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

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ABSTRACT

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.



## 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 ($H_2S$) as a metabolic byproduct. Furthermore, this type of bacteria has the

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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) linepipe 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 by 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 (1.0 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).

**Table 1**
The chemical composition of API-5L X80 carbon steel coupons.

| C | Mn | Ti | S | P | Fe |
|---|---|---|---|---|---|
| 0.07 | 1.36 | 0.008 | 0.003 | 0.004 | Balance |

### 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 $mm^2$. 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 $10^8$ cell/ml. The electrochemical cells were purged

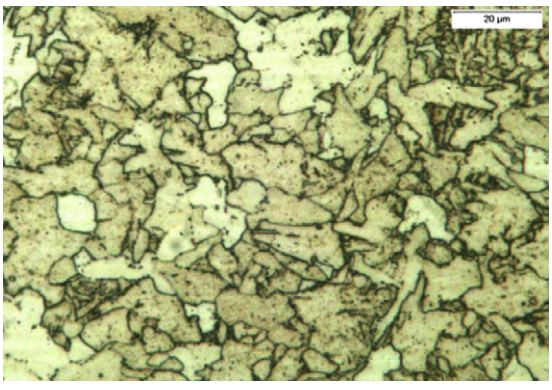


**Fig. 1.** API X80 linepipe steel micrograph showing an irregular fine-grained ferritic microstructure.