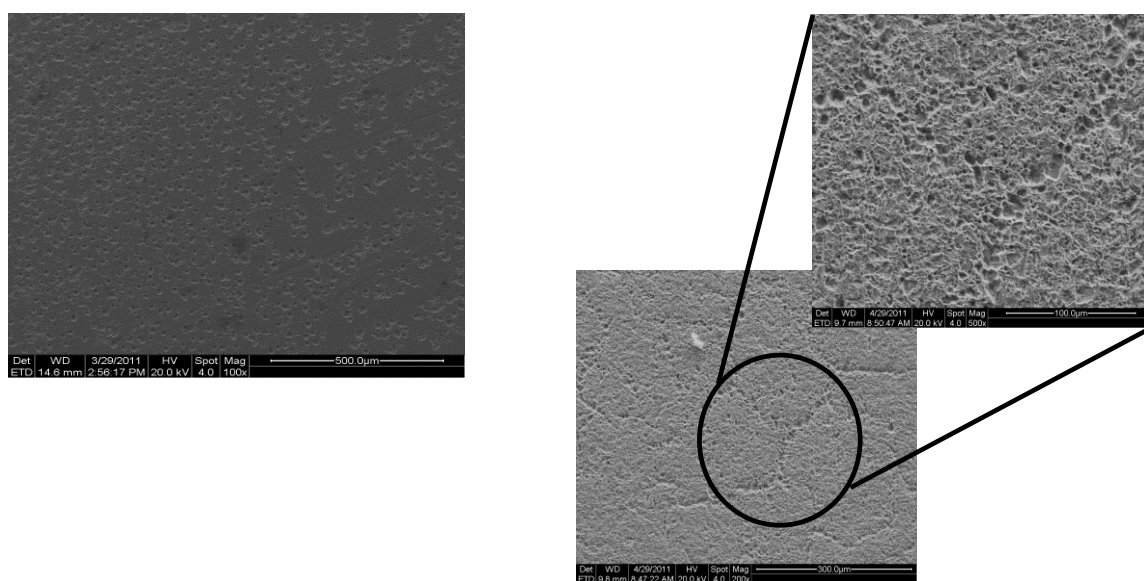


Figure 4. FESEM analysis for the coupon surface after cleaning for the system under biotic conditions after 14 days exposure

3.2. Influence of SRB Metabolic Reactions on the corrosion process

According the cathodic depolization classical theory introduced by Khur and Vlught in 1934 [1-4], SRB consume the cathodic hydrogen via an enzyme known by hydrogenase to obtain the eight electrons required to reduce sulfate to hydrogen sulfide. The SRB production of hydrogen sulfide supports following cathodic reaction that would be further enhanced with the presence and activity of SRB in the media at pH range between 1 and 7 [1,3,7].



SRB have different strategies to obtain the hydrogen from the media: (a) direct consumption of the hydrogen produced by water dissociations reactions or by (b) converting the carbon source (lactate) through pyruvate to acetate with the production of hydrogen molecules [2-4] and has been illustrated in Figure 5. Some hydrogen sulfide ions will convert to hydrogen sulfide especially at acidic pH as follows [7]:



Reaction (2) is rapidly facilitated by the presence of SRB. The production of hydrogen sulfide and the oxidation of iron (anodic reaction), leads to the formation of iron sulfide as follows [2-4, 7]:

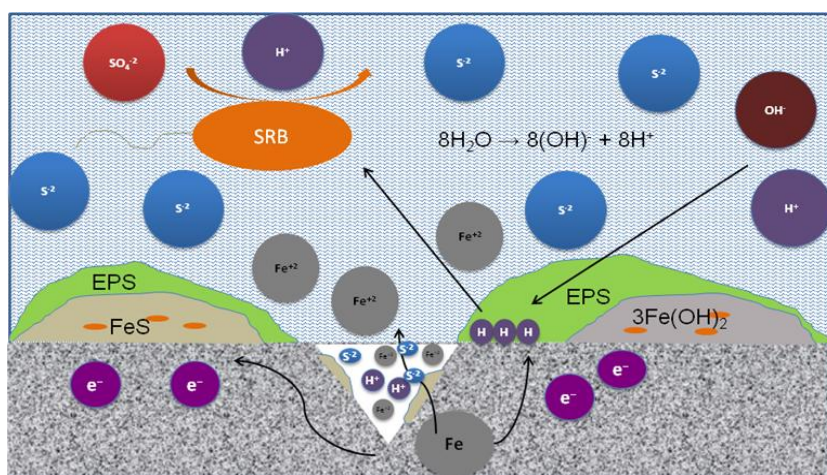


Figure 5. Schematic representation of Corrosion mechanism by *Desulfovibrio* sp.

3.3. Open circuit potential / linear polarization potential

The open circuit potentials (OCP) variations for biotic and abiotic systems are shown in Figure 6. The E_{corr} as function of time data revealed that in biotic medium, a substantial shift of E_{corr} towards noble values (-590 mV/SCE) occurred for the first 120 hours and then remained stable at a value of -600 mV/SCE throughout the period of exposure. The shift to positive potential is correlated with the growth of the SRB species. The shift reaches stable value at the stationary phase of the growth cycle. The potential shift clearly supports that the activity and the growth of the SRB species have enhanced the redox quality of the medium and accelerated the iron dissolution. SRB attached to the coupon surface, colonized and reproduced to form a biofilm. The aggressiveness factors of the biofilm and the active metabolisms of the sessile bacteria alter the electrochemical process; subsequently, changing the pH level, producing more H_2S and introducing multiple cathodic reactions, reactions 1 and 2. These factors collectively enhanced the reduction quality of the system and accelerated the anodic dissolutions [9-11].

On the other hand, in abiotic system, the E_{corr} remained more or less steady at approximately -700 mV/SCE. There is a difference in noble direction of approximately 100 mV/SCE between the biotic and abiotic systems. This positive shift in E_{corr} is known by ennoblement. The ennoblement has been acknowledged by different investigators as probably the most notable phenomenon in the MIC studies [3]. The ennoblement has been attributed to the microbial colonization and biofilm formation, which collectively result in organometallic catalysis and acidification of the electrode surface [3]. It promotes pitting corrosion, which is more critical for passive alloys [3-4].

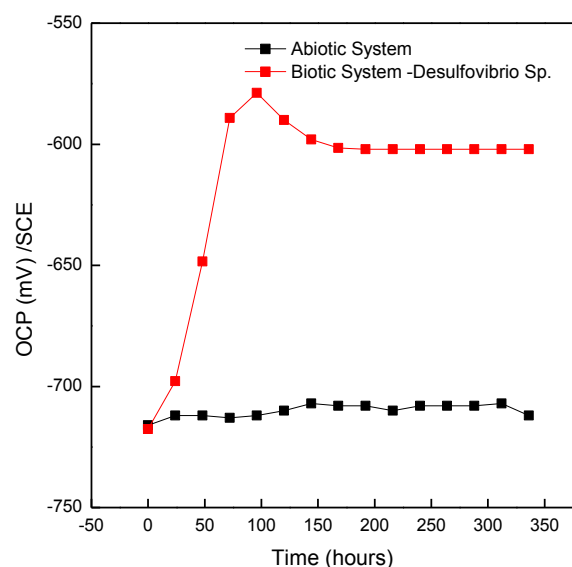


Figure 6. Open Circuit Potential (OCP) variations under biotic and abiotic conditions.

The polarization resistance (R_p) variations for the biotic and abiotic systems are shown in Figure 7(a). R_p as a function of time data revealed that in the biotic system, a substantial decrease of polarization resistance (R_p) to $1000 \Omega \cdot \text{cm}^2$ was followed by another decrease to approximately 250

$\Omega\cdot\text{cm}^2$ at 250 hours which then remained stable throughout the period of exposure. The decrease in the R_p is attributed to different factors: the production of hydrogen sulfide by SRB species and the formation of organic compound such as EPS and acetate at the metal/biofilm interface [7, 12-14]. These factors create an aggressive environment leading to a decrease of polarization resistance. The polarization resistance is inversely proportional to the corrosion current density, which means high corrosion rate at low resistance. On the other hand, in abiotic medium, there is a gradual decrease of the R_p which remained more or less steady at approximately $1000 \Omega\cdot\text{cm}^2$.

The corrosion rate plots over time for biotic and abiotic systems are shown in Figure 7(b). The corrosion rate for the biotic medium reached a value over 60 mpy after 150 hours whereas the corrosion rate for the abiotic system for the same interval is approximately 15 mpy. The high corrosion rate in the biotic system agrees with the OCP and R_p results as already described.

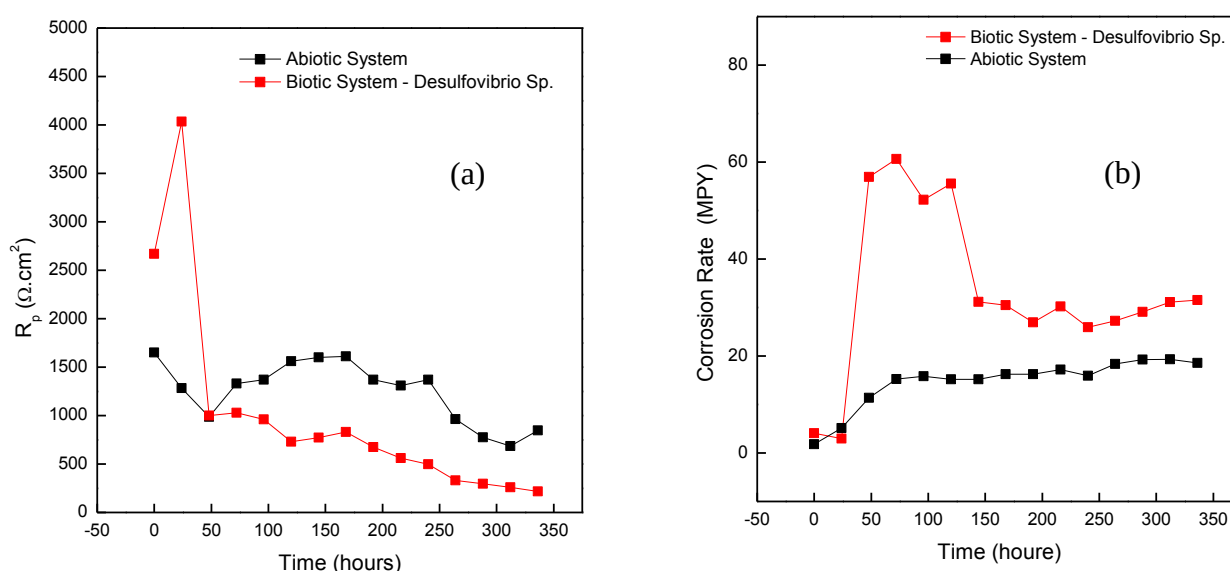
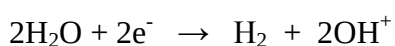


Figure 7. (a) Polarization (R_p) and (b) Corrosion rate variations under biotic and abiotic conditions.

3.4. Electrical impedance spectroscopy results

Figure 8(a) displays the Nyquist plots for a carbon steel coupon exposed to sterilized culture medium over time. The steady state was reached at 144 hours. At low frequencies, shown in Figure 8(a), the magnitude of the capacitive loop represented by the semicircle diameter decreased with time.

These low frequency magnitudes represent the change in charge transfer resistance (R_{ct}) that describes the evolution of the anodic reaction that is controlled by charge transfer processes [7]. In abiotic system, kinetic of anodic reaction is represented by reaction (4) while the cathodic reaction can be shown by reaction shown underneath;



The decrease of R_{ct} with time indicates an increase in corrosion rate, possibly due to the effect of a formation of a mixed layer of sodium chloride, sulfide, potassium and carbon-based compounds on the electrode surface [7,16]. This layer was confirmed by the phase angle spectra, Figure 8(b), that shows two time constants at intermediate frequency.

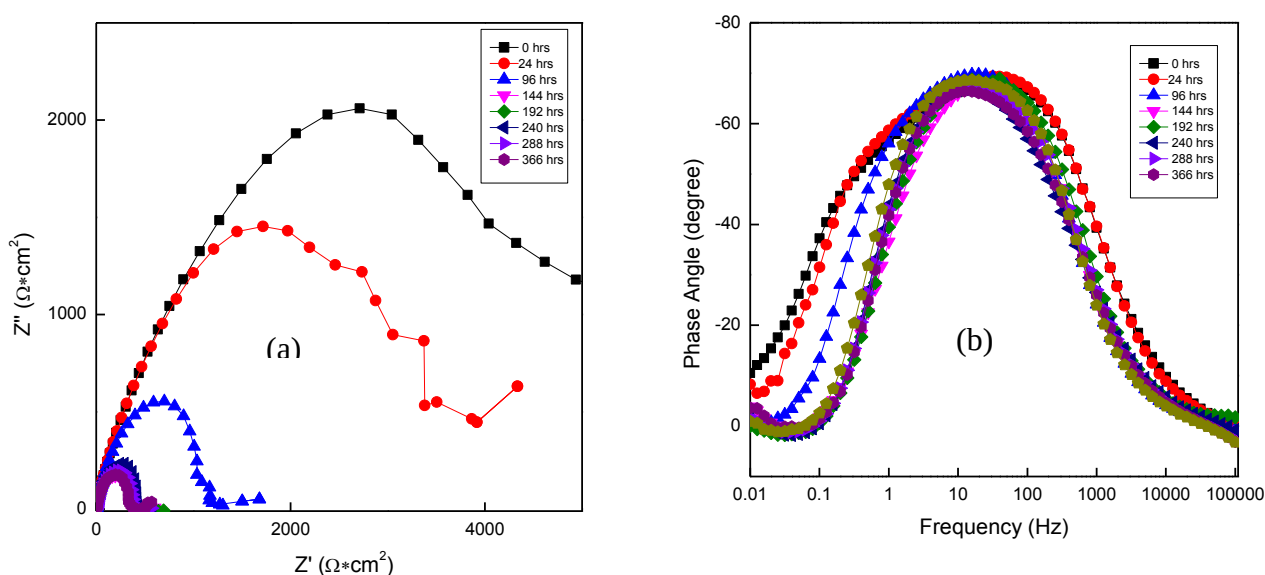


Figure 8. EIS data for the abiotic system; (a) Nyquist Plots (b) Phase angle plots.

The electrical circuit representation for the abiotic condition is shown in Figure 9(a). These fits were based on the minimum deviation between the measured and fitted data.

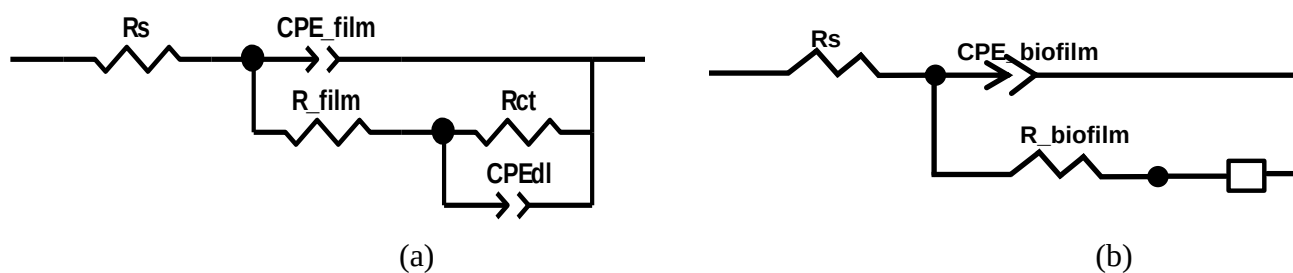


Figure 9. (a) Circuits models used to fit for the EIS data (a) Randle with CPE, and (b) Randle w/RC and finite Warburg diffusion impedance (W_o).

The circuit includes charge transfer resistance (R_{ct}) for steel surface, constant phase element (CPE) associated with the formation of a heterogeneous layer and R_s representing the solution resistance. The heterogeneous layer composed of corrosion products along with other compounds deposited from the growth media. The impedance of CPE is defined by the following equation:

$$Z_{CPE} = \frac{1}{CPE \cdot (j\omega)^\alpha}$$

6)

In which, CPE and α are not frequency-dependent values, and α value of less than 1 for CPE. When the carbon steel was exposed to biotic system, the EIS spectra varied significantly with exposure time as shown in Figure 10(a). The low frequency magnitude, represented by the semicircle diameter, significantly decreased with time indicating an increase in corrosion rate and decrease in R_{ct} as supported by Figure 11(b). The SRB bio-catalytic activities promote the corrosion rate via formation of biofilm, production of hydrogen sulfide and biotic reduction of phosphates and subsequent formation of iron phosphides. For the first 24 hours, the intermediate frequency response presented in the phase diagram in Figure 11(a) shows one time constant that indicates activation control process. This behavior is attributed to the formation of an unstable conditioning layer based on a mixture of inorganic/ organic compounds [7,15]. When mature biofilm formed two time constants were observed. However, when steady state is reached, mass transfer limitations overcome the interfacial activation, which is reflected in a change from a semicircle behavior to a straight line shown in Figure 10(b) at low frequency. It is speculated that the formation of an adherent biofilm along with iron sulfide layer influenced the mass transfer processes control in the electrochemical cell.

The equivalent circuit for biotic conditions is presented in Figure 9(b). It consists of a constant phase element (CPE) that is associated with the behavior of this film and a distributed generalized finite Warburg element that was for described the diffusional influence of the biofilm.

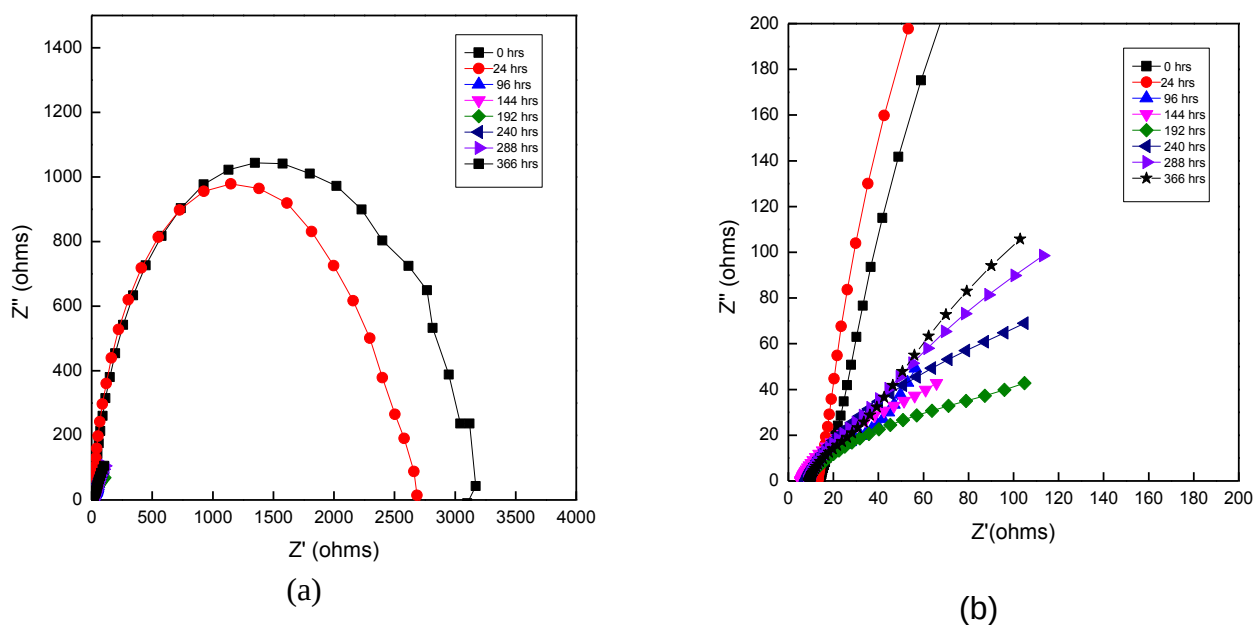


Figure 10. Impedance diagrams in the Nyquist representation (a) at different times of the biotic system with (b) detailed representation at high frequencies.

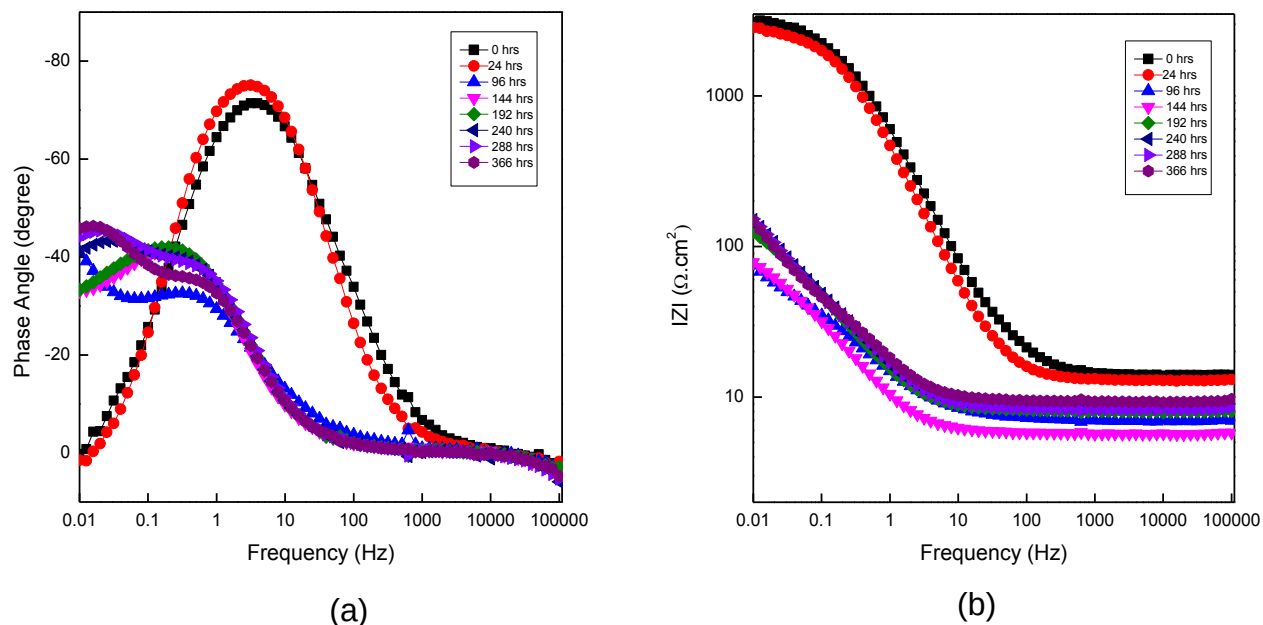


Figure 11. (a) Phase angle diagram, and (b) Modulus plots of biotic system.

The increase of the dissolution kinetics of the metallic surface is evidenced by the decrease of the magnitude of charge transfer resistance with time as shown in Figure 11(b).

In fact, protective iron sulfide films are found in hydrogen sulfide or sour environments. In these environments, there are always thin films adhered to the surface [16]. However, in bacteria containing media, the sulfide films are not stable. They are disrupted by bacteria metabolic actions and other reactants, such as acetic acid.

The presence of acetic acid has recently been suggested to inhibit the protectiveness of iron sulfide corrosion product [16]. Therefore, with the proliferation of the SRB and metabolic products, the protective iron sulfide film decomposed to other polysulfide products [7,16]. The integrity of the protective film will be then degraded and become loose and porous [16]. Subsequently, the steel surface exposed to the aggressive medium, will have accelerated the corrosion rate significantly (50 mpy). At the last stage, when the SRB activity declined their metabolic activity decline gradually, it would lead to a reduction of the iron/sulfide ratio with reduction of hydrogen sulfide. This behaviour leads to the formation of a protective layer of corrosion products that subsequently decreases the corrosion rate gradually.

4. CONCLUSION

In this study the microbiologically Influenced corrosion (MIC) by *Desulfovibrio sp.* on API 5L grade X52 carbon steel coupons was investigated. The most important results are:

1. The bio-catalytic activities at the biofilm/surface interface increased the corrosion rate significantly. The corrosion rate increased three times from the abiotic to the biotic system.

2. The biofilm and the active metabolisms of the attached bacteria alter the electrochemical process; subsequently producing more H_2S and introducing multiple cathodic reactions. These factors collectively enhanced the redox quality of the system and accelerated the anodic dissolution.

3. EDS revealed the formation of different sulfide compounds such as mackinawite and other biogenetic iron sulfide (FeS).

4. The corrosion damage is localized pitting.

5. The anodic dissolution of carbon steel is a control process under the abiotic system over time, while under the presence of the SRB the biofilm formation shifted the active charge transfer reactions to a diffusion-limited process.

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