

Split Chamber Zero Resistance Ammetry Technique (SC-ZRA) for Detecting and Quantifying Microbially Influenced Corrosion in Oil and Gas Systems

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ABSTRACT

Microbiologically influenced corrosion (MIC) is a common problem in the oil and gas industry. MIC is an immense threat especially inside of pipelines, especially by Sulfate Reducing Bacteria (SRB). For this purpose, MIC by SRB was investigated in experiments using split-cell zero resistance ammetry (SC-ZRA) measurements. To understand the extent and activity of MIC by SRB, various experiments were conducted where carbon steel coupon was deployed in laboratory synthesized media, which was formulated from the oil and gas pipeline sludge using elemental analysis. SC-ZRA incubations were inoculated with SRB enrichment isolated from contaminated pipeline sludge. One side of the SC-ZRA is inoculated with SRB enrichment (WE1) and the other side of the SC-ZRA is kept sterile (WE2). Current and potential between the two coupons was measured. Series of batch experiments are also conducted with the same enrichment and synthetic media. When SC-ZRA was conducted for 30-days, biofilm attachment was not observed under scanning electron microscopy (SEM) as the biofilm was heavily encrusted by corrosion products. When the experiment was conducted for 17-days, some microbial attachment was observed but the organisms were covered with iron sulfide (corrosion product), which was formed as a byproduct due to microbial metabolism. Consequently, intense corrosion and pitting was observed on the coupon exposed to the SRBs in the 30-days run. Weight loss for WE2 was higher than WE1 at the end of the experiment. For the 17-days run, no pitting was observed for the inoculated coupons, and the WE1 side had a higher mass loss when compared to the WE2 side. SC-ZRA technique can be used to characterize microbial activity, in this work with SRB. This technique can also be used as a quick monitoring tool for detecting the extent of microbial corrosion for the oil and gas systems.

INTRODUCTION

Microbiologically influenced corrosion (MIC) is one of the most aggressive forms of corrosion leading to infrastructure and equipment damage in various industries, including but not limited to the oil and gas industry,¹ water systems,² medical devices,³ marine environments,⁴ nuclear waste storage facilities,⁵ and aviation fuel systems⁶ and storage.⁷ MIC received great attention because of the increasing cost associated with corrosion damage, particularly in oil and gas industry. The overall cost of corrosion has been estimated to be approximately 3.4% in the global Gross National Product⁸ which equates to approximately \$2.9 trillion in 2018.⁹ A conservative estimate shows that 20% of all corrosion in aqueous system is MIC,¹⁰ which accounts to billions of dollars in the US.

The common types of bacteria associated with MIC are sulfate-reducing bacteria (SRB), iron and CO₂ reducing bacteria and iron and manganese oxidizing bacteria.^{11,12} SRB are generally considered the most aggressive group of bacteria in pipeline systems that causes MIC and pitting, especially of steel in the oil and gas industry.^{13,14} Therefore, SRBs are the most widely studied bacteria because of their practical importance.¹⁵ The sulfide formed by SRB activity is reported as a chemically aggressive^{16–19} compound resulting in CMIC (chemically microbially influenced corrosion) where the SRB acts indirectly on the iron surface by chemical reaction.²⁰ By definition, all SRB can influence corrosion through excretion of chemical H₂S if sulfate and suitable electron donors are present. SRB biofilms on metal surfaces are usually composed of sessile cells, extracellular polymeric

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substances (EPS) and corrosion products.^{21–23} Underneath the SRB biofilm there may be a local shortage of organic carbon (such as lactate), so SRB may switch to elemental iron in carbon steel for the reduction of sulfate.²⁴ Chen et al. also showed that SRB in biofilm when trapped under the FeS crust could survive longer in the absence of organic electron donors by harvesting electron from the carbon steel surface.²⁵ The study further suggested that SRB causes serious pitting corrosion, and the biofilm was protective against uniform corrosion. Furthermore, the attached cells are mostly distributed around or inside the pits.²⁵ Gu et al. suggested that this oxidation of iron occurs extracellular, while sulfate reduction occurs intracellular under enzyme catalysis in SRB cytoplasm.^{13,24,15} Gu et al. suggested the biocatalytic cathodic sulfate reduction (BCSR) theory [need reference], which explains the use of extracellular electrons in carbon steel by SRB MIC for energy generation. Furthermore, the electron transfer direction in this case is the same as that between a cathodic biofilm and the cathode surface in a Microbial Fuel Cell that uses a biocathode for electron utilization, it is also known as the biocathode theory.²⁶ Furthermore, Gu and Xu they introduced the extracellular electron transfer (EET) is required to transport electrons across the cell SRB cell wall,²⁶ calling the SRB MIC as an example of EET-MIC.¹⁹ To transport electrons between an external solid surface and a cell's outer surface, cells use two forms of electron transfer methods:^{27,28} direct electron transport (DET) and mediated electron transport (MET).

Most of the laboratory-based MIC related corrosion studies are previously conducted in the same vial or chamber and the chemical compositions were altered to understand microorganism behavior. There are few work done using different chambers (such as Microbial Fuel Cell(MFC)), to characterize MIC.^{29,15} Previously, two compartment cell was used to study MIC,³⁰ where the compartments were biologically isolated and electrically continuous. Similar idea was also used to create a bacterial battery induced by SRB and study MIC of steel.³¹ However, the mechanism was derived based on the classic cathodic depolarization theory was questioned by a lot of the authors and has recently been deemed as disputable. Also, zero resistance ammetry (ZRA) technique approach was used by many authors elucidating mechanisms of MIC and proposed as MIC detection and monitoring approaches.^{32–35} However, a "preconditioning current" was used in many of these cases to polarize the electrodes and add an artificial galvanic current between the cathode and anode, resulting in the anode being protected and just slightly corroded.^{33,34} Electrochemical noise analysis, which involves both similar electrodes and environments and does not require preconditioning of electrodes, has been suggested as a MIC monitoring technique, where ZRA-measured electrochemical noise is indicative of metal surface alteration.^{33,34,36,37} Electrochemical noise analysis, which involves both similar electrodes and conditions and does not need preconditioning of electrodes, has been suggested as a MIC control technique, where ZRA-measured electrochemical noise is representative of metal surface change.³² The split-chamber setup has the advantage of distinguishing various conditions and allowing one to track the passage of electrons from a biofilm-protected region of metal to an exposed area of metal by allowing the biofilm to shield the metal in the inoculated chamber (WE1) and not preconditioning the metals. By using the ZRA technique, Miller et al. discovered that the actions of *S. oneidensis* can both prevent and accelerate steel corrosion based on the presence and distribution of biofilm as previously the influence of Fe(III) reducing bacteria on corrosion of carbon steel was inconclusive.³⁸ They also used the ZRA technique to show findings that suggested, under nitrate-reducing conditions, uniform and pitting carbon steel corrosion can occur due to nitrite accumulation and the use of steel-Fe(0) as an electron donor, but conditions of prolonged nitrite accumulation can lead to more aggressive corrosive conditions.³⁹ The author in another work using ZRA technique discovered a new mechanism of MIC called aqueous/non-aqueous MIC (ANI-MIC), where fungi isolated from contaminated B20 biodiesel storage tanks, incubations with a fungus whose filaments penetrated the diesel fuel coating caused the most corrosion on both coupons, while species that did not investigate the fuel caused corrosion only on the coupon immersed in water and not on the coupon immersed in the fuel.⁷ Following ZRA studies, it was discovered that current between steel coupons could only be accomplished when fungal hyphae reached the non-aqueous substrate, resulting in the most severe corrosion of the coupons.

The previously derived SRB mechanism can be further studied by having the inoculated environment electrically connected with the sterile environment, in a separate chamber, to understand the SRB corrosion. In this work we are using split cell zero resistance ammetry (SC-ZRA) technique to characterize the mechanism of SRB by altering the environments in each chamber to understand the SRB behavior. ZRA and potential measurements can be used in combination with microbiological activity assessments. The current magnitude and direction may be representative of the mechanisms and scope of MIC. We propose to use the SC-ZRA setup to determine the electrochemical signatures of MIC, which mimics the heterogeneous biofilm coverage of metal surfaces that leads to MIC. As a result, this technique may include a mechanistic understanding and a monitoring tool for corrosion of metals that are exposed to a variety of redox regimes, geochemical conditions (e.g., pipes), or alternate O₂ supply (e.g., coastal structures).

MATERIALS AND METHOD

SRB enrichment cultivation

In order to grow the Sulfate-Reducing Bacteria (SRB), we made use of a pigging sludge sample obtained from the Pipelines and Hazardous Materials Safety Administration (PHMSA) and used it as an inoculum for a brine-based media that reflected the water chemistry of the pipeline interior, with several additional components to allow for optimal growth of Sulfate-Reducers. This media consisted of (900 mL Milli-Q water, 350 mM NaCl, 30 mM NaHCO₃, 25 mM CaCl₂, 15 mM MgCl₂, 2mM KCl, 10 mM Na₂SO₄, 0.05 mM K₂HPO₄, 20 mM Sodium Lactate, Wolfe's Vitamin and Mineral Solution (10 mL each), Yeast Extract (0.7 grams/L), (NH₄)₂SO₄ (0.3 grams/L), CaSO₄ (0.04 grams/L), C₃H₈ClNO₂S (0.2 grams/L), MgSO₄·7H₂O (0.2 grams/L), C₆H₈O₆ (0.1 grams/L)). We systematically transferred the enrichment to fresh stock for several months, in order to ensure that there were no lingering organisms in the solution that might affect the experiment, and that we would inoculate the ZRA with a pure SRB culture. In preparation for a SC-ZRA experiment, we prepare 100 mL of the pure bacteria that is suspended in 1000 mL of the brine-based media and grown to the beginning of the stationary phase: taking approximately 4 days. Cells are then harvested by centrifugation and washed 3-4 times with a brine that acts as the water chemical backbone to the brine-based media minus the components that allow for optimal growth of Sulfate-Reducers. We then resuspend the cells in the brine and use optical density to determine whether the cell density is appropriate for addition into the SC-ZRA system. This is done using a Helios UV/VIS spectrophotometer (Thermo-Fisher Scientific, Waltham, MA) and taking measurements at a wavelength of 600 nm. For our SC-ZRA experiments, cells are grown to the beginning of the stationary phase (O.D = ~1.000) with a final volume of 50 mL (10 % by volume inoculum).

Corrosion Incubations

Carbon Steel (UNS G10180) coupons were prepared for SC-ZRA experiments by first polishing them progressively finer SiC papers, including 240, 320, 400, and 600 grits as described in ASTM standard E1558-09.⁴⁰ Approximately the total surface area exposed to the media is 652 mm² for the carbon steel coupon. After obtaining an initial weight, the carbon steel coupons were placed in the SC-ZRA apparatus. Air was removed from chambers by vacuum and replaced with nitrogen gas. Next, the SC-ZRA apparatus with steel coupons was placed in an oven at 160 °C for 4 hours. This process sterilizes the metal while minimizing alteration of the surface metal, which occurs during other common sterilization approaches (e.g., autoclaving).⁴¹

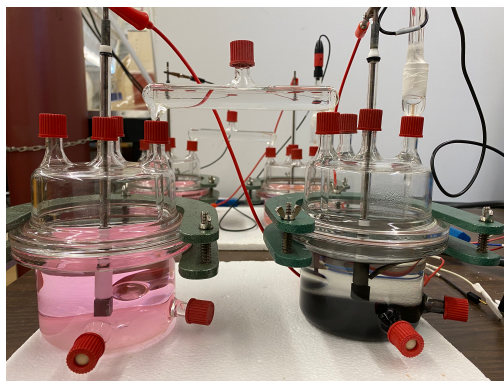


Figure 1: Split-Cell Zero Resistance Ammetry (SC-ZRA) Setup

To evaluate steel corrosion in the split chamber format, 450 mL of synthetic media was added to both the split cells in a glove bag to maintain anaerobic environment where the chambers were sealed tight to avoid any contact of oxygen. The synthetic media consists of (per liter): 350 mM NaCl, 30 mM NaHCO₃, 25 mM CaCl₂, 15 mM MgCl₂, 2mM KCl, 10 mM Na₂SO₄, 0.05 mM K₂HPO₄, 20 mM Sodium Lactate, Wolfe's Vitamin and Mineral Solution (10 mL each). This synthetic media was formulated to mimic the fluid chemistry of the sludge retrieved from pigging sludge of oil and gas pipelines. The two chambers of the SC-ZRA are separated by a salt bridge filled with saturated potassium chloride solution. Polished working electrodes (referred as WE1 and WE2) were included in the two cells, with a saturated calomel electrode (SCE) reference electrode deployed in the cell containing WE1.

Two sets of experiments were conducted: one set contains H₂/N₂ mixture in the headspace and the other set of

experiments have CO₂/N₂ mixture in the headspace. Current and potential were recorded using a Biologic potentiostat/galvanostat, Model VSP-300 (SN 0001). Samples were removed periodically from both chambers to measure pH, sulfate/Sulfide concentration and lactate concentration. Samples taken from the split cells were used to compare the bacterial growth with the evolution of the electrical parameters. In this configuration, a positive current represents flow of electrons from WE1 to WE2.³⁸ Galvanic potential readings were collected in fifteen-minute intervals during operation of the split-chamber experiments. For experiments that included cells, chamber WE1 received an inoculum of concentrated SRB cells that were prepared as described above. At the conclusions of batch and ZRA experiments, the steel coupons were removed subjected to weight loss analysis (described below).

Batch experiments were conducted using flat carbon steel coupons that have been prepared by polishing using progressively fine SiC papers including 240, 320, 400 and 600 grit, as described in ASTM standard in E1558. Batch experiments were run in triplicates where the coupons were immersed in serum tubes of 20 ml with synthetic media solution. A 10% volume of inoculum of synthetic media water with nutrients and SRB bacteria was added to the bottles and chambers at an optical density of 1.17 at 600 nm, after being washed with synthetic media water three times. The set-up was prepared in a glove compartment and the headspace was filled with nitrogen to maintain anaerobic conditions and mimic the same environment as the ZRA experiments. These batch experiments were incubated for 32 days, and samples were periodically recovered. Cells were removed from suspension by centrifugation, and the supernatant was removed for subsequent measurements of lactate and anion concentrations.

Corrosion rates were determined by weight loss analysis (WLA) using ASTM method G1-03.⁴² Samples were rinsed in deionized water, wire brushed, and then, immersed in Clarke's Reagent (1000 mL 12.1 M HCl, 20 g antimony trioxide, and 50 g stannous chloride) for 30 seconds to remove surface oxides. After the Clarke's Reagent bath, the coupons were rinsed with DI water, dried, and weighed. The Clarke's reagent wash, DI wash, and weighing were repeated until no mass was lost between wash cycles, indicating that all oxides were removed.⁴² The total mass loss was recorded indicating the physical weight that was lost due to corrosivity of the environment. Corrosion rate was calculated using the following equation:

$$CR = \frac{W \cdot K}{D \cdot A \cdot t}$$

where CR represents the corrosion rate in mm/yr., K (8.76 × 10⁴) is a dimensionless constant, W is the mass loss in grams, A is the exposed surface area in cm², T is exposure time in hours and D is the density of carbon steel UNS G10180 in g/cm³.⁴²

Lactate was quantified by high-performance liquid chromatography^{†1} by using an equipped with an HPX-87H column (300 mm × 7.8 mm)^{†2} with UV (254 nm) detection (SPD- 10A). A mobile phase of 0.008N H₂SO₄ was used at a flow rate of 0.6 mL/min. pH was measured using STARA2150.^{†3}

Sulfate concentration was quantified using ICS-1100^{†1} with AS-DV Autosampler equipped with liquid eluent, a high-pressure pump, a sample injector, a guard and separator column, a chemical suppressor, a conductivity cell, and a data collection system. Analyzation is processed by using a software.^{†4} Samples for measurement of sulfide were preserved with equal volume of a zinc acetate solution (100 g/L) and sulfide was quantified. ZnS was separated from the fluid by centrifugation, solids were resuspended in deionized water, and sulfide was measured using the methylene blue assay.⁴³ The measured results for all the liquid samples were averaged from 4 to 6 measurements, 2 for each system.

A scanning electron microscope was used to test coupons retrieved from incubations (both with and without the SRB biofilm).^{†5} Coupons were immersed in anoxic glutaraldehyde solution (2 percent w/w) for 1 day at 4 °C, dehydrated in an ethanol series (0 percent, 20 percent, 50 percent, 75 percent, and 100 percent), and placed in a desiccator for 1 day at room temperature to fix biofilms. After performing weight loss analysis, coupons are analyzed for surface characterization (pitting) under IFM.^{†6}

^{†1} Agilent 1200 system, Agilent Technologies, Inc. Santa Clara, CA

^{†2} Aminex, Bio-Rad Laboratorios, Inc. Hércules, CA

^{†3} Thermo Scientific's Orion Laboratory instrument

^{†4} Chromeleon Chromatography Management System

^{†5} Tescan LYRA-3 XMU FIB-FESEM system

^{†6} Alicona InFocus G5 instrument

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RESULTS AND DISCUSSION

SRB is the most studied and investigated group of bacteria causing severe corrosion inside of pipelines especially the oil and gas industry. To understand the mechanism of SRB corrosion with carbon steel, a series of SC-ZRA experiments with different environments along with biotic and abiotic controls were tested (Table 6-1). As previously mentioned, most of previous studies were conducted in the same chamber on the same coupon under different circumstances such as electron donors, electron acceptors, pH, headspace, flow and length of experimental run. Conducting experiments in the same reaction chamber does not allow to study the anodic and cathodic sites exclusively. Furthermore, the mechanistic idea of SRB activity is highly debated among many scholars.^{20,44,9} The benefit of conducting SRB studies with the SC-ZRA setup provided the ability to study how the heterogeneous biofilm formation of SRB providing a better mechanistic idea of SRB by analyzing the inoculated (WE1) and sterile (WE2) region under continuous monitoring of current and potential signals. In another words, comparing how the biofilm formed region acts with an adjacent area which is not covered by biofilm in real-time. Since the SC-ZRA technique connects the two chambers (WE1 and WE2) electrically but separates them chemically, this allows to study the “real world” scenario, where biofilm coverage is likely to be heterogeneous.²⁵ To have a better understanding of the mechanism of SRB observed in the SC-ZRA setup, figure 4 will be used to explain the current signals in correlation with the activity of SRB into 3 different regions. Corrosion rates of each coupon were reported as well as the ratio of corrosion rates between WE2 and WE1 (Table 6-1).

During SC-ZRA studies that did not involve cells in either chamber (ZRA 1), similar rates and extents of corrosion were reported for WE1 and WE2, and the corrosion rate ratio (CR_{WE2}/CR_{WE1}) was about 1, showing that the same anaerobic corrosion reactions were occurring in both chambers. Likewise, for the electrochemical signature from the SC-ZRA, minimal current was observed indicating little or no electron transfer between the two chambers (Figure 2). This result indicated that when both chambers were under identical environmental conditions, there was little or no electrochemical driving force to measure a net current between the two electrodes.

Table 1: Various environments of SRB incubation, showing the run-time, and corrosion rate.

ZRA Experiment	Chamber/Electrode	SRB	Run Time (days)	Lactate	Sulfate	I_{max} (uA)	Corrosion Rates (mils/year)	CRR WE2/WE1
ZRA 1	WE 1	Sterile	21	Lactate	Sulfate	2	2.02	0.98
	WE 2	Sterile		Lactate	Sulfate		1.98	
ZRA 2	WE 1	SRB	30	Lactate	Sulfate	15.3	3.81	1.77
	WE 2	Sterile		Lactate	Sulfate		6.75	
ZRA 3	WE 1	SRB	17	Lactate	Sulfate	19.4	1.28	0.16
	WE 2	Sterile		Lactate	Sulfate		0.21	
ZRA 4	WE 1	SRB	30	No Lactate	Sulfate	-8.8	1.10	4.41
	WE 2	Sterile		No Lactate	Sulfate		4.85	
ZRA 5	WE 1	Sterile	14	Lactate	No Sulfate; added Sulfide	-10.2	1.98	4.19
	WE 2	Sterile		Lactate	No sulfate		8.29	
ZRA 6	WE 1	SRB	50	lactate	Sulfate	13.6	-1.00	-0.99
	WE 2	SRB		No Lactate	Sulfate		0.99	
ZRA 7	WE 1 (Dialysis Bag)	SRB	50	Lactate	Sulfate	20.2	5.70	0.39
	WE 2 (Dialysis Bag)	Sterile		Lactate	Sulfate		2.23	

Upon delayed inoculation (48 hours) of SRB to WE1 side of the SC-ZRA (ZRA 2), a positive galvanic current ($I_{max} = 15.3 \mu A$) was observed (Table 6-1) indicating that electrons were flowing from the WE1 side to the WE2 side. After 14 days of the ZRA 2 run, the galvanic current flow switched, indicating that the electrons now were flowing from the WE2 side to the WE 1 side for the rest of the 30 days run of the experiment. This can be further indicated that for the first half of the experimental run, the WE1 side is acting as the anode and the WE2 side is acting as the cathode. After 14 days, the anodic and cathodic behavior of the electrodes switched. This phenomenon will be explained further with perspective from both experimental run and possible mechanistic point of view. Furthermore, it was observed that the corrosion rate of WE2 side was 6.75 mils/year and 3.81 mils/year for the WE1 side. The corrosion rate ratio (CR_{WE2}/CR_{WE1}) was 1.77 when WE1 was inoculated with SRB in the presence of lactate and sulfate, with a maximum coupled current was 7.2 times higher than control trials. Consecutively the depletion of lactate was observed at 0.55 mM/day and the depletion of sulfate at a rate of 0.29 mM/day (Table 6-2). The ratio of lactate depletion to sulfate was approximately 2:1. SRB growth and corrosion was evident in anerobic environment as the pH was observed to be in the neutral region, which are consistent with previous studies.⁴⁵ Furthermore, SRB metabolism was evident by the formation of FeS resulting in the solution turning black and the metal coupons were