Influence of sulfate reducing bacterial biofilm on corrosion behavior of low-alloy, high-strength steel (API-5L X80)

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A B S T R A C T
The utilization of high strength carbon steels in oil and gas transportation systems has recently increased. This work investigates microbiologically influenced corrosion (MIC) of API 5L X80 linepipe steel by sulfate reducing bacteria (SRB). The biofilm and pit morphology that developed with time were characterized with field emission scanning electron microscopy (FESEM). In addition, electrochemical impedance spectroscopy (EIS), polarization resistance ($R_p$) and open circuit potential (OCP) were used to analyze the corrosion behavior. Through circuit modeling, EIS results were used to interpret the physicoelectric interactions between the electrode, biofilm and solution interfaces. The results confirmed that the extensive localized corrosion activity of SRB is due to a formed biofilm and a porous iron sulfide layer on the metal surface. Energy Dispersive Spectroscopy (EDS) revealed the presence of different sulfide and oxide constituents in the corrosion products for the system exposed to SRB.

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1. Introduction

In the last decade, oil and gas demand has increased drastically, and efficient, cost-effective steel pipe grades are required to transport this increased demand. High-strength steel pipeline technology development provides significant economic benefit to pipeline operators through an increased pressure (i.e., more volume and thus revenue) of transmitted oil/gas while decreasing wall thickness of the pipe (Jin et al., 2010). Recently, high strength steel grades (API5L X80 and above) have been installed in pipeline projects in Northern Canada, the North Sea and the Japanese Sub-Sea (Jin et al., 2010). Microbiologically influenced corrosion (MIC) is one of the most damaging mechanisms to pipeline steel materials. Microorganisms are thought to be responsible for greater than 20% of pipeline systems failures (Javaherdashti, 2008). MIC is not a distinct type of corrosion form, but rather is the synergistic interaction of microorganisms, with resulting biofilms and metabolic products that enhances corrosion processes. In most cases MIC morphologies are localized types of corrosion that manifest as pitting, crevice corrosion, under deposit corrosion, cracking, enhanced erosion corrosion and dealloying (Little and Lee, 2007).

Pipelines are considered suitable environments in which microorganisms (from all three domains of life, Bacteria, Archaea and Eucarya) live because the essential components for their metabolism are present in these environments. Heterotrophic microorganisms need nutrition comprising primarily of four main components to thrive: a carbon source, water, an electron donor and an electron acceptor (Madigan, 2009). Hydrocarbons act as an excellent food source (both a carbon source and an electron donor) for a wide variety of bacteria. The main types of bacteria associated with metals in pipeline systems are sulfate-reducing bacteria (SRB), iron and CO$_2$ reducing bacteria and iron and manganese oxidizing bacteria (Little and Lee, 2007; Javaherdashti, 2008). Among them, SRB have been recognized as the major MIC causative bacteria in pipeline systems. Typically, MIC results from synergistic interactions of different microorganisms acting in consortia, which coexist in the environment and are able to affect the corrosion process through co-operative metabolisms. SRB are facultative anaerobes and live in oxygen free environments and utilize sulfate as a terminal electron acceptor and produce hydrogen sulfide (H$_2$S) as a metabolic byproduct. Furthermore, this type of bacteria has the
ability to reduce both nitrate and thiosulfate and obtain their energy from organic nutrients, such as lactate. They can grow in a pH range from at least 4.0 to 9.5 and tolerate pressure up to 500 atm (Javaherdashti, 2008). Most SRB exist in a temperature range of 25–60 °C (Little and Lee, 2007; Javaherdashti, 2008; Madigan, 2009). SRB can be found everywhere in oil and gas production facilities, from the deep subsurface throughout the refinery and through the delivery system. The environments inside pipeline systems typically have anaerobic and/or low oxygen concentrations, thus allowing SRB to be the main contributor to bio-corrosion (Little and Lee, 2007; Javaherdashti, 2008).

The objective of this study is to investigate the impact of environmental SRB (cultivated from oil field samples rather than obtained from a culture collection) on the corrosion behavior of low alloy high strength (API 5L X80) pipeline steel. The SRB consortia used in this study were cultivated from an oil well in Louisiana, USA. The nature and kinetics of chemical and electrochemical reactions introduced by SRB activities on API 5L grade X80 carbon steel coupons were characterized using electrochemical impedance spectroscopy (EIS), polarization resistance ($R_p$) and open circuit potential (OCP). The biofilm and corrosion morphology were investigated by scanning field emission scanning electron microscopy (FESEM) coupled with energy dispersive spectroscopy (EDS).

2. Materials and methods

2.1. Organisms and testing medium

The SRB consortium used in this study was cultivated from water samples obtained from an 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 (2004). The SRB were cultivated in modified Baar's medium (ATCC medium 1250). Baar's medium is reported to be suitable when studying the influence of mixed bacterial communities on steel corrosion (Antony et al., 2008). 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 (10.0 g). All components were per liter of distilled water. The pH of the medium was adjusted to 7.5 using 5 M sodium hydroxide. The growth medium was then sterilized in an autoclave at 121 °C for 20 min. 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 0.1–5.0 ml respectively. The bacteria were incubated for 72 h at 37 °C under an oxygen-free nitrogen headspace.

2.2. Identification of the sulfate-reducing consortium

DNA was extracted from cultivars using the MoBio Powersoil DNA extraction kit (MoBio, Carlsbad, CA); the 10-min vortexing step was replaced by 1 min of bead beating. 16S rRNA gene amplification was carried out using the ‘universal’ polymerase chain reaction (PCR) primers 515F and 1391R (Lane, 1991). PCR, cloning and transformation were carried out as described by Sahl et al. (2010). Unique restriction fragment length polymorphisms (RFLP) were sequenced on an ABI 3730 DNA sequencer at Davis Sequencing Inc. (Davis, CA). Sanger reads were called with PHRED (Ewing et al., 1998; Ewing and Phil, 1998) via XplorSeq (Frank, 2008). Sequences were compared to the GenBank database via BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Frank, 2008).

2.3. Specimen preparation

Steel coupons were cut from a 6-inch (12.5 mm) section of API-5L X80 carbon steel pipe with chemical compositions (in weight%) shown in Table 1. Microstructure characterization of the material was performed by optical microscopy. Optical micrographs were obtained after polishing and etching with 2% nital reagent. Fig. 1 reveals a mixed ferrite microstructure with dispersed small carbides and inclusions accumulated at the grain boundaries and grain sizes ranging from 4 to 20 μm. The coupons were machined to a size of 10 mm × 10 mm × 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 progressively finer sand paper to a final grit size of 600 microns. After polishing, the coupons were rinsed with distilled water, ultrasonically degreased in 100% acetone followed by 100% ethanol and sterilized by exposure to 100% ethanol for 24 h.

2.4. Electrochemical tests

Electrochemical impedance spectroscopy (EIS), open circuit potential (OCP) and polarization resistance ($R_p$) measurements were carried out simultaneously under both biotic and abiotic (control) conditions for 30 days consecutively 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 graphite electrode as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All glassware was autoclaved at 121 °C for 20 min and dried prior to experiment initiation. Graphite electrodes, purging tubes, rubber stoppers and needles were sterilized by immersing in 70 vol.% ethanol for 24 h followed by exposure to a UV lamp for 20 min. 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 inoculated with 5 ml of SRB consortium at 10⁸ cell/ml. The electrochemical cells were purged...
for 1 h 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 up to 30 days. The frequency sweep was applied from $10^2$ to $10^{-2}$ Hz with an AC amplitude of 10 mV. The polarization resistance ($R_p$) was measured on the system at scanning amplitude of ±10 mV with reference to the open circuit potential for various time intervals.

2.5. Sulfide measurements

The sulfide level in the growth medium was monitored over the test period to monitor the growth of the SRB consortium. Samples of the test medium were extracted from the electrochemical cell using a sterile syringe. The procedure detailed under the American Public Health Association (APHA, 1989) standard method was followed.

2.6. 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 h in a 2% glutaraldehyde solution, serially dehydrated in ethanol (15 min each in 25, 50, 75 and 100% ethanol), and then gold sputtered. Afterward, electron microscopy, using the 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 G1-03 (2009) and pit morphology and density were examined using FESEM.

3. Results and discussions

3.1. Identification of the sulfate-reducing consortium

16S rRNA gene analysis indicated that the mixed bacterial culture consortium contained three phylotypes: members of the Proteobacteria (Desulfovibrio sp.), Firmicutes (Clostridium sp.) and Bacteroidetes (Anaerophaga sp.). Desulfovibrio has been isolated previously from different oil fields in the North Sea (Leu et al., 1999). Also, the presence of similar SRB species in five of six different Alberta oil fields in Canada has been demonstrated by Voordouw et al. (1992). Desulfovibrio sp. are anaerobic, Gram-negative, rod-shaped, sulfate-reducing bacteria that grow on different carbon source substrates including lactate, pyruvate, glycerol, and ethanol with optimal growth temperatures between 25 °C and 35 °C. They are capable of using sulfate, thiosulfate or sulfite electron acceptors (Leu et al., 1999; Madigan, 2009). It has been reported that these microorganisms can play a significant role in oil field reservoir souring by generation of hydrogen sulfides (Leu et al., 1999). Clostridium is an anaerobic Gram-negative, spore-forming bacterium. This type of bacteria is capable of surviving high temperatures due to heat-resistant endospores (Little and Lee, 2007; Madigan, 2009). Anaerophaga sp. has also been previously identified in samples from a produced water obtained from the high-temperature Troll oil formation in the North Sea (Dahle et al., 2008).

3.2. Morphology and composition of interfacial surfaces

The morphology observations and elemental analysis of corrosion products of API X80 carbon steel exposed to abiotic conditions over 30 days are shown in Fig. 2. There is one coherent, homogenous layer of corrosion product formed on the surface. Quantitative EDS analysis shows that this layer is composed of iron oxides mixed with phosphates and chlorides in addition to carbon-based compounds that accumulated from the growth medium (Table 2). The presence of di-potassium hydrogen orthophosphate (a constituent in the growth medium) might lead to the formation of phosphorous-based compounds at the steel surface. Crystalline and amorphous iron phosphides are expected products as the growth medium is supplemented with phosphates along with sulfates (Castaneda and Benetton, 2008).

The characteristics of the layer that developed in the presence of the sulfate-reducing microbial consortium over 30 days are shown in Fig. 3. FESEM and EDS analysis display the morphological and chemical characteristics of the developed biofilm on the carbon steel surface, as shown in Fig. 3A–C, respectively. In Fig. 3A, two distinctive areas are shown in a FESEM image: the light outer layer that is composed mainly of high amounts of sulfides, carbon based compounds, iron oxides and low amounts of sodium chloride and phosphates (Table 2); and the dark inner layer with different compositions composed of low amounts of sulfides and high amounts of carbon-based compounds, iron oxides, sodium chloride and phosphates. The dark area in Fig. 3A is a highly cohesive, cracked mass with homogeneous consistency. These cracks are not the result of sample/image processing as they are evident on the
coupons after the experiment, and they might be induced by intrinsic physical growth stresses. The light region in Fig. 3A shows heterogeneous distribution with thicker mass layers.

The corrosion products for biotic and abiotic systems showed significant differences in appearance, structure and composition (Figs. 2 and 3 and Table 2). There is evidence of accumulation of sulfide-based compounds in the presence of SRB in the biotic systems (Fig. 3B and C). The interface in the biotic system exhibits thick, hard and high mass corrosion products while the abiotic interface appears to have a flat, homogenous and rigid corrosion layer (Fig. 3A compared to Fig. 2A).

The significant amount of products observed in the biotic system is primarily due to the production of a biofilm matrix. Fig. 4 displays different surface magnifications of the biofilm structure. Microscopy confirms bacterial attachment to the metal surface (Fig. 4). The chemical composition variations of the API 5L X80 together with the presence of discrete inclusion and carbide structures (Fig. 1) produce localized electrochemical potential gradients. These gradients may promote SRB attachment and biofilm development. Addition of alloying elements, such as sulfur, which is also a microbial nutrient, has been reported to increase the low-alloy carbon steel susceptibility to MIC (Little and Lee, 2007).

Reports show sulfide inclusion sites were the most favorable sites for bacterial colonization (Little and Lee, 2007; Javaherdashti, 2008). The morphology of the SRB cells is rod-shaped with different length and sizes. The cells are of 2–20 μm in length and they interlink to form elongated thread-like structures (Fig. 4A), and Desulfomicrobium sp. can have rod or oval morphology (Javaherdashti, 2008; Madigan, 2009). Some of the SRB species possess an array of organic filaments and are potentially interconnected via putative bacterial nanowire structures (Fig. 4B). Studies have reported that bacterial nanowires are conductive and could facilitate various electron transport reactions (Sherar et al., 2011). It may be possible that these structures can be used as a method to capture electrons from the steel directly, or to capture molecular hydrogen to supplement their energy in the absence of a carbon source. Such behavior has been reported for metal-reducing bacteria such as Geobacter sulfurreducens (Gorby et al., 2006) and Shewanella oneidensis MR-1 (Reguera et al., 2005). Here, we report the potential for such as phenomenon under effectively ‘real-world’ conditions.

As shown in Fig. 4A, the biofilm contains bacterial cells and extracellular polymeric substance (EPS) as well as corrosion products. Most commonly, the EPS and corrosion products occupied 75–95% of produced biofilm volume, while 5–25% is occupied by

<table>
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<tr>
<th>wt% element</th>
<th>C</th>
<th>O</th>
<th>Na</th>
<th>Si</th>
<th>Fe</th>
<th>S</th>
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<tr>
<td>Abiotic system</td>
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<td>Whole Region</td>
<td>2.35</td>
<td>43.31</td>
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<td>1.71</td>
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<td>Dark Region</td>
<td>9.52</td>
<td>31.21</td>
<td>8.66</td>
<td>1.24</td>
<td>23.85</td>
<td>11.49</td>
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<tr>
<td>Light Region</td>
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<td>1.79</td>
<td>0.89</td>
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Table 2: Comparison of EDS analysis corresponding to the abiotic and the biotic systems, respectively.
The growth cycle in... number of cells and the growth is limited by insuf... decrease. The high production of sul... reactions, promotes the formation of iron sul... level with time. As shown in Fig. 6C, some pits resemble actual bacterial cell morphologies, which could be attributed to the potential direct contact between individual cells and steel surface. It is quite possible that SRB acquire their energy by obtaining electrons through direct contact of the steel via their apparent nanowire connections (Fig. 4B). Steel has a negative redox potential, $E_{\text{SHE}} = -0.44V$ that makes it an electron donor (Duan et al., 2008).

Some biologically produced sulfide ions will convert to hydrogen sulfide especially at acidic pH as follows:

$$\text{HS}^- + \text{H}^+ \rightarrow \text{H}_2\text{S}$$

$$(3)$$

Fig. 5 shows the dissolved sulfide variations over the period of exposure. As shown in Fig. 5, the level of dissolved sulfide increased drastically for the first 12 days and remained stable until the 20th day followed by sharp decrease. The high production of sulfide occurred during the cellular exponential growth phase until it reached a maximum value. At that stage, the bacterial growth slowed as it reached a stationary phase followed by a death phase. The growth cycle influenced the decline in sulfide level with time. During the stationary phase, there will be no increase in the number of cells and the growth is limited by insufficient nutrients and waste product accumulations (Madigan, 2009). It is postulated that the exponential phase promotes MIC because of the high bacterial activities and subsequent metabolic products. The production of hydrogen sulfide and the oxidation of iron (anodic reaction), promotes the formation of iron sulfide as follows (Javaherdashti, 2008):

$$\text{Fe} \rightarrow \text{Fe}^{2+} + 2\text{e}$$

$$\text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS} + 2\text{H}^+$$

$$(5)$$

Fe$^{2+}$ and $\text{H}_2\text{S}$ can precipitate on the steel surface depending upon the local supersaturation of iron sulfide (Singer et al., 2011). When more ferrous ions are released from the steel surface another form of iron sulfide (i.e., cubic ferrous sulfide and pyrite) could be precipitated on the steel surface depending upon the local supersaturation of iron sulfide (Singer et al., 2011).

The coupons immersed in the sulfate-reducing consortium exhibit aggressive and deeper pitting in comparison with abiotic controls as shown in Figs. 6 and 7 respectively. The metabolic activities of the sulfate-reducing consortium and associated biofilm induce higher concentrations of sulfide, phosphate-based compounds and other potentially biologically-generated, corrosion-influencing compounds that collectively enhance the corrosion process. As shown in Fig. 6C, some pits resemble actual bacterial cell morphologies, which could be attributed to the potential direct contact between individual cells and steel surface. It is quite possible that SRB acquire their energy by obtaining electrons through direct contact of the steel via their apparent nanowire connections (Fig. 4B). Steel has a negative redox potential, $E_{\text{SHE}} = -0.44V$ that makes it an electron donor (Duan et al., 2008).

$$\text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS} + 2\text{H}^+$$

$$(5)$$

In the presence of sulfide and hydrogen sulfide, a porous mackinawite layer followed by a thicker mackinawite layer develops on the steel surface (Singer et al., 2011). It appears that the mackinawite layer that initially formed on the steel surface by a solid-state reaction can then become cracked easily (Fig. 3A). When more ferrous ions are released from the steel surface another form of iron sulfide (i.e., cubic ferrous sulfide and pyrite) could be precipitated on the steel surface depending upon the local supersaturation of iron sulfide (Singer et al., 2011).

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It was reported that SRB species could use a putative pathway that involves a membrane-associated cytochrome and intracellular hydrogenase-mediated electron transfer system to acquire energy directly from iron (Duan et al., 2008; Sherar et al., 2011). The chemical and small grains associated with the microstructural carbides of the API X80 steel induce greater susceptibility to corrosion. On the other hand, less pitting corrosion was observed in the abiotic system where the polishing marks are still evident, Fig. 7.

3.3. Open circuit potential ($E_{corr}$)/polarization resistance measurements

The $E_{corr}$ as a function of time data for biotic and abiotic systems are shown in Fig. 8A. The $E_{corr}$ as function of time data revealed that in biotic medium, a shift of $E_{corr}$ toward active value at (−720 mV/SCE) occurred in the first 2 days followed by rapid positive shift of $E_{corr}$ at a value of (−580 mV/SCE) between 1 and 7 days followed by a reasonably stable region. This potential shift clearly supports that the activity and the growth of the sulfate-reducing consortium 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 sulfide and introducing multiple cathodic reactions as supported by literature (Beech and Sunner, 2004; Castaneda and Benetton, 2008; Javaherdashti, 2008). Further, in the abiotic control system, there was a notable increase of the $E_{corr}$, which then remained more or less steady at approximately −700 mV/SCE. This potential shift in the abiotic system, might be attributed to the accumulation of the growth medium constituents such as organic compounds, potassium, sulfate, sodium chloride and phosphorous on the coupon surface (Castaneda and Benetton, 2008). It is interesting to note the difference of $E_{corr}$ values of the carbon steel coupons in both systems, there was a positive shift observed (approximately 100 mV/SCE) in medium containing SRB as compared to the one in the abiotic system. This positive shift in $E_{corr}$ is known as ennoblement. It is probably the most notable phenomenon in the MIC investigations (Little and Lee, 2007). The exact mechanism of ennoblement remains unsolved (Little and Lee, 2007). Complex
deposits of microbial cells, extracellular polymers, and organic and inorganic compounds that accumulate on the metal surface accelerate corrosion by changing the electrochemical behavior of the metal. These factors collectively could result in ennoblement (Little and Lee, 2007). Different studies have attempted to understand if there is a direct link between biofilm formation and ennoblement (Dickinson et al., 1997). Others correlate ennobled potential with cell density and biological activity in the biofilm, by measuring the ATP accumulation, electron transport activity and lipopolysaccharide content (Dickinson et al., 1997). The polarization resistance ($R_p$) variations for biotic and abiotic systems are shown in Fig. 8. The $R_p$ as a function of time data for the biotic system revealed an increase to 5000 Ω cm$^2$ for the first 2 days followed by a substantial decrease to 200 Ω cm$^2$ which then remained stable throughout the period of exposure. The initial increase in the $R_p$ is correlated to development of conditioning film composed of macromolecules and other corrosion products, possibly this layer could be protective in nature. This film is important for the initial adhesion of bacterial cells (Beech and Sunner, 2004; Castaneda and Benetton, 2008). The production of sulfide by SRB species and the formation of organic compound such as extracellular polymeric substance (EPS) at the metal/biofilm interface create an aggressive environment that leads to a decrease of polarization resistance (Beech and Sunner, 2004; Kuanga et al., 2007; Castaneda and Benetton, 2008). The polarization resistance is inversely proportional to the corrosion current, which means high corrosion rate at low resistance. The corrosion rate plots over time for biotic and abiotic systems are shown in Fig. 8C. The corrosion rate for the biotic system reached a value over 60 mpy after 200 h. In the abiotic system, $R_p$ remained more or less steady at approximately 1000 Ω cm$^2$ and the corrosion rate was significantly lower and remained constant at 10 mpy.

3.4. Electrical impedance spectroscopy results

Fig. 9A displays the impedance response for a carbon steel coupon exposed to abiotic medium over time. The steady state was reached at 240 h. At low frequencies (LF), shown in Fig. 9A, the magnitude of the capacitive loop represented by the semicircle diameter decreased with time. These LF magnitudes represent the change in the charge transfer resistance ($R_{ct}$) that describes the evolution of the anodic reaction that is controlled by the charge transfer process. In the abiotic experiment, the kinetics of the anodic reaction is represented by reaction (4) with the cathodic reaction shown by reaction (2). The decrease of the $R_{ct}$ with time indicates an increase in corrosion rate (Fig. 8C) possibly due to the effect of the formation of a mixed, thin layer of sodium chloride, sulfide, potassium and carbon-based compounds as well as other corrosion products on the electrode surface (Kuanga et al., 2007; Castaneda...
and Benetton, 2008). The electrical circuit representation for the abiotic system is shown in Fig. 10A. Curve fits were based on the minimum deviation between the measured and fitted data. The circuit includes a charge transfer resistance ($R_{ct}$) for the steel surface, a constant phase element (CPE) associated with the electrical double layer and $R_s$ representing the solution resistance. This one time constant RC circuit is confirmed by the phase angle spectra, Fig. 9B, that shows peak at medium frequency (MF).

Significant changes were observed when the carbon steel was exposed to the sulfate-reducing consortium, the EIS spectra varied significantly with exposure time as shown in Fig. 11A. The low frequency (LF) magnitude represented by the semicircle diameter (Fig. 11A), significantly decreased with time. These shifts indicate substantial decrease in the charge transfer resistance ($R_{ct}$) that demonstrates that the sulfate-reducing consortium accelerated the corrosion (Fig. 8C). The bio-catalytic activities of the sulfate-reducing bacteria likely increased the corrosion rate via biofilm formation, production of sulfide and subsequent formation of conductive iron sulfide layers (Beech and Sunner, 2004; Javaherdashti, 2008). For the first 240 h, the MF response presented in the phase diagram in Fig. 11B shows one time constant, which indicates the system was under activation control processes. This behavior is attributed to the formation of an unstable conditioning layer based on a mixture of inorganic/organic compounds.

Fig. 9. EIS data for abiotic system; (A) Nyquist Plots (B) Phase angle plots.

Fig. 10. (a) Circuits models used to fit for the EIS data (A) single layer circuit R(RC) for abiotic medium (B) double layer circuit for biotic medium R(C(R(RC))).

Fig. 11. EIS data for biotic system; (A) Nyquist Plots (B) Phase angle plots.
However, after mature biofilm formation a steady state was reached, and two time constants appeared in the phase response (Fig. 11B). The equivalent circuit for biotic system is presented in Fig. 10B. It consists of two constant phase elements (CPE) that are associated with the behavior of the biofilm and the double layer capacitor, $R_{\text{film}}$ denotes the biofilm resistance, a charge transfer resistance ($R_t$) for steel surface and a $R_s$ representing the solution resistance.

The formation of conductive iron sulfide film on the surface enhanced the corrosion kinetics. This kinetic enhancement is evidenced by the decrease in the magnitude of charge transfer resistance ($R_t$) as shown in Fig. 11A. The shifts in the phase response at low frequencies revealed a higher electrical capacitance value, which confirmed the high conductivity of corrosion products. The enhanced corrosion kinetics could be attributed to the formation of a porous, conductive iron sulfide layer in the presence of SRB. Another possible reason could be due to the direct consumption of electrons from the steel surface by SRB via their electron transport pathways (Duan et al., 2008; Sherar et al., 2011).

4. Conclusions

In this study the microbiologically influenced corrosion (MIC) of API 5L grade X80 carbon steel coupons by a sulfate-reducing consortium cultivated from an oil well produced water was investigated. Elemental analysis with EDS detected phosphorous-based corrosion products on both biotic and abiotic experimental systems; however, in the presence of a SRB-biofilm consortium cultivated from an oil well produced water was investigated. Elemental analysis with EDS detected phosphorous-based corrosion products in terms of composition, levels of sulfur, and conductivity. More importantly the corrosion of the steel surface by SRB via their electron transport pathways (Duan et al., 2008; Sherar et al., 2011).

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